Biota Neotropica 14(3): e20140069, 2014
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
thematic revision

A review on macrobenthic trophic relationships along subtropical sandy shores in southernmost Brazil

Raphael Mathias Pinotti[1,2,3]*, Diogo Marroni Minasi*[1]*, Leonir André Colling*[1] *& Carlos Emílio Bemvenuti*[1]

[1]*Laboratório de Ecologia de Invertebrados Bentônicos, Instituto de Oceanografia, Universidade Federal do Rio Grande - FURG, Rio Grande, RS, Brazil.*
[2]*Programa de Pós-Graduação em Oceanografia Biológica, Instituto de Oceanografia, Universidade Federal do Rio Grande - FURG, Rio Grande, RS, Brazil. CNPq Postgraduate grant.*
[3]*Corresponding author: Raphael Mathias Pinotti, e-mail: pinottirm@gmail.com*

PINOTTI, R.M., MINASI, D.M., COLLING, L.A., BEMVENUTI, C.E. **A review on macrobenthic trophic relationships along subtropical sandy shores in southernmost Brazil.** Biota Neotropica. 14(3): e20140069. http://dx.doi.org/10.1590/1676-06032014006914

Abstract: Main trophic relationships that occur along the exposed sandy shores in southernmost Brazil (~33° S) are established taking into account several biological compartments operating at morphodynamically distinct environments. Beaches are predominantly of the intermediate type but some stretches of coastline are truly dissipative (*Cassino Beach*) or tending-to-reflective (*Concheiros Beach*), presenting thus diverse biological compartments and inhabitant macrobenthic assemblages. Dense concentrations of the surf-zone diatom *Asterionellopsis glacialis* are responsible - at least for the intermediate shorelines - for the most year-round primary production, sustaining several consumers up to tertiary level. Among them, bivalves *Amarilladesma mactroides*, *Donax hanleyanus* and the hippid crab *Emerita brasiliensis* can account for more than 95% of all the surf-zone secondary production, in addition to the elevated biomass of the suspension-feeder polychaete *Spio gaucha* and the scavenger isopod *Excirolana armata*. Crabs, whelks, carnivorous polychaetes, seabirds and surf-zone fishes may also be present and occupy superior trophic levels depending on the beach morphodynamics. Based on the high species richness, abundance and the role of macrobenthic fauna in transferring matter and energy to seabirds and the surf-zone fish assemblages, we address this important issue on the Southwestern Atlantic ecology. Conservation efforts should be implemented for the southernmost Brazilian sandy shores, at least for those non urbanized areas.

Keywords: *exposed beach, intermediate states, primary production, secondary consumers, conservation.*

PINOTTI, R.M., MINASI, D.M., COLLING, L.A., BEMVENUTI, C.E. **Revisão sobre as relações tróficas macrozoobentônicas ao longo da costa arenosa subtropical do extremo sul do Brasil.** Biota Neotropica. 14(3): e20140069. http://dx.doi.org/10.1590/1676-06032014006914

Resumo: As principais relações tróficas observadas ao longo da costa arenosa exposta do extremo sul do Brasil (~33° S) são estabelecidas levando-se em consideração os vários compartimentos biológicos que operam em ambientes morfodinamicamente distintos. Estas praias são predominantemente do tipo intermediário, embora alguns trechos sejam dissipativos (Praia do Cassino) ou apresentem tendências reflectivas (Praia dos Concheiros), possuindo compartimentos biológicos diversificados e associações macrobentônicas residentes. Densas concentrações da diatomácea *Asterionellopsis glacialis* são responsáveis - pelo menos para as praias intermediárias - por grande parte da produtividade primária anual, sustentando vários consumidores até o nível terciário. Entre estes, os bivalves *Amarilladesma mactroides*, *Donax hanleyanus* e o crustáceo *Emerita brasiliensis* são responsáveis por mais de 95% de toda a produção secundária da zona de surfe, em adição à elevada biomassa do poliqueta suspensívoro *Spio gaucha* e do isópode necrófago *Excirolana armata*. Caranguejos, gastrópodes, poliquetas carnívoros, aves marinhas e peixes da zona de surfe também podem estar presentes, ocupando níveis tróficos superiores dependendo da morfodinâmica praial. Baseado na alta riqueza de espécies, na abundância e no papel da macrofauna bentônica em transferir matéria e energia para as aves marinhas e as assembléias de peixes da zona de surfe, esta importante questão ecológica do Atlântico Sudoeste foi investigada. Esforços de conservação devem ser implementados nas praias arenosas do extremo sul do Brasil, pelo menos naquelas áreas não urbanizadas.

Palavras-chave: *praia exposta, estágios intermediários, produção primária, consumidores secundários, conservação.*

Introduction

Coastlines around the globe are dominated by sandy shores, dynamic environments largely controlled by physical factors like waves, tides, sand grain size and slope (McLachlan & Brown 2006). Processes and interactions of such factors can result in a wide range of morphodynamic states - from reflective to dissipative - although most beaches are intermediate between these extremes (Short 1996): reflective beaches are coarse-grained, steep and narrow environments whereas dissipative beaches present finer sediments, flat slopes, large wave periods and extensive surf zones.

Species richness, total abundance and biomass of the macrobenthic fauna along sandy shores tend to increase from reflective to dissipative states (Defeo & McLachlan 2005, 2011, McLachlan & Dorvlo 2005). Both *Swash Exclusion Hypothesis* (SEH: McLachlan et al. 1993) and latter the *Habitat Harshness Hypothesis* (HHH: Defeo et al. 2001, 2003) have already predicted such variations in population dynamics and life history traits. The *Habitat Favorability Hypothesis* (HFH) further suggests that benign (i.e. dissipative) environments or sites undisturbed by human activities may significantly favor intraspecific interactions and density-dependent processes (Caddy & Defeo 2003) being therefore considered more suitable to the inhabiting macrofauna (McLachlan et al. 1995).

Threats to sandy beach ecosystems are amplified by human over-exploitation of resources and population shifts towards the cost (Roberts & Hawkins 1999), inducing a wide source of stressors ranging from local (e.g. trampling, sewer discharge) to global impacts (e.g. sea-level rise, global warming) acting at multiple spatial-temporal scales (Defeo et al. 2009). Such human-induced threats can significantly endanger the sandy beach macrobenthic fauna and subsequently affect their complex food webs (McLachlan et al. 1981, Lercari et al. 2010) given their critical role in transferring energy and regenerating nutrients (Soares et al. 1997, Cisneiros et al. 2011).

Food webs at sandy environments are mainly based on marine sources like wrack and carrion (Colombini & Chelazzi 2003, Dugan et al. 2003), microphytobenthos (Delgado 1989) and surf-zone diatoms (Odebrecht et al. 2013) which support (*i*) an independent interstitial food web; (*ii*) a surf-zone microbial loop; and (*iii*) a macroscopic food web (McLachlan et al. 1981, Heymans & McLachlan 1996). The knowledge about the food web structure and trophic relationships on sandy shores has growing with the implementation of stable isotopes (SI) techniques (Colombini et al. 2011) and recent studies have evidenced that the number of trophic pathways and food web complexity are strongly linked to morphodynamics, increasing significantly from reflective to dissipative environments (Lercari et al. 2010, Bergamino et al. 2011, 2013).

Early quali-quantitative studies on both intertidal and surf zones indicated that the sandy beaches from southernmost Brazil are among the richest and highly productive sandy environments (Gianuca 1997a) but secondary production data are still lacking to build trophic pathways similar to those reported nearby e.g. the Uruguayan coastline (Lercari et al. 2010). This comprehensive review includes both published and unpublished data aiming to evidence the major trophic relationships and empirically trace the flows of matter through the food web compartments along the southern Brazilian sandy shores, serving as background information for further SI research or management of stakeholders.

Study area overview

1. *Beach morphodynamics*

Subtropical exposed sandy shores in southernmost Brazil extend along 220 km of a continuous NE-SW coastline between Rio Grande (32°10' S) and Chuí (33°45' S) and present distinct morphodynamic patterns (Calliari et al. 1996, Pereira et al. 2010): (*i*) immediately south of 32° S, *Cassino Beach* presents fine sandy substrates and dissipative characteristics; (*ii*) along ~40 km of coastline - between 33°20' S and 33°35' S - *Concheiros Beach* presents bimodal substrates (shell fragments and medium/fine sands) and tending-to-reflective beach states; and (*iii*) the remaining coastline, presenting extensive beach widths (40-120 m), large swash zones (10-50 m), gentle slopes (1/15-100), fine quartz sands (~3.0 phi) and intermediate morphodynamic states with intermediate/moderate low mobility (Figure 1).

2. *Coastal occupation & sandy shore threats*

The Brazilian southern state of Rio Grande de Sul (RS) has one of the least developed shores in the country and less than 10% of the state population (~11 10^6 habitants) living along 620 km of an almost continuous coastline: most of developed coastal cities are concentrated in its northern region while just a few balnearios-like-towns are found southwards separated by long and "wild" shore segments (Esteves et al. 2000). The southern urbanized beaches of *Cassino*, *Hermenegildo* and *Barra do Chuí* are moderately used by local habitants and fishermen the year-round but heavily frequented by tourists between late austral spring and early autumn (December-April) (Figure 2).

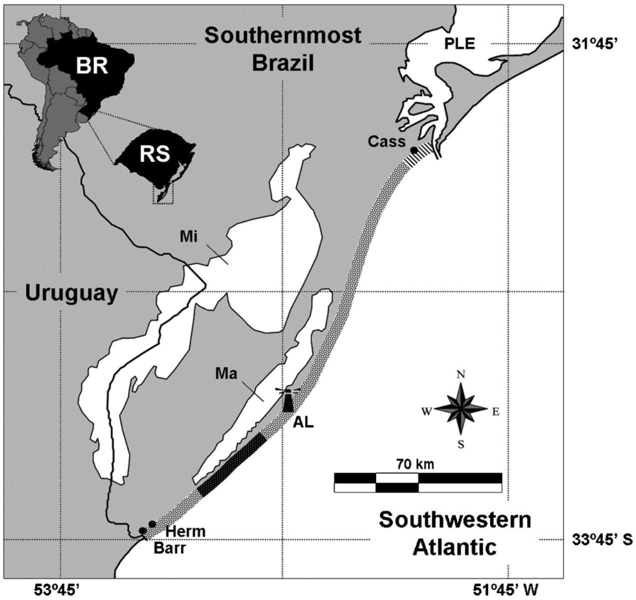


Figure 1. Subtropical sandy shores along the southernmost Brazil, presenting three sectors with distinct morphodynamic patterns: northern dissipative beaches (striped coastline); tending-to-reflective environments southwards the Albardão Lighthouse (AL), near the Concheiros Beach (dark coastline); and the remaining intermediate sandy shores (grainy coastline). The urbanized areas of Cassino Beach (Cass), Hermenegildo (Herm) and Barra do Chuí (Barr) are located. PLE: Patos Lagoon Estuary; Mi: Mirim Lagoon; Ma: Mangueira Lagoon.

http://dx.doi.org/10.1590/1676-06032014006914

Figure 2. Contrasting degrees of morphodynamic characteristics and human occupation along the exposed sandy shores of southernmost Brazil: at the dissipative Cassino Beach, an intense traffic of vehicles and tourists occurs throughout the warmer months (a) in opposition to those "wild" southward segments of intermediate (b) and tending-to-reflective shores like the Concheiros Beach (c).

Despite the relatively low human occupation, several human-induced threats have exponentially endanger the macrobenthic fauna along this coastal area: artisanal overfishing, mainly over the yellow clam *Amarilladesma* (= *Mesodesma*) *mactroides* and the ghost shrimp *Sergio mirim*; grooming and tourist trampling during the summer months; heavy traffic of vehicles including regular cars, ORVs and heavy trucks; inland freshwater discharge from agricultural and ranching activities; silviculture of exotic trees like *Pinus* sp.; intense harbor activities and dredge operations within the nearby Patos Lagoon estuary, increasing respectively the potential risk of biological invasions and mud depositions on *Cassino Beach*; and offshore oil-drilling operations to be implemented in the near future.

In addition, the sea-level rise due to global warming and the risk of macrobenthic strandings due to extreme weather events can potentially increase the list of local threats. Exotic macrobenthic species were not reported yet in the Southwestern Atlantic sandy shores but this may reflect a poor sampling coverage rather than lack of biological invasions (Orensanz et al. 2002, Defeo et al. 2009).

3. *Wave climate & weather events*

The southern Brazilian beaches are exposed to moderate/strong wave action (*Hb*: 0.5-5.5 m; *T*: 6-18 s) under the influence of mixed-diurnal microtides (< 1 m; annual average ~ 0.5 m) and thus classified as wave-dominated (Relative Tide Range < 3; *sensu* Masselink & Short 1993).

Wave climate can be divided into three major groups (Tomazelli & Villwock 1992, Figueiredo 2013): (*i*) swell waves of high amplitude and long period (mainly from S-SE); (*ii*) sea waves of short period (mainly from NE-E); and (*iii*) rare storm waves of high energy generally associated to storm surges. A bi-seasonal behavior is found superimposed on those overall morphodynamic patterns owing to the wave climate (Calliari & Klein 1993): spring/summer accretion profiles (low swell) versus autumn/winter erosive profiles (storm waves).

Extreme weather events such as storm surges and waves associated or not to cyclogenesis (Parise et al. 2009) are responsible for significant beach erosion rates (40-63 $m^3 m^{-1}$) and dune field retractions (up to ~ 5 m), raising temporally the sea-level to the base of dunes (> 2.0 m) especially when associated to spring tides (Calliari et al. 1998, Barletta & Calliari 2003, Tabajara et al. 2004). The wind regime over the region (Krusche et al. 2003) presents predominance of NE winds during the spring/summer seasons (October-March) and strong southerly winds during the autumn/winter seasons (April-September). The rainfall regime is highly influenced by frontal systems and cyclogenesis (Rao & Hada 1990, Diaz et al. 1998) and positively improved by ENSO *El Niño* phenomena (Grimm et al. 1998).

Macrobenthic compartments

1. *Zonation patterns*

The vertical distribution of the macrobenthic fauna along the southern Brazilian coast follows a typical zonation pattern (Borzone & Gianuca 1990, Gianuca 1997a): foredunes, as the terrestrial/marine interface; supralittoral zone (or supratidal), dry-sand areas only inundated during extreme weather events (e.g. storm surge/waves); mesolittoral zone (or intertidal) including here the swash zone, frequently inundated by regular tides; and the infralittoral (or surf zone), divided into the inner surf zone (from the lower swash towards the 2 m isobath) and the outer surf zone (2-10 m isobaths).

Macrobenthic zonation may change according to the type of beach as the number of zones increase from reflective to dissipative states (Defeo et al. 1992, Jaramillo et al. 1993). Nevertheless, temporal fluctuations may difficult the recognition of such zonation schemes - especially along microtidal shores - given the unpredictable tidal movements and the seasonal distribution of species across the beach profile (Brazeiro & Defeo 1996).

Along the intermediate sandy shores of southernmost Brazil (e.g. *Cassino Beach*), macrobenthic zonation was characterized as very dynamic through seasons despite the general 3-zone pattern (Neves et al. 2007): (*i*) supralittoral, occupied by burrows of the ghost crab *Ocypode quadrata*; (*ii*) upper mesolittoral, inhabited by the polychaete *Thoracophelia furcifera* (= *Euzonus furciferus*) and the isopod *Excirolana armata*; and (*iii*) a large lower zone including the lower mesolittoral and the inner surf zone, which was characterized by the intertidal migrants *Amarilladesma mactroides* and *Donax hanleyanus* in addition to several infralittoral species.

2. *Beach morphodynamics and macrobenthic assemblages*

The beach and surf zone of intermediate and dissipative coastlines may be characterized as a single semi-closed and self-sustaining ecosystem (*sensu* McLachlan 1980) in which the relative importance of each food web component may differ significantly between the extremes of a morphodynamic continuum (McLachlan & Brown 2006): whereas beaches with little or no surf zone (i.e. reflectives) are nutrient sinking environments with low *in situ* primary production, beaches with extensive surf zones (i.e. intermediate/dissipatives) are highly productive environments driven by an intense phytoplanktonic primary production. As a consequence, components of nekton, benthos, plankton and microbial communities tend to increase diversity and biomass along these latter habitats (McLachlan & Brown 2006).

The extensive southern Brazilian coast is predominantly of the intermediate type and presents high microalgae biomass mainly granted by accumulations of the surf-zone diatom *Asterionellopsis glacialis* (Castracane) Round throughout the year (Odebrecht et al. 2010, 2013). During high *A. glacialis* concentrations (10^7 to ^{9} cells L^{-1}), high primary production rates (6.4 mg C L^{-1} h^{-1}) and chlorophyll *a* values (up to 4.7 mg L^{-1}) can be registered (Odebrecht et al. 1995, Rörig & Garcia 2003). A decoupling mechanism between this surf-zone diatom and bacteria communities was also observed at *Cassino Beach* but, at least for this southern shore, the microbial loop is neither recycling nutrients nor serving as food source for other organisms in the food web (Abreu et al. 2003).

The primary producers within the surf zone constitute an important food source for intertidal species such as bivalves *Amarilladesma mactroides* and *Donax hanleyanus* (both filter-feeders), hippid crabs *Emerita brasiliensis* (suspension-feeder) and even other filter-feeding bivalves like *Amiantis purpurata*, *Tivela* spp. and *Mactra isabelleana* in the outer surf zone (Garcia & Gianuca 1997). Across the beach profile, accumulated annual production (ash-free dry weight; AFDW) of *A. mactroides* (3,251 g m^{-1}), *E. brasiliensis* (499 g m^{-1}) and *D. hanleyanus* (55 g m^{-1}) accounted for more than 95% of all the surf-zone secondary production (Gianuca 1983, 1997a), in addition to the biomass of the suspension-feeder polychaete *Spio* (= *Scolelepis*) *gaucha* (35 g m^{-2}) and the scavenger isopod *Excirolana armata* (8 g m^{-2}) (Gianuca 1997a, Santos 1994). Ultimately, such species may transfer substantial portions of organic matter to secondary consumers, which are finally preyed by intermediate/top predators such as resident and migratory birds (Vooren & Chiaradia 1990, Vooren 1997), supralittoral and infralittoral crabs (Wolcott 1978, Leber 1982) and also fish assemblages (Du Preez et al. 1990, Capítoli et al. 1994).

Besides the large predominance of intermediate beach states and the inhabiting macrobenthic assemblages, a singular stretch of dissipative coast is found northwards the *Cassino Beach*, near the Rio Grande western jetty (Calliari & Klein 1993), presenting ‘‘almost endemic’’ macrobenthic species like *Sergio mirim*, the only ghost shrimp (Thalassinidea: Callianassidae) reported for these sandy shores (Pezzuto 1998). Burrows of *S. mirim* can be found in relative high density across the dissipative lower intertidal/shallow infralittoral zones - especially during low tides - providing shelter and resources to crabs *Austinixa* (= *Pinnixa*) *patagoniensis* and several other symbiotic species (Gianuca 1997a, Alves & Pezzuto 1998). Exhibiting a (direct) deposit-feeding behavior, *S. mirim* storages organic matter within its burrow for later consumption on this enriched substrate (Coelho & Rodrigues 2001). Local fishermen have conducted an increasing catch of ghost shrimps as bait especially during the summer and thus should be managed to avoid overfishing (Pezzuto 1998). Other macrobenthic species inhabiting this dissipative shoreline have already experiencing declines in terms of density and biomass due to human-induced threats, given the proximity to the *Cassino* urbanized area (Queiroz 2008, Viana 2008, Girão 2009).

Lowest diversity, density and biomass of macrobenthic fauna are associated to that intermediate-to-reflective portion of the southern Brazilian coast - the *Concheiros Beach*, near the *Balneário Hermenegildo* - a pattern also found southwards for intermediate and truly reflective beaches in the Uruguayan coast e.g. Arachania and Manantiales (Defeo et al. 1992, Lercari et al. 2010). A snapshot survey conducted at *Concheiros Beach* reported that macrobenthic zonation was not clear and that macrobenthic richness (five species only) and biomass were both low when compared to the surrounding shores, given the high concentration of shell debris within the substrate: bivalve *Donax hanleyanus* (up to 43 g m^{-2}), hippid crab *Emerita brasiliensis* (11 g m^{-2}), carnivorous polychaete *Hemipodia californiensis* (= *Hemipodus olivieri*) (0.2 g m^{-2}) and isopods *Excirolana braziliensis* (0.5 g m^{-2}) and *E. armata* (< 0.1 g m^{-2}) (Barros et al. 1994). Recent seasonal and interannual surveys have significantly increased this short list of species: more than 21 macrobenthic taxa were so far identified for *Concheiros Beach* including polychaetes, flatworms, molluscs, nemerteans and crustaceans across the swash zone (Minasi 2013) whereas 28-75 burrows of ghost crabs *Ocypode quadrata* (1-4 mm to 13-16 mm) were seasonally registered within the upper mesolittoral/supralittoral zones (Brauer 2013).

Comparing the ecosystem structure and trophic networks of two sandy beaches with contrasting morphodynamics, significant differences were reported concerning the number of biological compartments operating at dissipative (20) and reflective beaches (9), with the former presenting a higher number of top predators and superior trophic levels (Lercari et al. 2010). In southernmost Brazil such dissimilarities between intermediate and reflective sandy environments become evident, based on qualitative records, stomach content analyzes, field samplings and laboratory experiments (Table 1). Higher species richness at intermediate shores coincided with distinct composition and number of biological compartments when compared to reflective shores; nevertheless, such difference may be biased by the fewer studies conducted along these latter environments.

Across-shore trophic relationships

Based on this comprehensive compilation of data (Table 1), major trophic relationships are highlighted and across-shore described taking into account the biological compartments of producers, macrobenthic fauna, seabirds and surf-zone fishes present at the intermediate shores in southernmost Brazil (Figure 3), as follows:

1. *Supralittoral zone*

By the action of strong SW winds, due to storm events or after the passage of frontal systems, vegetal detritus and high phytoplankton concentrations are both carried to the upper-shore forming extensive deposits of organic matter along the beach. Dense accumulations of the surf-zone diatom *Asterionellopsis glacialis* generally form dark-brown patches at the meso/supralittoral (Odebrecht et al. 2013), representing an important food source for terrestrial coleopterans (*Bledius* spp.) and the talitrid amphipod *Atlantorchestoidea brasiliensis*, a scavenger/detritivore species (Gianuca 1983). The abundant presence of the rove beetle *Bledius bonariensis* (2,350 ind. m^{-2})

Table 1. Species richness and biological compartments from supralittoral to the inner surf zone at intermediate (INT) and reflective (REF) sandy shores in southernmost Brazil. Taxonomic classification is presented when available. *p*: presence; —: absence; ?: unknown report / poor sampling. Anom: Anomura; Anopl: Anopla; Bival: Bivalvia; Brachy: Brachyura; Carab: Carabinidae; Gamma: Gammaridea; Gastr: Gastropoda; Isop: Isopoda; Polyc: Polychaeta; Staph: Staphynelidae; Talitr: Talitridae.

Marine and "terrestrial" species	**Taxonomy**	**INT (a)**	**REF (b)**
CRUSTACEA			
Arenaeus cribrarius (Lamarck, 1818)	Brachy	*p*	?
Atlantorchestoidea brasiliensis (Dana, 1853)	Talitr	*p*	?
Bathyporeiapus bisetosus Escofet, 1970	Gamma	*p*	*p*
Chiriscus giambiagiae (Torti & Bastida, 1972)	Isop	*p*	*p*
Emerita brasiliensis Schmitt, 1935	Anom	*p*	*p*
Excirolana armata (Dana, 1853)	Isop	*p*	*p*
Excirolana braziliensis Richardson, 1912	Isop	*p*	?
Macrochiridothea lilianae Moreira, 1972	Isop	*p*	—
Ocypode quadrata (Fabricius, 1787)	Brachy	*p*	*p*
Phoxocephalopsidae amphipods	Gamma	*p*	*p*
Platyischnopidae amphipods	Gamma	*p*	*p*
MOLLUSCA			
Amarilladesma mactroides (Reeve, 1854)	Bival	*p*	*p*
Buccinanops duartei Klappenbach, 1961	Gastr	*p*	?
Donax gemmula Morrison, 1971	Bival	*p*	—
Donax hanleyanus Philippi, 1847	Bival	*p*	*p*
Olivancillaria auricularia (Lamarck, 1811)	Gastr	*p*	?
Olivancillaria orbignyi (Marrat, 1868)	Gastr	*p*	?
ANNELIDA			
Hemipodia californiensis (Hartman, 1938)	Polyc	*p*	*p*
Nephtys simoni Perkins, 1980	Polyc	*p*	*p*
Sigalion cirrifer Orensanz & Gianuca, 1974	Polyc	*p*	—
Spio gaucha Orensanz & Gianuca, 1974	Polyc	*p*	*p*
Thoracophelia furcifera Ehlers, 1897	Polyc	*p*	*p*
INSECTA			
Bledius bonariensis Bernhauer, 1912	Staph	*p*	?
Bledius fernandezi Bernhauer, 1939	Staph	*p*	?
Bledius microcephalus Fauvel, 1901	Staph	*p*	?
Cicindela conspersa Dejean, 1825	Carab	*p*	?
Cicindela patagonica Horn,1895	Carab	*p*	?
Tetragonoderus variegatus Dejean, 1825	Carab	*p*	?
NEMERTEA	Anopl	*p*	*p*
SEABIRDS	**(c)**	*p*	—
FISHES	**(d)**	*p*	?
ZOOPLANKTON	**(e)**	*p*	?
PHYTOPLANKTON	**(f)**	*p*	?

Based on references for southern Brazilian **(a)** intermediate beaches: Gianuca (1983, 1997), Girão (2009), Neves et al. (2007, 2008, 2012), Queiroz (2008), Silva et al. (2008), Viana (2008); **(b)** reflective beaches: Barros et al. (1994), Brauer (2013), Minasi (2013); **(c)** seabirds: Vooren & Chiaradia (1990), Vooren (1997); **(d)** fishes: Capítoli et al. (1994), Monteiro-Neto et al. (2003), Lima & Vieira (2009), Rodrigues & Vieira (2010); **(e)** zooplankton: Bersano (1994); **(f)** phytoplankton: Odebrecht et al. (1995, 2010, 2013).

and the tiger beetle *Cicindela conspersa* may attract coleopteran predators like the ground beetle *Tetragonoderus variegatus*, being all ultimately preyed by resident birds like the collared plover *Charadrius collaris* and the pipit *Anthus correndera* (Garcia & Gianuca 1997).

The supralittoral zone of exposed sandy beaches are typically inhabited by several species of insects, amphipods, isopods and crabs of the genus *Ocypode*, this latter worldwide distributed in tropical and subtropical sandy shores (McLachlan & Brown 2006). The ghost crab *Ocypode quadrata* presents a generalist feeding behavior and actively moves from the supralittoral down to the upper swash zone during low tides to prey on live intertidal migrants (e.g. *Donax* and *Emerita* species), flesh of large dead animals (e.g. fishes) or even deposited phytoplankton from the substrate (Wolcott 1978, Leber 1982, Robertson & Pfeiffer 1982, Trott 1999).

Ocypode quadrata excavates burrows across the mesolittoral (juveniles) and supralittoral zones (adults) of southern Brazil (Alberto & Fontoura 1999, Girão 2009) and scavenges macrobenthic strandings after storm surge events, when

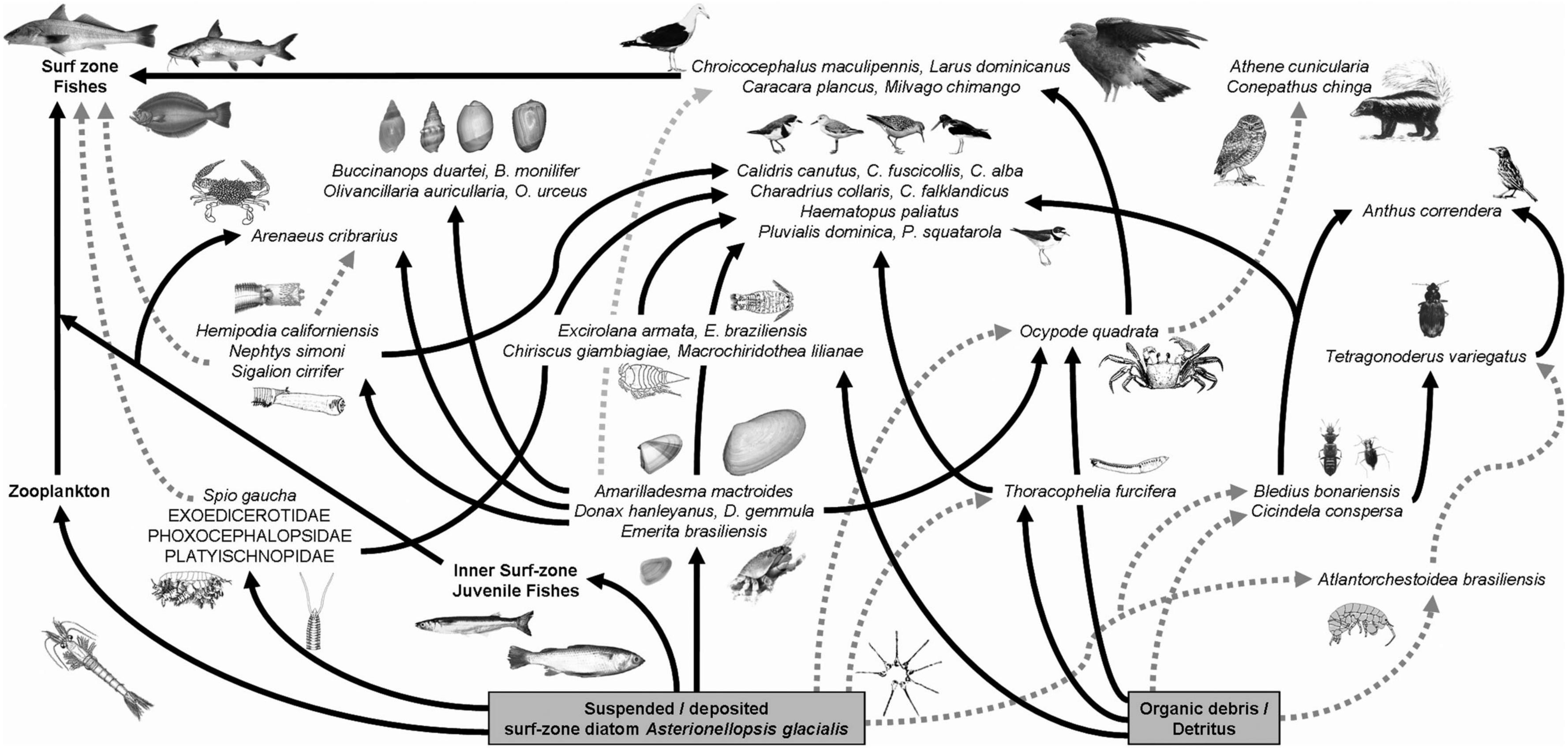


Figure 3. Trophic relationships (solid lines: evidenced; dashed lines: suggested) reported for the intermediate sandy shores in southernmost Brazil. Sources of primary production and species richness within biological compartments can differ significantly through time (seasonally) and according to the morphodynamic beach state. Draws are presented in arbitrary scale and are copyrighted to respective owners.

Amarilladesma mactroides and *Donax hanleyanus* empty shells and *Emerita brasiliensis* debris are registered around large burrows of the ghost crab (*pers. obs.*). Macrobenthic strandings result from the passive transport of several intertidal/infralittoral species to the upper parts of the beach due to strong hydrodynamics and SW winds, determining massive mortality due to their trapping in the dry sand and dying by desiccation, starvation, predation or human-induced factors (Neves et al. 2008, Pinheiro 2013, Pinotti & Bemvenuti *in prep.*).

Nocturnal activities of *Ocypode quadrata* are commonly reported for populations inhabiting the southern coast of Rio Grande do Sul state, even under favorable daytime temperatures e.g. 25 °C (Alberto & Fontoura 1999). Along Moçambique Beach (Florianópolis, SC, Brazil) such night-time behavior was considered an important strategy to keep away from diurnal predators like seagulls and falconid species (Blankensteyn 2006). Avoid daylight exposure may decrease predation rates over *O. quadrata* if taken into account the high diversity and abundance of predatory birds foraging alone or in flocks during the day along the southern Brazilian shores (Vooren & Chiaradia 1990). On the other hand, such behavior does not prevent the nocturnal predation of other animals like the burrowing owl *Athene cunicularia* and the skunk *Conepathus chinga*, both inland predators alternatively feeding on this crab (Gianuca 1997b).

Other inland top predators found over the entire beach all year round are seagulls (*Larus dominicanus* and *Chroicocephalus maculipennis*) and hawks (*Milvago chimango* and *Caracara plancus*) which also feed on stranded debris, wind-blown insects and discards of coastal fishery (Vooren 1997).

2. *Mesolittoral zone*

The shore exposure to strong winds from S-SW quadrants (mainly through the cold seasons) determine an abundant availability of food sources (suspended/deposited) to the inhabiting intertidal macrofauna, mostly granted by high concentrations of the surf-zone diatom *Asterionellopsis glacialis* (Garcia & Gianuca 1997).

The infaunal polychaete *Thoracophelia furcifera* is commonly registered along the upper/mid-intertidal zones from sandy beaches of southern Brazil and northern Uruguay, from the surface up to 20 cm deep (Defeo et al. 1992, Brazeiro & Defeo 1996, Neves et al. 2007). This small Opheliidae (< 2 cm long, ~3 mm wide), usually classified as a deposit-feeder (Fauchald & Jumars 1979), may utilize that organic matter derived from *A. glacialis* deposits within the substrate whilst exploiting bacteria and other microbes as carbon source (Kemp 1986). Under severe insolation and consequent drying of the sand surface, elevated concentrations of *T. furcifera* are found deeper in the substrate; conversely, when the water table is high and the sand is wet, higher polychaete densities are found near the surface (Viana 2008), occasion in which *T. furcifera* and abundant intertidal migrants may constitute significant food sources for the northern migrant sandpipers *Calidris* spp. (Vooren & Chiaradia 1990, Vooren 1997).

The scavenger isopod *Excirolana armata* is generally characterized as a primary consumer exposed to high predation pressure (Lercari et al. 2010). Inhabiting the intermediate to lower intertidal, *E. armata* swims and burrows faster than other Cirolanid found in upper levels - *E. braziliensis* - but both are capable of actively pursuit prey and search for carrion under a wide range of swash currents (Yannicelli et al. 2002). Several macrobenthic species and even large dead organisms (e.g. fishes, seabirds) are voraciously consumed by hundreds of *Excirolana* isopods which literally swarm the carcasses while feeding (*pers. obs.*), thus accelerating the recycling of organic matter (McLachlan & Brown 2006). During the summer months, dense flocks of sandpipers *Calidris canutus*, *C. fuscicollis* and *C. alba* can be found foraging on *E. armata* isopods within the upper swash zone, on slightly submerged substrates or even on dry sand (Vooren 1997).

Probing the substrate with their fine bills and localizing the prey through touch and taste (and even visually), *Calidris* sandpipers also adopt this forage strategy to efficiently prey on *Amarilladesma mactroides* (recruits to juveniles), *Donax hanleyanus* and *Emerita brasiliensis* (Vooren & Chiaradia 1990, Vooren 1997). Such intertidal migrants may achieve high densities across the beach profile especially during recruitment peaks, between late spring and early autumn (Neves et al. 2007). The above species and *E. armata* are also significantly preyed in a solitary manner by charadriid plovers *Charadrius falklandicus* (southern migrant), *C. collaris* (resident species), *Pluvialis dominica* and *P. squatarola* (both northern migrants), which search their prey exclusively by visual means at the upper swash zone and to a lesser extent on slightly submerged sands (Vooren 1997). As a result, intertidal migrants can account for 90 % of the stomach content of sandpipers and plovers by the end of summer (Garcia & Gianuca 1997).

Omnivorous-detritivorous amphipods like *Bathyporeiapus bisetosus* and *B. ruffoi* (Exoedicerotidae), *Phoxocephalopsis zimmeri* and *Puelche orensanzi* (Phoxocephalopsidae) and Platyischnopidae amphipods may utilize part of primary (or even secondary) production for nourishment along the swash zone, where suspended/deposited diatoms and detritus are important food sources (Escofet 1973, Lercari et al 2010). Linking the primary production to higher trophic levels, such amphipod species may be characterized as potential food items in the diet of those migrant/resident sandpipers and plovers, given the macrobenthic distribution across the beach profile and the shorebird feeding strategies (Vooren 1997, Neves et al. 2007).

Endemic in southern Brazil, Uruguay and northern Argentina exposed sandy beaches, the polychaete *Spio gaucha* is commonly found across the entire mesolittoral zone, reaching elevated mean annual biomass (up to 31 g AFDW m^{-1}) and production (up to 113 g AFDW m^{-1} $year^{-1}$) due to extremely high densities (up to 100,000 ind. m^{-2}), especially during spring and early summer (Santos 1991, 1994). Such infaunal species constructs a weak cohesive tube within the first 20 cm of sand, exhibiting a suspension-feeding behavior near the substrate surface (Santos 1991). Indeed, *Scolelepis* sp. feed predominantly on suspended particles within the intertidal zone but may opportunistically change to a deposit-feeding strategy under still environmental conditions (Pardo & Amaral 2004). Regardless the feeding behavior, *S. gaucha* may utilize that abundant offer of suspended and/or deposited diatoms (mainly *Asterionellopsis glacialis*) for nourishment, linking such primary production to higher trophic levels and thus playing an important role in sandy beach ecosystem functioning (Speybroeck et al. 2007).

Sandpipers and plovers are shorebirds with short bills well adapted to prey on small macrobenthic fauna living shallowly nearby the substrate surface (Santos 1991, Vooren 1997). On the other hand, the shape and size of the bill and the specialized searching behavior of the American oystercatcher *Haematopus* *palliatus* allow the species to feed preferentially on large yellow clams *Amarilladesma mactroides* (Vooren & Chiaradia 1990). This intertidal migrant exhibits a shallow behavior within the first cm layers during early stages of development, but reaches 15-40 cm deep in the sediment when adult (> 50 mm) using the strong foot to dig and anchor (Narchi 1981, Penchaszadeh 2004). Buried across the swash zone at depths around 5 cm, large clams are available neither to other birds (e.g. sandpipers, plovers) nor to marine predators (e.g. fish assemblages) except when emerging to migrate with tides; then, they are also preyed by the resident seagulls *Larus dominicanus* (Vooren 1997). Dense beds of *A. mactroides* (2-6 10^3 ind. m^{-2}) are broadly found across the swash zone especially between late spring and early autumn (Neves et al. 2007) when they are heavily preyed, reinforcing that important link of trophic transference between macrobenthic species and bird compartments (Garcia & Gianuca 1997).

3. *Infralittoral (inner and outer surf zones)*

Under the influence of regular wave action and strong currents, the inner surf zone of southernmost Brazil is characterized as a turbulent environment, in which the frequent winter storms further increase such stressful hydrodynamics (Gianuca 1997). During extremely low tides, sandpipers and plovers may alternatively feed on *Donax gemmula* (highly abundant), isopods *Chiriscus giambiagiae* and *Macrochiridothea lilianae*, and also on polychaetes *Sigalion cirrifer*, *Hemipodia californiensis* and *Nephtys simoni*, given their frequency and abundance within the interface lower swash zone/inner surf zone (Gianuca 1997). The infaunal polychaetes do not reach those high densities of the intertidal *Spio gaucha* but attain a much larger body size and consequently higher biomass per individual (*pers. obs.*), preying actively on the meiofauna (e.g. nemerteans, ostracods, harpacticoid copepods) or ingesting post-settled recruits of the macrofauna (e.g. bivalves, polychaetes, crustaceans) specially during recruitment peaks (Neves et al. 2007).

Several marine decapods, whelks and juvenile fishes from the surf zone are generally reported moving into the lower mesolittoral during high tides, promoting significant predation excursions (Garcia & Gianuca 1997). The speckled swimming crab *Arenaeus cribrarius* is a voracious predator, being frequently registered across the swash zone searching for living prey (e.g. bivalves, gastropods and other invertebrates) or meat in decomposition. Both juveniles and adults of *A. cribrarius* display a generalist carnivorous behavior in which prey are selected according to the capture capacity and swimming ability of different size classes (Warner 1977). Intertidal migrant species like *Amarilladesma mactroides* (recruits to juveniles), *Donax hanleyanus* and *Emerita brasiliensis* are recognized as important food sources for *A. cribrarius* given their abundance near the substrate surface as shallow filter-/suspension-feeders, a predation-prey relationship also reported elsewhere (Leber 1982). Furthermore, we can not disregard the inner surf zone polychaetes *Sigalion cirrifer*, *Hemipodia californiensis* and *Nephtys simoni* as recurrent food sources in the swimming crab diet given the high burrowing capacity of portunid crabs (Warner 1977).

Surf zone whelks like *Buccinanops duartei* and *Olivancillaria auricularia* (0-5 m depth), *B. monilifer* and *O. urceus* (5-8 m) are either predators or scavengers frequently registered in predatory excursions across the swash zone/inner surf zone (Borzone & Gianuca 1990, Garcia & Gianuca 1997), expanding their large foot muscle to burrow the sand (anchoring) or efficiently ride the swash waves (surfing) up and down the beach (Penchaszadeh 2004). Employing an accurate chemical perception to detect debris of dead animals or living prey, *Buccinanops* species take advantage of their strong foot to capture food sources, including young/small *Donax hanleyanus* and *Emerita brasiliensis* (Rios 2009). Similar displacement and hunt strategies are also exhibited by *Olivancillaria* species when preying over small clams, especially *D. hanleyanus* and juveniles of *Amarilladesma mactroides* (Penchaszadeh 2004).

The ichthyofauna is usually dominated by benthic feeders moving into shallow waters to feed on benthos and alternatively on zooplankton, being therefore characterized as higher predators (Lasiak & McLachlan 1987; Du Preez et al. 1990). The zooplankton community in southernmost Brazil is broadly dominated by mysids (highly abundant) and copepods (more diverse) in addition to several meroplanktonic larvae, accounting for a high biomass within the surf zone (up to 8 10^3 mg C m^{-3}; Bersano 1994). Such elevated zooplankton biomass is likely to exert a significant grazing pressure over the phytoplankton community.

The shallow depth in the inner surf zone generally limits the access of large fishes whilst may provide an important nursery area for juveniles (Lasiak 1981). Along the southern Brazilian coast, Monteiro-Neto et al. (2003) reported 43 fish species across this shallow zone (0-1.2 m), with juveniles ranging between 15-150 mm of total length. A more recent study within this zone also revealed a significant differentiation between resident and seasonal species (Lima & Vieira 2009). Among some of those surf zone inhabitants, juveniles of pampo *Trachinotus marginatus* do not feed on macrobenthic fauna whilst the silverside *Odonthestes argentinensis* alters the main food source from diatoms to zooplankton during its development (Bemvenuti 1990). The mullets *Mugil platanus* and *M. gaimardianus* ingest zooplankton and deposited material over the substrate while offshore, including the diatom *Asterionellopsis glacialis* (Vieira 1991, Garcia & Gianuca 1997).

The vertical distribution of the macrobenthic fauna across the southern Brazilian surf zone is a function of depth, where 126 species form three distinct assemblages (Borzone & Gianuca 1990): a shallow assemblage (2-5 m of depth), characterized by whelks *Olivancillaria auricularia* and *Buccinanops duartei*, bivalves *Corbula* sp. and *Donax gemmula*, polychaetes *Sigalion cirrifer* and *Diopatra viridis*, the isopod *Macrochiridothea lilianae* and the sand dollar *Mellita quinquiesperforata*; an intermediate assemblage (5-8 m) with shared composition and thus higher diversity; and a deeper assemblage (8-10 m) dominated by abundant polychaetes *Kinbergonuphis difficilis* and *Paraprionospio pinnata*, bivalves *Mactra isabelleana* and *Tellina* spp., the gastropod *Duplicaria gemmulata* and the polychaetes *Pherusa capitata*, *Glycinde multidens* and *Glycera americana*. Several other species like the cumacean *Diastylis sympterygiae*, isopods *Synidotea marplatensis* and *Ancinus gaucho*, molluscs *Abra lioica* and *Anachis isabellei* and the polychaete *Parandalia* sp. present a wider distribution and also higher density throughout the outer surf zone (Borzone & Gianuca 1990).

This high macrobenthic richness/density and the high abundance of juvenile fishes in deeper waters of the outer surf zone (2-10 m depth) distinguishes this zone as an important feeding ground to fishes, comprising secondary or even higher consumers: adults of *T. marginatus*; kingcroakers *Menticirrhus americanus* and *M. littoralis*; banded croaker *Paralonchurus* *brasiliensis*; flatfishes *Oncopterus darwini* and *Paralichthys orbignyanus*; whitemouth croaker *Micropogonias furnieri*; and catfishes *Genidens barba* and *G. planifrons* (Monteiro-Neto & Cunha 1990; Capítoli et al. 1994, Rodrigues & Vieira 2010). Large individuals of *Pogonias cromis* (black drum) may feed abundantly on the filter-feeding bivalves *A. purpurata*, *Tivela* spp. and *M. isabelleana* (Garcia & Gianuca 1997).

From the inner shelf to the upper slope, even more diverse and abundant macrobenthic assemblages are found in the southwestern Atlantic (Absalão 1990, 1991; Capítoli & Bemvenuti 2004, 2006) supporting demersal and benthic teleost fish assemblages, many of them commercially valuable (Haimovici 1997); nevertheless, such trophic relationships within/between deeper marine zones are beyond the scope of the present revision.

Summary & conclusions

The southernmost Brazilian sandy shore is characterized as primarily intermediate, despite those dissipative (*Cassino Beach*) and tending-to-reflective (*Concheiros Beach*) stretches of coast (Calliari et al. 1996). Along this shoreline, the surf-zone diatom *Asterionellopsis glacialis* is the most important primary producer, sustaining intermediate (secondary to tertiary) macrobenthic consumers like filter-feeding bivalves, hippid crabs and scavenger isopods, forming short trophic chains (Heymans & McLachlan 1996, Lercari et al. 2010, Colombini et al. 2011).

During short-term high tides, the elevated abundance and consequent high biomass of intertidal species along the southern Brazilian shores may attract several top-predators like swimming crabs, whelks and fishes (upshore) whilst the predation threats are mainly exerted by ghost crabs and seabirds (downshore) at low tides (Gianuca 1983). Only ghost crabs, some carnivorous gastropods and polychaetes (mainly *Hemipodia californiensis*) may conversely integrate the superior trophic levels on *Concheiros Beach*, given the virtual absence of bird and fish assemblages along reflective shorelines due to limited swash/surf zone widths (Lercari et al. 2010). Dissipative beaches have higher number of species occupying higher trophic levels whilst reflective environments exhibit higher levels of connectance and omnivory, increasing the trophic network robustness to secondary extinctions (due to primary species loss e.g. by biological invasions and/or habitat loss) and therefore enhancing the ecosystem stability (Bergamino et al. 2013).

Given its role as nursery and growth area to juvenile/adult fish assemblages and as strategic feeding area for resident/migrant seabirds, conservation efforts should be heavily implemented for the southernmost Brazilian intermediate shores, at least for those non urbanized segments. Such strategies could be focused on preserve the dune fields and their natural vegetation, prohibiting the traffic of vehicles along the beach, limiting the access of tourists and regulate the invertebrate fisheries over the yellow clam *Amarilladesma mactroides* and the ghost shrimp *Sergio mirim*.

Acknowledgments

We would like to thank the anonymous reviewers for their critics and improvements on the original manuscript. This research was financially supported by the National Council for Scientific and Technological Development – CNPq (141132/2010-8).

References

ABREU, P.C., RÖRIG, L.R., GARCIA, V.M., ODEBRECHT, C. & BIDDANDA, B. 2003. Decoupling between bacteria and the surf-zone diatom *Asterionellopsis glacialis* at Cassino Beach, Brazil. Aquat. Microb. Ecol. 32:219–228, http://dx.doi.org/10.3354/ame032219

ABSALÃO, R.S. 1990. Ophiuroid assemblages off the Lagoa dos Patos outlet, Southern Brazil. Ophelia. 31(2):133–143, http://dx.doi.org/10.1080/00785326.1990.10430857

ABSALÃO, R.S. 1991. Environmental discrimination among soft-bottom mollusk associations off Lagoa dos Patos, south Brazil. Est. Coast. Shelf Sci. 32:71–85, http://dx.doi.org/10.1016/0272-7714(91)90029-B

ALBERTO, R.M.F. & FONTOURA, N.F. 1999. Distribuição e estrutura etária de *Ocypode quadrata* (Fabricius, 1787) (Crustacea, Decapoda, Ocypodidae) em uma praia arenosa do litoral sul do Brasil. Rev. Bras. Biol. 59(1):95–108, http://dx.doi.org/10.1590/S0034-71081999000100013

ALVES, E.S. & PEZZUTO, P.R. 1998. Population dynamics of *Pinnixa patagoniensis* Rathbun, 1918 (Brachyura, Pinnotheridae) a symbiotic crab of *Sergio mirim* (Thalassinidea: Callianassidae) in Cassino Beach, southern Brazil. Mar. Ecol. 19(1):37–51, http://dx.doi.org/10.1111/j.1439-0485.1998.tb00452.x

BARLETTA, R.C. & CALLIARI, L.J. 2003. An assessment of the atmospheric and wave aspects determining beach morphodynamic characteristics along the central coast of RS state, Southern Brazil. J. Coast. Res. 35(SI):300–308.

BARROS, F.C.R., CALLIARI, L.J. & GIANUCA, N.M. 1994. Estudo preliminar da macrofauna bentônica da Praia dos Concheiros, RS. Not. Téc. UFRGS. 7:33–37.

BEMVENUTI, M.A. 1990. Hábitos alimentares de peixes-rei (Atherinidae) na região estuarina da Lagoa dos Patos, RS, Brasil. Atlântica. 12(1):79–102.

BERGAMINO, L., LERCARI, D. & DEFEO, O. 2011. Food web structure of sandy beaches: temporal and spatial variation using stable isotope analysis. Est. Coast. Shelf Sci. 91(4):536–543, http://dx.doi.org/10.1016/j.ecss.2010.12.007

BERGAMINO, L., GÓMEZ, J., BARBOZA, F.R. & LERCARI, D. 2013. Major food web properties of two sandy beaches with contrasting morphodynamics, and effects on the stability. Aquat. Ecol. 47(3):253–261, http://dx.doi.org/10.1007/s10452-013-9440-5

BERSANO, J.G. 1994. Zooplâncton da zona de arrebentação, de praias arenosas situadas ao sul de Rio Grande, RS. Primavera de 1990 / Verão de 1991. MSc. Dissertation in Biological Oceanography, Federal University of Rio Grande - FURG, Rio Grande, Brazil.

BLANKENSTEYN, A. 2006. O uso do caranguejo maria-farinha *Ocypode quadrata* (Fabricius) (Crustacea, Ocypodidae) como indicador de impactos antropogênicos em praias arenosas da Ilha de Santa Catarina, Santa Catarina, Brasil. Rev. Bras. Zool. 23(3):870–876, http://dx.doi.org/10.1590/S0101-81752006000300034

BORZONE, C.A. & GIANUCA, N.M. 1990. A zonação infralitoral em praias arenosas expostas. Pub. ACIESP. 71(3):280–287.

BRAUER, B. 2013. Distribuição e abundância do caranguejo *Ocypode quadrata* nas praias arenosas do extremo sul do Brasil. Monograph in Oceanography, Federal University of Rio Grande - FURG, Rio Grande, Brazil.

BRAZEIRO, A. & DEFEO, O. 1996. Macroinfauna zonation in microtidal sandy beaches: is it possible to identify patterns in such variable environments? Est. Coast. Shelf Sci. 42:523–536, http://dx.doi.org/10.1006/ecss.1996.0033

CADDY, J.F. & DEFEO, O. 2003. Enhancing or restoring the productivity of natural populations of shellfish and other marine invertebrate resources. FAO Fish. Tech. Paper 448. FAO, Rome.

CALLIARI, L.J. & KLEIN, A.H.F. 1993. Características morfodinâmicas e sedimentológicas das praias oceânicas entre Rio Grande e Chuí, RS. Pesq. Geoc. 20(1): 48–56.

CALLIARI, L.J., KLEIN, A.H.F. & BARROS, F.C.R. 1996. Beach differentiation along the Rio Grande do Sul coastline (Southern Brazil). Rev. Chil. Hist. Nat. 69:485–493.

CALLIARI, L.J., TOZZI, H.A.M. & KLEIN, A.H.F. 1998. Beach morphology and coastline erosion associated with storm surge in Southern Brazil - Rio Grande to Chuí, RS. An. Acad. Bras. Cienc. 70(2):231–247.

CAPÍTOLI, R.R. & BEMVENUTI, C.E. 2004. Distribuição batimétrica e variações de diversidade dos macroinvertebrados bentônicos da plataforma continental e talude superior no extremo sul do Brasil. Atlântica. 26(1):27–43.

CAPÍTOLI, R.R. & BEMVENUTI, C.E. 2006. Associações de macroinvertebrados bentônicos de fundos inconsolidados da plataforma continental e talude superior no extremo sul do Brasil. Atlântica. 28(1):47–59.

CAPÍTOLI, R.R., BAGER, A. & RUFFINO, M.L. 1994. Contribuição ao conhecimento das relações tróficas bentônico-demersais nos fundos de pesca do camarão *Artemesia longinaris* Bate, na região da barra da Lagoa dos Patos, RS, Brasil. Nauplius, 2:53–74.

CISNEROS, K.O., SMIT, A.J., LAUDIEN, J. & SCHOEMAN, D.S. 2011. Complex, dynamic combination of physical, chemical and nutritional variables controls spatio-temporal variation of sandy beach community structure. PloS ONE. 6(8):e23724, http://dx.doi.org/10.1371/journal.pone.0023724

COELHO, V.R. & RODRIGUES, S.A. 2001. Setal diversity, trophic modes and functional morphology of feeding appendages of two callianassid shrimps, *Callichirus major* and *Sergio mirim* (Decapoda: Thalassinidea: Callianassidae). J. Nat. Hist. 35:1447–1483, http://dx.doi.org/10.1080/002229301317067638

COLOMBINI, I. & CHELAZZI, L. 2003. Influence of allochthonous input on sandy beach communities. Oceanogr. Mar. Biol. Ann. Rev. 41:115–159.

COLOMBINI, I., BRILLI, M., FALLACI, M., GAGNARLI, E. & CHELAZZI, L. 2011. Food webs of a sandy beach macroinvertebrate community using stable isotopes analysis. Acta Oecol. 37:422–432, http://dx.doi.org/10.1016/j.actao.2011.05.010

DEFEO, O. & MCLACHLAN, A. 2005. Patterns, processes and regulatory mechanisms in sandy beach macrofauna: a multi-scale analysis. Mar. Ecol. Prog. Ser. 295:1–20, http://dx.doi.org/10.3354/meps295001

DEFEO O. & MCLACHLAN, A. 2011. Coupling between macrofauna community structure and beach type: a deconstructive meta-analysis. Mar. Ecol. Prog. Ser. 433:29–41, http://dx.doi.org/10.3354/meps09206

DEFEO, O., JARAMILLO, E. & LYONNET, A. 1992. Community structure and intertidal zonation of the macroinfauna on the Atlantic coasts of Uruguay. J. Coast. Res. 8(4):830–839.

DEFEO, O., GÓMEZ, J. & LERCARI, D. 2001. Testing the swash exclusion hypothesis in sandy beach populations: the mole crab *Emerita brasiliensis* in Uruguay. Mar. Ecol. Prog. Ser. 212:159–170, http://dx.doi.org/10.3354/meps212159

DEFEO, O., LERCARI, D. & GÓMEZ, J. 2003. The role of morphodynamics in structuring sandy beach populations and communities: what should be expected? J. Coast. Res. 35(SI):352–362.

DEFEO, O., MCLACHLAN, A., SCHOEMAN, D.S., SCHLACHER, T.A., DUGAN, J., JONES, A., LASTRA, M. & SCAPINI, F. 2009. Threats to sandy beach ecosystems: A review. Est. Coast. Shelf Sci. 81(1):1–12, http://dx.doi.org/10.1016/j.ecss.2008.09.022

DELGADO, M. 1989. Abundance and distribution of microphytobenthos in the bays of Ebro Delta (Spain). Est. Coast. Shelf Sci. 29(2):183–194, http://dx.doi.org/10.1016/0272-7714(89)90007-3

DIAZ, A.F., STUDZINSKI, C.D. & MECHOSO, C.R. 1998. Relationships between precipitation anomalies in Uruguay and Southern Brazil and sea surface temperature in the Pacific and Atlantic oceans. J. Clim. 11:251–271, http://dx.doi.org/10.1175/1520-0442(1998)011<0251:RBPAIU>2.0.CO;2

DU PREEZ H.H, MCLACHLAN A, MARAIS, J.F.K, & COCKCROFT, A.C. 1990. Bioenergetics of fishes in a high-energy surf-zone. Mar. Biol. 106:1–12, http://dx.doi.org/10.1007/BF02114669

DUGAN, J.E., HUBBARD, D.M., MCCRARY, M.D. & PIERSON, M.O. 2003. The response of macrofauna communities and shorebirds to macrophyte wrack subsidies on exposed sandy beaches of southern California. Est. Coast. Shelf Sci. 58S:25–40, http://dx.doi.org/10.1016/S0272-7714(03)00045-3

ESCOFET, A. 1973. Observaciones sobre alimentación y hábitos cavadores en *Bathyporeiapus ruffoi* y *Bathyporeiapus bisetosus* (Amphipoda: Oedicerotidae). Physis. 32(84):95–103.

ESTEVES, L.S., PIVEL, M.A.G., SILVA, A.R.P., BARLETTA, R.C., VRANJAC, M.P., OLIVEIRA, U.R. & VANZ, A. 2000. Beachfront owners perception of erosion along an armored shoreline in Southern Brazil. Pesq. Geoc. 27(2):93–109.

FAUCHALD, K. & JUMARS, P.A. 1979. The diet of worms: a study of polychaete feeding guilds. Oceanogr. Mar. Biol. Ann. Rev. 17:193–284.

FIGUEIREDO, S.A. 2013. Modelling climate change effects in southern Brazil. J. Coast. Res. 65(SI):1933–1938.

GARCIA, V.M.T. & GIANUCA, N.M. 1997. The Beach and Surf Zone. In Subtropical Convergence Environments: The Coast and Sea in the Southwestern Atlantic (Seeliger, U., Odebrecht, C. & Castello, J.P. eds.). Springer-Verlag, Berlin, p. 166–170.

GIANUCA, N.M. 1983. A preliminary account of the ecology of sandy beaches in Southern Brazil. In Sandy Beaches as Ecosystems (Mclachlan, A., & Erasmus, T., eds.). Springer, Netherlands, p.413–419.

GIANUCA, N.M. 1997a. Benthic Beach Invertebrates. In Subtropical Convergence Environments: The Coast and Sea in the Southwestern Atlantic (Seeliger, U., Odebrecht, C., & Castello, J.P. eds.). Springer-Verlag, Berlin, p.114–117.

GIANUCA, N.M. 1997b. A fauna das dunas costeiras do Rio Grande do Sul. Oecol. Bras. 3:121–133, http://dx.doi.org/10.4257/oeco.1997.0301.08

GIRÃO, M.M.L. 2009. Variação espaço-temporal de *Ocypode quadrata* sob a influência de um gradiente antrópico na praia do Cassino, Rio Grande do Sul - Brasil. MSc. Dissertation in Biological Oceanography, Federal University of Rio Grande - FURG, Rio Grande, Brazil.

GRIMM, A.M., FERRAZ, S.E.T. & GOMES, J. 1998. Precipitation anomalies in Southern Brazil associated with El Niño and La Niña events. J. Clim. 11:2863–2880, http://dx.doi.org/10.1175/1520-0442(1998)011<2863:PAISBA>2.0.CO;2

HAIMOVICI, M. 1997. Demersal and Benthic Teleosts. In Subtropical Convergence Environments:The Coast and Sea in the Southwestern Atlantic (Seeliger, U., Odebrecht, C, Castello, J.P. eds.). Springer-Verlag, Berlin, p.129–136.

HEYMANS, J.J. & MCLACHLAN, A. 1996. Carbon budget and network analysis of a high-energy beach/surf-zone ecosystem. Est. Coast. Shelf Sci. 43:485–505, http://dx.doi.org/10.1006/ecss.1996.0083

JARAMILLO, E., MCLACHLAN, A., COETEZEE, P. 1993. Intertidal zonation patterns of macroinfauna over a range of exposed sandy beaches in south-central Chile. Mar. Ecol. Prog. Ser. 101:105–117, http://dx.doi.org/10.3354/meps101105

KEMP, P.F. 1986. Direct uptake of detrital carbon by the deposit-feeding polychaete *Euzonus mucronata* (Treadwell). J. Exp. Mar. Biol. Ecol. 99:49–61, http://dx.doi.org/10.1016/0022-0981(86)90020-1

KRUSCHE, N., SARAIVA, J.M.B. & REBOITA, M.S. 2003. Normais Climatológicas Provisórias de 1991 a 2000 para Rio Grande, RS. Editora FURG, Rio Grande.

LASIAK, T.A. 1981. Nursery grounds of juvenile teleosts:evidence from the surf zone of King's Beach, Port Elizabeth. S. Afr. J. Sci. 77:388–390.

LASIAK, T. & MCLACHLAN, A. 1987. Opportunistic utilization of mysid shoals by surf-zone teleosts. Mar. Ecol. Prog. Ser. 37:1–7, http://dx.doi.org/10.3354/meps037001

LEBER, K.M. 1982. Seasonality of macroinvertebrates on a temperate, high wave energy sandy beach. Bull. Mar. Sci. 32(1):86–98.

LERCARI, D., BERGAMINO, L. & DEFEO, O. 2010. Trophic models in sandy beaches with contrasting morphodynamics:comparing ecosystem structure and biomass flow. Ecol. Model. 221:2751–2759, http://dx.doi.org/10.1016/j.ecolmodel.2010.08.027

LIMA, M.S.P. & VIEIRA, J.P. 2009. Variação espaço-temporal da ictiofauna da zona de arrebentação da Praia do Cassino, Rio Grande do Sul, Brasil. Zoologia. 26(3):499–510, http://dx.doi.org/10.1590/S1984-46702009000300014

MASSELINK, G. & SHORT, A.D. 1993. The effect of tide range on beach morphodynamics and morphology:a conceptual beach model. J. Coast. Res. 9:785–800.

MCLACHLAN, A. 1980. Exposed sandy beaches as semi-closed ecosystems. Mar. Environ. Res. 4:59–63, http://dx.doi.org/10.1016/0141-1136(80)90059-8

MCLACHLAN, A. & BROWN, A.C. 2006. The Ecology of Sandy Shores. Academic Press, New York.

MCLACHLAN, A. & DORVLO, A. 2005. Global patterns in sandy beach macrobenthic communities. J. Coast. Res. 21(4):674–687, http://dx.doi.org/10.2112/03-0114.1

MCLACHLAN, A., ERASMUS, T., DYE, A.H., WOOLDRIDGE, T., VAN DER HORST, G., ROSSOUW, G., LASIAK, T.A. & MCGWYNNE, L. 1981. Sand beach energetics:an ecosystem approach towards a high energy interface. Est. Coast. Shelf Sci. 13:11–25, http://dx.doi.org/10.1016/S0302-3524(81)80102-8

MCLACHLAN, A., JARAMILLO, E., DONN, T.E. & WESSELS, F. 1993. Sand beach macrofauna communities and their control by the physical environment:a geographical comparison. J. Coast. Res. 15(SI):27–38.

MCLACHLAN, A., JARAMILLO, E., DEFEO, O., DUGAN, J., DE RUYCK A & COETZEE, P. 1995. Adaptations of bivalves to different beach types. J. Exp. Mar. Biol. Ecol. 187:147–160, http://dx.doi.org/10.1016/0022-0981(94)00176-E

MCLACHLAN, A., WOOLDRIDGE, T. & DYE, A.H. 1981. The ecology of sandy beaches in southern Africa. S. Afric. J. Zool. 16:219–231.

MINASI, D.M. 2013. Composição e variabilidade espaço-temporal da macrofauna bentônica da Praia dos Concheiros, extremo sul do Brasil. Monograph in Oceanography, Federal University of Rio Grande - FURG, Rio Grande, Brazil.

MONTEIRO-NETO, C. & CUNHA, L.P.R. 1990. Seasonal and ontogenetic variation in food habits of juvenile *Trachinothus marginatus* Cuvier, 1832 (Teleostei, Carangidae) in the surf zone of Cassino Beach, RS, Brazil. Atlântica. 12(1):45–54.

MONTEIRO-NETO, C., CUNHA, L.P.R. & MUSICK, J.A. 2003. Community structure of surf-zone fishes at Cassino Beach, Rio Grande do Sul, Brazil. J. Coast. Res. 35(SI):492–501.

NARCHI, W. 1981. Aspects of the adaptive morphology of *Mesodesma mactroides* (Bivalvia:Mesodesmatidae). Malacologia. 21(1-2):95–110.

NEVES, L.P., SILVA, P.S.R. & BEMVENUTI, C.E. 2007. Zonation of benthic macrofauna on Cassino Beach, Southernmost Brazil. Braz. J. Oceanogr. 55(4):293–307, http://dx.doi.org/10.1590/S1679-87592007000400006

NEVES, L.P., SILVA, P.S.R. & BEMVENUTI, C.E. 2008. Temporal variability of benthic macrofauna on Cassino Beach, Southernmost Brazil. Iheringia (Zoo). 98:36–44, http://dx.doi.org/10.1590/S0073-47212008000100005

ODEBRECHT, C., SEGATTO, A.Z. & FREITAS, C.A. 1995. Surf-zone chlorophyll *a* variability at Cassino Beach, Southern Brazil. Est. Coast. Shelf Sci. 41:81–90, http://dx.doi.org/10.1006/ecss.1995.0054

ODEBRECHT, C., BERGESCH, M., RÖRIG, L.R. & ABREU, P.C. 2010. Phytoplankton interannual variability at Cassino Beach, southern Brazil (1992–2007) with emphasis on the surf zone diatom *Asterionellopsis glacialis*. Est. Coast. 33:570–583, http://dx.doi.org/10.1007/s12237-009-9176-6

ODEBRECHT, C., DU PREEZ D.R, ABREU, P.C & CAMPBELL, E.E. 2013. Surf zone diatoms:a review of the drivers, patterns and role in sandy beaches food chains. Est. Coast. Shelf Sci. http://dx.doi.org/10.1016/j.ecss.2013.07.011.

ORENSANZ, J.M., SCHWINDT, E., PASTORINO, G., BORTOLUS, A., CASAS, G., DARRIGRAN, G., ELÍAS, R., GAPPA, J.J.L., OBENAT, S., PASCUAL, M., PENCHASZADEH, P., PIRIZ, M.L., SCARABINO, F., SPIVAK, E.D. & VALLARINO, E.A. 2002. No longer the pristine confines of the world ocean:a survey of exotic marine species in the southwestern Atlantic. Biol. Invasions. 4:115–143, http://dx.doi.org/10.1023/A:1020596916153

PARDO, E.V. & AMARAL, A.C.Z. 2004. Feeding behavior of *Scolelepis* sp. (Polychaeta:Spionidae). Braz. J. Oceanogr. 52(1):75–79, http://dx.doi.org/10.1590/S1679-87592004000100007

PARISE, C.K., CALLIARI, L.J. & KRUSCHE, N. 2009. Extreme storm surges in the south of Brazil:atmospheric conditions and shore erosion. Braz. J. Oceanogr. 57(3):175–188, http://dx.doi.org/10.1590/S1679-87592009000300002

PENCHASZADEH, P.E. 2004. Caracoles, Almejas y Mejillones. In La Vida entre Mareas:Vegetales y Animales de las Costas de Mar del Plata, Argentina (Boschi , E.E. & Cousseau M.B. eds.). INIDEP, Mar del Plata, p. 253–270.

PEREIRA, P.S., CALLIARI, L.J. & BARLETTA, R.C. 2010. Heterogeneity and homogeneity of Southern Brazilian beaches:A morphodynamic and statistical approach. Cont. Shelf Res. 30:270–280, http://dx.doi.org/10.1016/j.csr.2009.11.007

PEZZUTO, P.R. 1998. Population dynamics of *Sergio mirim* (Rodrigues, 1971) (Decapoda:Thalassinidea:Callianassidae) in Cassino Beach, southern Brazil. Mar. Ecol. 19(2):89–109, http://dx.doi.org/10.1111/j.1439-0485.1998.tb00456.x

PINHEIRO, C. 2013. Embancamentos da macrofauna bentônica e sua relação com eventos meteorológicos em uma praia arenosa do extremo sul do Brasil. Monograph in Oceanography, Federal University of Rio Grande - FURG, Rio Grande, Brazil.

QUEIROZ, L.R. 2008. Variação espaço-temporal da biomassa macrofaunal bentônica da zona de varrido da praia do Cassino - RS, Brasil. MSc. Dissertation in Biological Oceanography, Federal University of Rio Grande - FURG, Rio Grande, Brazil.

RAO, V.B. & HADA, K. 1990. Characteristics of rainfall over Brazil:annual variations and connections with the Southern Oscillation. Theor. Appl. Clim. 42:81–91, http://dx.doi.org/10.1007/BF00868215

RIOS, E.C. 2009. Compendium of Brazilian Sea Shells. Evangraf, Rio Grande.

ROBERTS, C.M. & HAWKINS, J.P. 1999. Extinction risk in the sea. Trends Ecol. Evolut. 14(6):241–246, http://dx.doi.org/10.1016/S0169-5347(98)01584-5

ROBERTSON, J.R. & PFEIFFER, W.J. 1982. Deposit-feeding by the ghost crab *Ocypode quadrata* (Fabricius). J. Exp. Mar. Biol. Ecol. 56:165-177, http://dx.doi.org/10.1016/0022-0981(81)90187-8

RODRIGUES, F.L. & VIEIRA, J.P. 2010. Feeding strategy of *Menticirrhus americanus* and *Menticirrhus littoralis* (Perciformes:Sciaenidae) juveniles in a sandy beach surf zone of southern Brazil. Zoologia. 27(6):873–880, http://dx.doi.org/10.1590/S1984-46702010000600006

RÖRIG, L.R. & GARCIA, V.M.T. 2003. Accumulations of the surf-zone diatom *Asterionellopsis glacialis* (Castracane) Round in Cassino Beach, Southern Brazil, and its relationship with environmental factors. J. Coast. Res. 35(SI):167–177.

SANTOS, P.J.P. 1991. Morphodynamical influence of a temporary freshwater stream on the population dynamics of *Scolelepis gaucha* (Polychaeta:Spionidae) on a sandy beach in Southern Brazil. Bull. Mar. Sci. 48(3):657–664.

SANTOS, P.J.P. 1994. Population dynamics and production of *Scolelepis gaucha* (Polychaeta:Spionidae) on the sandy beaches of Southern Brazil. Mar. Ecol. Prog. Ser. 110:159–165, http://dx.doi.org/10.3354/meps110159

SHORT, A.D. 1996. The role of wave height, period, slope, tide range and embaymentisation in beach classifications:a review. Rev. Chil. Hist. Nat. 69:589–604.

SOARES, A.G., SCHLACHER, T.A. & MCLACHLAN, A. 1997. Carbon and nitrogen exchange between sandy beach clams (*Donax serra*) and kelp beds in the Benguela coastal upwelling region. Mar. Biol. 127:657–664, http://dx.doi.org/10.1007/s002270050056

SPEYBROECK, J., ALSTEENS, L., VINCX, M. & DEGRAER, S. 2007. Understanding the life of a sandy beach polychaete of functional importance - *Scolelepis squamata* (Polychaeta:Spionidae) on Belgian sandy beaches (northeastern Atlantic, North Sea). Est. Coast. Shelf Sci. 74:109–118, http://dx.doi.org/10.1016/j.ecss.2007.04.002

TABAJARA, L.L., MARTINS, L.R. & ALMEIDA, L.E.S.B. 2004. Respostas e recomposição das praias e dunas após sequência de ciclones extratropicais. Gravel. 2:104–121.

TOMAZELLI, L.J. & VILLWOCK, J.A. 1992. Considerações sobre o ambiente praial e a deriva litorânea de sedimentos ao longo do litoral norte do Rio Grande do Sul, Brasil. Pesq. Geoc. 19(1):3–12.

TROTT, T.J. 1999. Gustatory responses of ghost crab *Ocypode quadrata* to seawater extracts and chemical fractions of natural stimuli. J. Chem. Ecol. 25(2):375–388, http://dx.doi.org/10.1023/A:1020859115984

VIANA, M.G. 2008. Avaliação de efeitos antrópicos sobre a distribuição do poliqueta *Euzonus furciferus* (Eulhers, 1897) na Praia do Cassino - RS, Brasil. MSc. Dissertation in Biological Oceanography, Federal University of Rio Grande - FURG, Rio Grande, Brazil.

VIEIRA, J.P. 1991. Juvenile mullets (Pisces:Mugilidae) in the Estuary of Lagoa dos Patos, RS, Brazil. Copeia. 2:409–418, http://dx.doi.org/10.2307/1446590

VOOREN, C.M. 1997. Sea and Shore Birds. In Subtropical Convergence Environments:The Coast and Sea in the Southwestern Atlantic (Seeliger, U., Odebrecht, C. & Castello, J.P.., eds.). Springer-Verlag, Berlin, p. 154–159.

VOOREN, C.M. & CHIARADIA, A. 1990. Seasonal abundance and behavior of coastal birds on Cassino beach, Brazil. Ornit. Neotrop. 1:9–24.

WARNER, G.F. 1977. The Biology of Crabs. Elek Science, London.

WOLCOTT, T.G. 1978. Ecological role of ghost crabs *Ocypode quadrata* (Fabricius) on an ocean beach:scavengers or predators? J. Exp. Mar. Biol. Ecol. 31(1):67–82, http://dx.doi.org/10.1016/0022-0981(78)90137-5

YANNICELLI, B., PALACIOS, R. & GIMÉNEZ, L. 2002. Swimming ability and burrowing time of two cirolanid isopods from different levels of exposed sandy beaches. J. Exp. Mar. Biol. Ecol. 273:73–88, http://dx.doi.org/10.1016/S0022-0981(02)00146-6

Received 05/05/2014
Accepted 21/08/2014

Biota Neotropica 14(3): e20140114, 2014
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
article

Subterranean biodiversity in the Serra da Bodoquena karst area, Paraguay river basin, Mato Grosso do Sul, Southwestern Brazil

Lívia Medeiros Cordeiro[1,2], Rodrigo Borghezan[1] & Eleonora Trajano[1]

[1]Departamento de Zoologia, Instituto de Biociências da Universidade de São Paulo, CP: 11461, CEP: 05422-970, São Paulo, SP, Brasil. http://www.ib.usp.br/
[2]Corresponding author: Lívia Medeiros Cordeiro, e-mail: liviamc@hotmail.com

CORDEIRO, L. M., BORGHEZAN, R., TRAJANO, E. **Subterranean biodiversity in the Serra da Bodoquena karst area, Paraguay river basin, Mato Grosso do Sul, Southwestern Brazil.** Biota Neotropica. 14(3): e201400114. http://dx.doi.org/10.1590/1676-06032014011414

Abstract: We present herein a synthesis of faunistic data from caves in the Serra da Bodoquena karst area, middle Paraguay River basin, Mato Grosso do Sul State. Those include phreatic, submerged and dry caves. Emphasis is given to troglobites (exclusively subterranean species), potentially threatened due to their morphological, physiological and behavioral specializations, associated to generally small distributions. The Bodoquena karst area distinguishes as a spot of high diversity of troglobites, such as trichomycterid and heptapterid catfishes, aquatic planarians and gastropods, arachnids (*Eusarcus* opilionids, ctenid spiders), Polydesmida diplopods, several collembolans, some insects, and Peracarida crustaceans, which include interesting phylogenetic relicts as spelaeogriphaceans. Four geographic compartments corresponding to microbasins, seemingly with biogeographic importance for the subterranean fauna, are recognized. Phreatobic troglobites (*Trichomycterus* catfishes, aquatic peracarids, planarians) distribute widely across these compartments and some well beyond (the spelaeogriphacean *Potiicoara brasiliensis* reaches Mato Grosso state), while those living in base-level streams (*Rhamdia* and *Ancistrus* catfishes) and the terrestrial ones, that live in the vadose (aerated zone) are generally restricted to one compartment. Many subterranean ecosystems in Mato Grosso do Sul are endangered by several threats described here and urgent actions for effective protection are required to guarantee a sustainable use of the land and the karst aquifers.
Keywords: *Subterranean biodiversity, troglobites, distribution, Serra da Bodoquena karst area, Mato Grosso do Sul, Biota-MS Program.*

CORDEIRO, L. M., BORGHEZAN, R., TRAJANO, E. **Biodiversidade subterrânea na área cárstica da Serra da Bodoquena, bacia do Rio Paraguai, Estado do Mato Grosso do Sul, Sudoeste do Brasil.** Biota Neotropica. 14(3): e201400114. http://dx.doi.org/10.1590/1676-06032014011414

Resumo: Neste trabalho, são reunidos dados faunísticos sobre os táxons registrados em cavernas, freáticas, submersas e secas, da área cárstica da Serra da Bodoquena, na bacia do médio Rio Paraguai, Estado do Mato Grosso do Sul. É dada maior ênfase às espécies exclusivamente subterrâneas (troglóbias), potencialmente ameaçadas de extinção devido às especializações morfológicas, fisiológicas e comportamentais, e a distribuição geralmente restrita. A Serra da Bodoquena destaca-se por sua alta diversidade de troglóbios, entre peixes siluriformes, planárias aquáticas e gastrópodes, aracnídeos (opiliões *Eusarcus*, aranhas Ctenidae), diplópodes Polydesmida, diversos colêmbolos e alguns insetos, e crustáceos Peracarida, que incluem destacados relictos filogenéticos, como os crustáceos Speleogriphacea e anfípodes *Megagidiella*. São reconhecidos quatro compartimentos de aparente relevância biogeográfica para cavernícolas, correspondendo a microbacias na área. Troglóbios freáticos (bagres *Trichomycterus*, crustáceos Peracarida aquáticos, planárias) apresentam distribuições amplas através desses compartimentos, atingindo áreas distantes, como o Mato Grosso no caso dos Spelaeogriphacea *Potiicoara brasiliensis*, enquanto as espécies aquáticas que vivem em riachos (siluriformes como *Rhamdia e Ancistrus*) e os terrestres em geral, que vivem na zona vadosa, descontínua, tendem a apresentar distribuições restritas aos respectivos compartimentos. Muitos ecossistemas subterrâneos do Mato Grosso do Sul estão vulneráveis às várias ameaças descritas aqui e ações urgentes de proteção efetiva são necessárias para garantir uma utilização sustentável da terra e dos aquíferos cársticos.
Palavras-chave: *biodiversidade subterranean, troglóbios, área cárstica da Serra da Bodoquena, Mato Grosso do Sul, Programa Biota-MS.*

Introduction

The Serra da Bodoquena karst area has ca. 200 caves so far recorded (Sallun-Filho et al. 2010), with a great variety of subterranean habitats, including phreatic (never exposed at the vadose zone – Ford & Williams 2007) and submerged (found beneath the water level after a period at the vadose zone) cave systems, and dry caves. It distinguishes as a high diversity spot for troglobites (species with exclusively subterranean source populations – Trajano 2012) in South America. Among these, five siluriform fishes and several invertebrates have been recorded, such as spelaegriphaceans and bogidiellid amphipods, planarians, Pomatiopsidae gastropods, polydesmid diplopods, opilionids and onychophorans, including phylogenetic and geographic relicts. In addition, a rich troglophilic (also found in epigean habitats) fauna is observed, which coexists and interacts with the several troglobitic populations. Such diversity points to intricate evolutionary patterns related to the complex geological history of the area, interacting with present-day ecological factors, including the epigean (surface) biodiversity and biomass allowing for a significant input of nutrients into the subterranean realm.

1 History of speleobiology in the Mato Grosso do Sul State

The speleobiological interest in the area started in the mid-1980′s, with the discover, in a submerged cave (the Lago Azul Cave), followed by description of *Potiicoara brasiliensis* Pires 1987, the second known species of living Spelaeogriphacea – the first one was *Spelaeogriphus lepidops* Gordon 1957, from Table Moutains, South Africa (there are recent reports from caves other than the type locality - S. Craven & A.L.F. Guil, pers. comm.). Later on, two other species were found in Australia, confirming the Gondwanic distribution for the taxon and its relictual character. Further studies showed a wide distribution for spelaeogriphaceans in Mato Grosso do Sul, and also brought into light another phylogenetically and biogeographically important crustacean, the *Megagidiella* amphipods.

The period from mid-1990′s to mid-2000′s was marked by cave diving by biologists, who found the first troglobitic fishes from Serra da Bodoquena karst area, Bonito County, in the southern plateau: the armored catfish *Ancistrus formoso* Sabino & Trajano 1997 and *Trichomycterus dali* Rizzato, Costa-Jr., Trajano & Bichuette 2011 (this fish was first collected in 1998, but its description took more than one decade to be published).

In the early 1990′s, extensive biologic surveys were made, focusing mainly on terrestrial cave communities, which resulted in the first comprehensive faunal lists for caves in the southern plateau of the Bodoquena karst area, Bonito, Jardim and Porto Murtinho counties (Gnaspini & Trajano 1994). By then, the high potential for the occurrence of specialized terrestrial and aquatic subterranean animals was clear.

The presence of several spectacular submerged and phreatic caves, for which the Bonito region is notorious worldwide, implied an increasingly strong touristic pressure over the area. Brazilian legislation requires management plans for caves destined for tourism and their surroundings. Hence, environmental studies were designed to base such plans, beginning with the most famous touristic point, the flooded cave Lago Azul, where *Potiicoara brasiliensis* was discovered. Such studies allowed for a better knowledge of the aquatic communities in some caves, such as Mimoso, Buraco das Abelhas, São Miguel and Anhumas, besides the Lago Azul.

In 2002, N. Moracchioli finished her Ph. D. thesis centered on Brazilian spelaeogriphaceans, which proved to be quite abundant and widespread in Serra da Bodoquena, also occurring in Ricardo Franco Cave, Forte Coimbra area, Corumbá Co. (also in Mato Grosso do Sul, but on the opposite margin of the Paraguay river, distant ca. 200 km from Bodoquena), and in Curupira cave, Serra das Araras, Rosário do Oeste County, in Mato Grosso state, to the north of Mato do Grosso do Sul. She also gathered data on *Megadiella* amphipods, which co-occur with spelaeogriphaceans in most localities, including Ricardo Franco, but not Curupira cave. Ricardo Franco Cave is historically important because it was the first Brazilian cave mentioned in a written document, a poem by Dom Aquino Corrêa, from the late 1700′s (Martin 1980) – *Harta figueira à porta. Entrada bruta./ Desço. Horrido hypogeum! Antro sombrio!/ Mas, de repente, um mágico arrepio,/ À luz das tochas. Tímida, a alma escruta.../*.... In 2000, that big fig tree was still at the cave entrance. As far as we know, this cave was not studied by biologists before their visit.

The studies above were carried out basically by researchers from the Universidade de São Paulo - USP, since there was no autochthonous academic speleobiology in Mato Grosso do Sul. In 2008, L. M. Cordeiro finished her M.Sc. dissertation, presented at the Universidade Federal do Mato Grosso do Sul - UFMS, which significantly enlarged the faunistic knowledge on the Bodoquena subterranean communities, in terms of new records for previously studied caves and new caves visited, including several in the northern plateau, reaching higher altitudes. She also performed the first detailed ecological study on cave terrestrial invertebrate communities in the area. As the result of a joint effort with Rodrigo Borghezan, student at the UNIDERP (MS), two additional new troglobitic fishes were found by these pioneers works on speleobiology in Mato Grosso do Sul, the heptapterid *Rhamdia* Bleecker 1858, (description is in progress) and the second troglobitic *Ancistrus* Kner 1845, known for the area. These species have been first studied by R. Borghezan for his undergraduate monograph (Borghezan 2008) and, later on, for his M.Sc. dissertation at the Universidade de São Paulo (Borghezan 2012).

We present here a synthesis of the faunal knowledge on the subterranean ecosystems in two karst areas of Mato Grosso do Sul, the intensively studied Serra da Bodoquena and the less known Forte Coimbra, based on published and unpublished data from the last two decades, as well as studies in progress, discussing the main threats for this highly relevant fauna.

Study site

The Serra da Bodoquena is one of the most extensive continuous Brazilian karst areas, located in the Mato Grosso do Sul state, in central-western Brazil. It consists of a north-south plateau extending for approximately 200 km, which forms an important water divide with an altitude around 800 m (Figure 1). It is unique in the context of the Brazilian Platform, because tectonic activities have been recognized in the area in the context of the Paraguay Belt and related to the development of the Cenozoic Pantanal Basin, which is still subsiding (Sallun-Filho & Karmann 2009, Campanha et al. 2011). Rains are concentrated in November to February, with a mean average

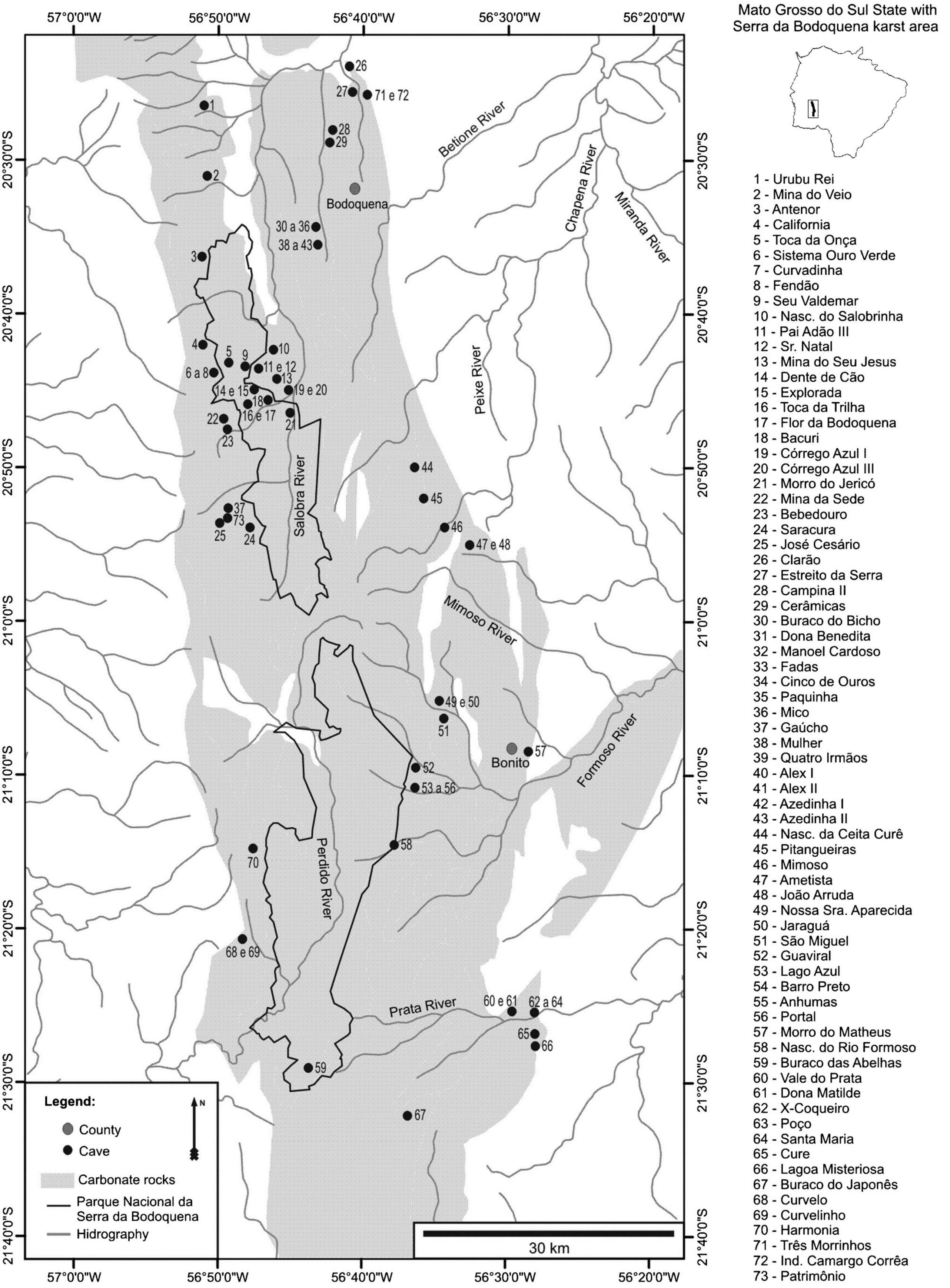


Figure 1. Map of the Serra da Bodoquena karst area, showing the localization of the studied caves (Modified from Bizzi et al. 2001). / Figura 1: Mapa da área cárstica da Serra da Bodoquena com a localização das cavernas estudadas (Modificado de Bizzi et al. 2001).

precipitation of about 1,300 mm per year and a mean annual temperature of 24°C. The natural vegetation consists of savanna in contact with semi-deciduous seasonal forest (Boggiani & Clemente 1999, Scremin-Dias et al. 1999).

At the eastern highland (greater than 500 m a.s.l.) the Bodoquena Plateau is divided into two main compartments, well defined by geomorphological contrasting features: the southern sector (Perdido River area), which has mainly karst landforms; and the northern part (Salobra River area), which has more fluvial characteristics. In the western lowlands (less than 500 a.s.l.), the Miranda river Depression forms exposed karst systems and part of a separate system of subjacent karst (Sallun-Filho & Karmann 2007). Geological evidence indicates that the karst aquifer is recharged mainly by autogenic waters, promoting the intense deposition of carbonates (as tufa deposits) as a consequence of resurgence of saturated waters (Sallun-Filho et al. 2004).

Most caves included in the present study are within the limits of the Parque Nacional da Serra da Bodoquena (PNSB), with 76.481 ha., created in 2000. Four rural settlements have been established in the surroundings of the PNSB at least 20 years ago, where several relevant caves harboring endemic troglobites are located. The livestock is currently the main economic activity in the region, although the tourism, including speleotourism, has an increasing economic importance, especially in Bonito, Bodoquena and Jardim counties.

Methods

Part of the data herein gathered were compiled from the literature, including publications such as the faunistic inventories (Gnaspini & Trajano 1994, Pinto da Rocha 1995, Pinto-da-Rocha & Sossegolo 2001, Galati et al. 2003, Costa-Jr. 2004, Eriksson & Gonçalves 2010) and taxonomy-oriented works (Trajano et al. 2000, Andrade et al. 2001, Mahnert 2001, Labruna & Venzal 2009, Pires-Vanin 2012), in addition to unpublished dissertations (Moracchioli 2002, Cordeiro 2008) and grey literature (Godoy 1986, Gnaspini et al. 1994). Original data came from collections by several speleobiologists in different occasions, including field courses, and more detailed identifications of material cited in the literature. For the sake of simplicity, we did not distinguish the source of data in the tables. Details on geographic coordinates of the caves listed in present work can be accessed at CNC/SBE link (Cadastro Nacional de Cavernas / Sociedade Brasileira de Espeleologia - www.cavernas.org.br/cnc).

In general, terrestrial organisms were collected by hand during visual inspections of all accessible substrates, using small brushes and tweezers. Pitfall-traps were used in some caves (Dente de Cão, Mateus, Córrego Azul I, Córrego Azul III, Sr. Natal, Fadas, Pitangueiras). Aquatic animals were collected with hand nets, bottles and baited minnow traps, from outside the water and during free and scuba diving. Surbers 30 x 30 cm were used for sampling benthonic organism in the Urubu Rei Cave.

Specimens were preserved in the proper solutions and brought to the laboratory for identification. Fishes were killed by over-anesthesia using benzocaine, fixed in formalin 4%, and preserved in alcohol 70%. Some samples were fixed in alcohol 100% for future molecular studies.

We did not use parataxonomy. Morphoespecies, when cited, have been identified by specialists on the taxonomy of the respective groups. Otherwise, the identifications are provided at the lowest level (usually supraespecific) reliable by non-specialists. Therefore, the number of species is probably much higher than shown in the tables.

The collecting efforts were highly heterogeneous. Most caves were sampled on a few occasions, generally during the dry seasons and on different years. A few caves were studied on several, consecutive occasions, both during dry and rainy seasons, such as the Córrego Azul I, Córrego Azul III, Fadas and Sr. Natal caves (4-5 occasions on 2006-2007; Cordeiro 2008); sampling with surber were carried out on eight occasions in the Urubu Rei Cave (2010-2012; L. M. Cordeiro, in progress). Therefore, differences in the number of taxa recorded in the tables do not necessarily reflect differences in biodiversity among caves.

The species/populations were classified according the Schiner-Racovitza system, following definitions in Trajano (2012): 1. **troglobites** correspond to exclusively subterranean source populations (a source populations has excess production and continues to grow if isolated); sink populations (a sink population, if cut off from all other migrants, eventually becomes extinct) may be found in surface habitats; 2. **troglophiles** include source populations both in hypogean and epigean habitats, with individuals regularly commuting between these habitats, promoting the introgression of genes selected under epigean regimes into subterranean populations (and *vice-versa*); 3. **trogloxenes** are instances of source populations in epigean habitats, but using subterranean resources.

Results

The taxa so far recorded for 84 caves visited in the Serra da Bodoquena karst area are presented in tables 1 and 2 (troglobites, found in 43 out of those caves) and in tables 3 to 9 (the remaining taxa); in addition, tables 1, 2 and 8 (Psychodidae dipterans) include caves in the poorly known Corumbá karst area, respectively Ricardo Franco and Forte Junqueira caves, situated ca. 200 km to the northwest of Bodoquena, at the border with Bolivia. The geographic location of studied caves is shown in Figure 1, except for 16 caves, for which there are no coordinates.

The diversity of some taxa, especially mites and ants, is not accurately represented due to taxonomic impediment: only the few identifications below the Order (for Acari) or Family (for the widespread formicids) levels were included in the tables.

As expected, the highest diversities were observed in the most intensively studied caves, in different occasions and seasons, and using different, complementary methods: Córrego Azul I e III, Fadas, Pitangueiras, João Arruda, Nossa Senhora Aparecida, São Miguel e Lago Azul.

Discussion

1 Distribution patterns and diversity

The subterranean fauna from the Bodoquena karst area has a unique taxonomic composition, combining typically subtropical (e.g., oniscodesmid diplopods) and tropical (e.g., Amblypygids, *Eusarcus* opilionids, isopterans, Bogidiellid amphipods), with (so far) exclusive elements (e.g., troglophilic and troglobitic onychophorans), including phylogenetic relicts, such as spelaeogriphaceans and *Megagidiella* amphipods. Geographic relicts include *Trichomycterus* catfish, not found

Table 1. Troglobitic taxa recorded in caves in the Salobra River Basin, Serra da Bodoquena karst area, Mato Grosso do Sul state. * Troglobitic *status* to be confirmed; ** at least six morphospecies recognized by specialists. / Tabela 1. Táxons troglóbios registrados em cavernas na Bacia do Rio Salobra, área cárstica da Serra da Bodoquena. * S*tatus* de troglóbio a ser confirmado; ** pelo menos seis morfoespécies identificadas pelos especialistas.

TAXA	Caves																					
	Salobra River - left margin																Salobra R. - right marg.					
	Urubu Rei	Mina do Véio	Califórnia	Toca da Onça	S. Valdemar	Pai Adão III	Sr. Natal	M. do Seu Jesus	Dente de Cão	Toca da Trilha	F. da Bodoquena	Bacuri	Morro do Jericó	Mina da Sede	Bebedouro	Saracura	Clarão	Buraco do Bicho	Dona Benedita	Manuel Cardoso	Fadas	Cinco de Ouros
AQUATIC																						
Teleostei Siluriformes																						
Heptapteridae *Rhamdia* sp. n.																				X	X	X
Loricariidae *Ancistrus* sp. n.																					X	
Trichomycteridae *Trichomycterus dali*													X			X						
Trichomycterus cf. *dali*	X	X	X	X	X						X			X	X							
Crustacea																						
Spelaeogriphacea																						
Spelaeogriphidae *Potiicoara brasiliensis*																X		X	X	X	X	X
Amphipoda																						
Bogidiellidae *Megagidiella azul*																	X	X				
Bathynellacea gen. n. sp. n.	X																					
Turbellaria Tricladida																						
Dugesiidae cf. *Girardia*								X										X	X			X
Annelida Oligochaeta																		X	X			
Gastropoda	X																					
Pomatiopsidae cf. *Spiripockia*																						
TERRESTRIAL																						
Onychophora																						
Peripatidae gen. n. sp. n.									X	X		X										
Arachnida																						
Araneae						X			X				X									X
Ctenidae						X			X				X									X
Opiliones																						
Gonyleptidae *Eusarcus* spp.**	X		X						X				X			X				X	X	X
Crustacea																						
Isopoda Oniscoidea	X										X											
Myriapoda																						
Diplopoda Paradoxosomatidae							X	X														
Hexapoda																						
Collembola																						
Paronellidae *Trogolaphysa* sp. n.	X																					
Coleoptera																						
Carabidae Paussinae *Pachytelis* sp.*							X															

Table 2. Troglobitic taxa recorded in the Ricardo Franco cave, in Corumbá area, and in other microbasins of the Serra da Bodoquena karst area, except Salobra River, both in Mato Grosso do Sul state. * troglobitic *status* to be confirmed. / Tabela 2. Táxons troglóbios registrados na caverna Ricardo Franco, área de Corumbá, e em outras microbacias da área cárstica da Serra da Bodoquena, exceto Rio Salobra, estado do Mato Grosso do Sul. * *Status* de troglóbio a ser confirmado.

TAXA	**CAVES**																					
	Corumbá	**Intermediate**					**Formoso River**					**Prata River**								**Perdido R.**		
	Ricardo Franco	Nasc. Ceita Curê	Pitangueiras	Mimoso	Abismo Opossum	Ametista	João Arruda	N. Sra. Aparecida	São Miguel	Lago Azul	Anhumas	N. Rio Formoso	B. das Abelhas	Dona Matilde	X – Coqueiro	Poço	Curê	Lagoa Misteriosa	B. do Japonês	Marcelo	Curvelinho	Harmonia
AQUATIC																						
Teleostei Siluriformes																						
Loricariidae *Ancistrus formoso*												X										
Trichomycteridae *Trichomycterus dali*													X									
Crustacea																						
Spelaeogriphacea																						
Spelaeogriphidae *Potiicoara brasiliensis*	X	X		X	X					X	X	X	X			X		X	X			
Amphipoda																						
Bogidiellidae *Megagidiella azul*		X		X	X					X	X		X			X						
Megagidiella cf. *azul*	X																					
Acari Oribata																						
Hidrozetidae *Hydrozetes* sp.*				X																		
Turbellaria Tricladida																						
Dugesiidae cf. *Girardia*				X	X					X												
Annelida Oligochaeta				X	X					X												
Gastropoda Pomatiopsidae		X										X										
TERRESTRIAL																						
Arachnida																						
Araneae																						
Ochyroceratidae *Speocera eleonorae*															X							X
Pseudoscorpiones																						
Chernetidae *Speleochernes dubius**			X							X	X											X
Myriapoda																						
Diplopoda Polydesmida																						
Oniscodesmidae *Katantodesmus* sp.							X	X	X								X					
Crypturodesmus sp.							X	X	X								X					
Paradoxosomatidae																				X		
Hexapoda																						
Collembola																						
Arrhopalitidae *Pararrhopalites papaveroi*							X			X												
Entomobryidae sp.1										X						X						
Entomobryidae sp.2								X														
Cyphoderidae						X								X			X					X
Paronellidae sp.*									X													
Hemiptera Dipsocoridae																					X	

Table 3. Non-troglobitic fishes recorded in caves of the Serra da Bodoquena karst area, Mato Grosso do Sul state. S. L. – Salobra River left margin; S. R. – Salobra River right margin; I. – Intermediate area; F. – Formoso river basin; Pr. – Prata river basin. / Tabela 3. Peixes não troglóbios registrados em cavernas da área cárstica da Serra da Bodoquena, estado de Mato Grosso do Sul. S. L. – margem esquerda do Rio Salobra; S. R. – margem direita do Rio Salobra; I – área intermediária; F. – bacia do rio Formoso; Pr. – bacia do Rio da Prata.

Taxa	**Caves**													
	S. L.		**S. R.**					**I.**	**F.**			**Pr.**		**I.**
	Antenor	N. Salobrinha	Nasc. Fadas	Dona Benedita	Manoel Cardoso	Fadas	Cinco de Ouros	Mimoso	Anhumas	N. Rio Formoso	N. Rio Formosinho	B. das Abelhas	Lagoa Misteriosa	B. do Japonês
Teleostei														
Synbranchiformes														
Synbranchidae *Synbranchus marmoratus*											X		X	
Perciformes														
Cichlidae *Bujurquina vittata*					X	X	X				X			
Crenicichla vittata											X			
Characiformes														
Characidae *Astyanax bimaculatus*									X			X	X	
Astyanax lineatus								X	X					
Astyanax fasciatus													X	
Astyanax sp.		X				X								
Jupiaba acanthogaster										X				
Moenkhausia bonita										X				
Hyphessobrycon eques										X				
Crenuchidinae *Characidium* sp.										X		X		
Curimatidae *Steindachneria* sp.										X				
Erythrinidae *Hoplias malabaricus*			X	X	X	X	X			X				
Iguanodectidae *Bryconops* sp.										X				
Gymnotiformes														
Sternopygidae *Sternopygus macrurus*		X									X			
Siluriformes														
Heptapteridae *Rhamdia quelen*	X		X								X	X		
Pimelodella sp.		X												
Loricariidae *Ancistrus* sp.											X	X		X
Hypostomus sp.											X			
Hypostomus cochliodon											X			

Table 4. Non-troglobitc invertebrates, except Psychodidae dipterans, recorded in caves of the left margin of the Salobra River basin, Serra da Bodoquena karst area, Mato Grosso do Sul state. * at least three species recognized by specialists. / Tabela 4. Invertebrados não troglóbios, exceto dípteros Psychodidae, registrados em cavernas da margem esquerda da bacia do Rio Salobra, área cárstica da Serra da Bodoquena, estado do Mato Grosso do Sul. * Três espécies reconhecidas pelos especialistas.

TAXA	CAVES																			
	Salobra River - left margin																			
	Urubu Rei	Antenor	Califórnia	Califórnia II	Sist. Ouro Verde	Curvadinha	Fendão	Sr. Valdemar	Pai Adão III	Sr. Natal	Mina do Seu Jesus	Dente de Cão	Explorada	Toca da Trilha	F. da Bodoquena	Bacurí	Córrego Azul I	Córrego Azul III	Morro do Jericó	Saracura
Turbellaria Continenticola																				
Turbellaria sp.3											X									
Nematomorpha Gordiacea							X											X		
Mollusca Gastropoda						X	X										X	X	X	
Pulmonata																	X	X		
Stylommatophora												X								
Onychophora																				
Peripatidae gen. n. sp. n.								X						X		X				
Epiperipatus sp.1												X	X	X						
Chelicerata Arachnida																				
Acari																				
Argasidae *Ornithodoros talaje*																	X	X		
Ornithodoros sp.																	X	X		
Ixodidae *Amblyomma cajennense*																		X		
Araneae																				
Araneidae *Alpaida negro*																	X			
Alpaida sp.1										X										
Cyclosa sp.	X																			
Caponiidae *Nops* sp.																		X		
Ctenidae			X		X	X		X				X						X		
Ctenus sp.										X							X	X		
Dipluridae												X								
Hahniidae																	X			
Lycosidae						X											X			
Allocosa sp.			X																	
Linyphiidae															X					
Mimetidae								X												
Ero sp.																	X			
Oonopidae																	X			
Philodromidae																		X		
Pholcidae *Mesabolivar* sp.2																	X			
Mesabolivar sp.3																	X			

Continued on next page

Table 4. Continued.

TAXA	**CAVES**																			
	Salobra River - left margin																			
	Urubu Rei	Antenor	Califórnia	Califórnia II	Sist. Ouro Verde	Curvadinha	Fendão	Sr. Valdemar	Pai Adão III	Sr. Natal	Mina do Seu Jesus	Dente de Cão	Explorada	Toca da Trilha	F. da Bodoquena	Bacurí	Córrego Azul I	Córrego Azul III	Morro do Jericó	Saracura
aff. *Spermophora*																	X	X		
Salticidae																	X			
Salticidae gen.1 sp.1																		X		
Salticidae gen.2 sp.1																	X			
Cory*thalia* sp.																	X			
Encolpius sp.																	X			
Noegus sp.																	X			
Marma nigritarsis																	X	X		
Segestriidae *Ariadna* sp.								X									X			
Sicariidae		X	X		X	X	X	X	X	X		X			X	X	X	X	X	
Loxosceles sp.1			X					X				X			X	X				
Theridiidae																	X	X		
Theridion sp.																	X	X		
Theridiosomatidae *Plato* sp.		X						X												
Titanoecidae																	X			
Trechaleidae			X				X										X	X		
Opiliones																				
Gonyleptidae *Eusarcus* spp.*			X		X	X	X												X	
Discocyrtus sp.	X		X					X				X		X			X		X	
Cosmetidae			X																	
Pseudoscorpiones																				
Chernetidae *Spelaeochernes* sp.									X								X	X	X	
Cheiridiidae																		X		
Scorpiones																				
Buthidae *Tityus confluens bodoquena*												X								
Amblypygi																				
Prhynidae *Heterophrynus vesanicus*	X	X	X	X	X		X	X		X		X		X	X	X	X	X	X	X
Crustacea																				
Isopoda										X		X					X	X		
Sphaeroniscidae *Circoniscus intermedius*												X								
Copepoda																				
Cyclopidae *Macrocyclops* cf. *albidus*	X																			

Continued on next page

Table 4. Continued.

TAXA	**CAVES**																			
	Salobra River - left margin																			
	Urubu Rei	Antenor	Califórnia	Califórnia II	Sist. Ouro Verde	Curvadinha	Fendão	Sr. Valdemar	Pai Adão III	Sr. Natal	Mina do Seu Jesus	Dente de Cão	Explorada	Toca da Trilha	F. da Bodoquena	Bacurí	Córrego Azul I	Córrego Azul III	Morro do Jericó	Saracura
Myriapoda																				
Chilopoda			X									X					X			
Diplopoda Spirostreptida																				
Pseudonannolenidae										X		X								
Spirostreptidae				X	X	X	X								X					
Polydesmida																				
Chelodesmidae						X		X				X								
Brasilodesmus sp.										X										
Paradoxosomatidae			X							X	X									
Oxidus gracilis			X																	
Oniscodesmidae	X		X		X	X				X		X			X				X	
Hexapoda																				
Archaeognatha																	X	X		
Blattaria					X		X	X												
Blaberidae aff. *Epilampra* sp.																	X			
Blattellidae *Cariblatta* sp.																	X			
Polyphagidae																	X	X		
Hypercompsa fenestrina																	X	X		
Coleoptera																				
Carabidae																		X		
Cerambycidae																	X	X		
Curculionidae																	X			
Dermestidae																	X	X		
Elateridae																		X		
Nitidulidae										X										
Pselaphidae																		X		
Scarabaeidae																	X			
Scolytidae										X								X		
Staphylinidae										X							X			
Leiodidae *Dissochaetus* sp.																				X
Diptera																				
Chaoboridae										X								X		
Drosophilidae *Drosophila* sp.																	X			

Continued on next page

Table 4. Continued.

TAXA	**CAVES**																			
	Salobra River - left margin																			
	Urubu Rei	Antenor	Califórnia	Califórnia II	Sist. Ouro Verde	Curvadinha	Fendão	Sr. Valdemar	Pai Adão III	Sr. Natal	Mina do Seu Jesus	Dente de Cão	Explorada	Toca da Trilha	F. da Bodoquena	Bacurí	Córrego Azul I	Córrego Azul III	Morro do Jericó	Saracura
Empididae																		X		
Mycetophilidae																		X		
Phoridae *Conicera* sp.1										X										
Conicera sp.2										X										
Megaselia sp.1																	X			
Megaselia sp.2																	X			
Dermaptera					X															
Orthoptera																				
Gryllidae	X		X	X	X			X			X				X					
Phalangopsidae *Endecous* sp.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Strinatia sp.					X					X										
Ephemeroptera Caenidae	X																			
Hemiptera																				
Cicadellidae																	X			
Cixiidae										X							X	X		
Heteroptera																				
Pyrrhocoridae																	X			
Reduviidae			X		X					X							X	X		
Mepraia sp.																		X		
Hymenoptera																				
Apidae Meliponinae																		X		
Bethylidae																	X			
Chalcidoidea sp.1																		X		
Chalcidoidea sp.2										X										
Ponerinae												X								
Lepidoptera																				
Tineidae	X	X	X		X		X	X				X			X		X	X	X	X
Noctuidae	X		X									X						X	X	
Neuroptera																				
Mantispidae																		X		
Trichoptera																		X		
Polycentropodidae *Polycentropus* sp.															X					
Thysanoptera																	X	X		

Table 5. Non-troglobitc invertebrates, except Psychodidae dipterans, recorded in caves of the right margin of the Salobra River basin, Serra da Bodoquena karst area, Mato Grosso do Sul state. * at least three species recognized by specialists. / Tabela 5. Invertebrados não troglóbios, exceto dípteros Psychodidae, registrados em cavernas da margem direita da bacia do Rio Salobra, área cárstica da Serra da Bodoquena, estado do Mato Grosso do Sul. * Três espécies reconhecidas pelos especialistas.

TAXA	**Caves**															
	Salobra River - right margin															
	Estreito da Serra	Campina II	Cerâmicas	Buraco do Bicho	Dona Benedita	Manoel Cardoso	Fadas	Cinco de Ouros	Paquinha	Mico	Mulher	Quatro Irmãos	Alex I	Alex II	Azedinha I	Azedinha II
Turbellaria Continenticola																
Turbellaria sp.1						X	X									
Turbellaria sp.2				X												
Nematomorpha Gordiacea								X								
Mollusca Gastropoda						X	X						X			
Onychophora Peripatidae																
Epiperipatus sp.2		X	X		X	X	X		X	X				X		
Chelicerata Arachnida																
Araneae																
Araneidae							X	X								
Alpaida sp.1													X			
Alpaida sp.2								X								
Micrathena sp.								X								
Eustala sp.								X								
Caponiidae *Nops* sp.				X												
Ctenidae			X		X	X	X						X			X
Ctenus sp.							X									
Corinnidae								X								
Linyphiidae													X			
Mimetidae *Ero* sp.							X									
Oonopidae													X			
Pholcidae *Mesabolivar* sp.2											X					
Salticidae *Chira* sp.1													X			
Chira sp.2								X								
Selenopsidae *Selenops* sp.								X								
Sicariidae							X	X					X			
Loxosceles sp.1								X								
Loxosceles sp.3													X			

Continued on next page

Table 5. Continued.

TAXA	**Caves**															
	Salobra River - right margin															
	Estreito da Serra	Campina II	Cerâmicas	Buraco do Bicho	Dona Benedita	Manoel Cardoso	Fadas	Cinco de Ouros	Paquinha	Mico	Mulher	Quatro Irmãos	Alex I	Alex II	Azedinha I	Azedinha II
Loxoceles gaucho												X				
Sparassidae							X									
Theridiidae							X									
Theridion sp.											X		X			
Thymoites sp.							X									
Theridiosomatidae *Plato* sp.					X	X	X	X					X			
Trechaleidae					X		X									
Uloboridae *Uloborus* sp.													X			
Opiliones																
Gonyleptidae *Eusarcus* spp.*			X								X		X			
Discocyrtus sp.						X		X								X
Cosmetidae						X										
Pseudoscorpiones																
Chernetidae							X									
Scorpiones Buthidae																
Tityus confluens bodoquena			X		X								X			
Amblypygi Phrynidae																
Heterophrynus vesanicus	X		X		X	X	X	X						X		
Crustacea																
Decapoda Palaemonidae																
Macrobrachium brasiliense							X	X								
Myriapoda																
Chilopoda					X			X					X			
Diplopoda																
Spirostreptida																
Pseudonannolenidae				X												
Spirostreptidae						X	X									

Continued on next page

Table 5. Continued.

TAXA	Caves															
	Salobra River - right margin															
	Estreito da Serra	Campina II	Cerâmicas	Buraco do Bicho	Dona Benedita	Manoel Cardoso	Fadas	Cinco de Ouros	Paquinha	Mico	Mulher	Quatro Irmãos	Alex I	Alex II	Azedinha I	Azedinha II
Polydesmida																
Chelodesmidae													X	X		
Paradoxosomatidae					X		X									
Oniscodesmidae			X		X	X	X									
Hexapoda																
Archaeognatha													X			
Coleoptera																
Cerambycidae							X									
Scydmaenidae							X									
Diptera																
Phoridae *Conicera* sp.1							X									
Megaselia sp.1							X									
Megaselia sp.2							X									
Orthoptera																
Gryllidae					X	X								X		
Phalangopsidae																
Endecous sp.						X	X	X	X		X		X		X	X
Ephemeroptera Caenidae						X										
Hemiptera Cixiidae							X									
Heteroptera Reduviidae							X									

Table 6. Non-troglobitc invertebrates, except Psychodidae dipterans, recorded in caves of the Formoso, Prata and Perdido river basins and intermediate areas of the Serra da Bodoquena karst area, Mato Grosso do Sul state. * Identified as *Blechroscelis* in Pinto-da-Rocha (1995); ** at least three species recognized by specialists. / Tabela 6. Invertebrados não troglóbios registrados em cavernas das bacias dos rios Formoso, Prata e Perdido e áreas intermediárias do carste da Serra da Bodoquena, estado de Mato Grosso do Sul. * Identificado como *Blechroscelis* em Pinto-da-Rocha (1995); ** pelo menos três espécies reconhecidas pelos especialistas.

TAXA	**Caves**									
	Formoso River									
	N. Sra. Aparecida	São Miguel	Jaraguá	Guaviral	Lago Azul	Barro Preto	Anhumas	Portal	Morro do Mateus	N. Rio Formoso
Annelida Oligochaeta					X					
Mollusca Gastropoda							X			
Pulmonata										
Stylommatophora										
Subulinidae		X								
Camaenidae *Solaropsis* sp.		X								
Megalobulimus cf. *oblongus*		X							X	
Chelicerata Arachnida										
Acari	X	X		X		X				
Argasidae *Carios fonsecai*		X								
Ornithodoros talaje		X	X							
Ornithodoros sp.									X	
Oribatida		X								
Araneae										
Amaurobiidae *Metaltella* sp.		X								
Araneidae				X	X					
Alpaida alto		X								
Ctenidae *Ctenus* aff. *griseolus*			X							
Ctenus sp.	X	X	X		X		X		X	
Isoctenus sp.		X							X	
Nothroctenus sp.		X								
Phoneutria nigriventer		X								
Lycosidae		X								
Mysmenidae *Mysmena* sp.					X					
Oonopidae *Oonops* sp.	X									
Orchestina sp.		X								
Pholcidae			X						X	
Mesabolivar sp.4							X			
Mesabolivar sp.5		X								
Mesobolivar sp.6*		X			X					
Scytodidae				X						
Scytodes globula				X						
Sicariidae	X				X		X		X	
Loxosceles sp.2									X	
Loxoceles gaucho	X	X								
Loxoceles grupo *gaucho*			X							
Loxoceles similis		X			X					
Theridiidae					X					
Achaearanea sp.	X									
Theridiosomatidae *Plato* sp.				X			X			
Titanoecidae		X								
Opiliones										
Gonyleptidae *Eusarcus* spp.**									X	
Parabalta sp.	X	X								
Cosmetidae							X			

Continued on next page

Table 6. Continued.

TAXA	Caves									
	Formoso River									
	N. Sra. Aparecida	São Miguel	Jaraguá	Guaviral	Lago Azul	Barro Preto	Anhumas	Portal	Morro do Mateus	N. Rio Formoso
Pseudoscorpiones										
Chernetidae *Spelaeochernes dubius*					X		X			
Spelaeochernes eleonorae	X	X		X						
Spelaeochernes sp.	X									
Amblypygi										
Prhynidae *Heterophrynus vesanicus*		X								
Crustacea										
Isopoda		X								
Armadillidae *Venezillo* sp.		X		X						
Diplopoda										
Spirostreptida										
Pseudonannolenidae	X			X	X					
Spirostreptidae	X			X						
Orthoporus sp.	X	X		X						
Polydesmida										
Chelodesmidae *Arthrosolaenomeris* sp.		X								
Oniscodesmidae	X				X			X	X	
Crypturodesmus sp.		X					X			
Cryptodesmidae *Cryptodesmus* sp.								X		
Hexapoda										
Collembola	X									
Arrhopalitidae *Arrhopalites* sp.		X								
Entomobryidae							X			
Paronellidae		X			X					
Coleoptera										
Carabidae *Galerita collaris*			X							
Dytiscidae					X					
Cholevidae *Dissochaetus murray*	X	X								
Scarabaeidae *Megasoma* sp.			X							
Staphylinidae	X									
Diptera										
Cecidomyiidae		X								
Chironomidae	X									
Culicidae Subf. Culicinae		X		X						
Drosophilidae *Drosophila eleonorae*	X									
Drosophila sp.				X						
Muscidae		X		X						
Mycetophilidae		X		X						
Keroplatidae				X						
Phoridae		X								
Conicera sp.3	X									
Megaselia sp.3				X						
Sphaeroceridae	X									
Tipulidae		X			X					
Orthoptera										
Phalangopsidae	X		X		X					
Endecous sp.		X					X			
Hemiptera										

Continued on next page

Table 6. Continued.

TAXA	Caves									
	Formoso River									
	N. Sra. Aparecida	São Miguel	Jaraguá	Guaviral	Lago Azul	Barro Preto	Anhumas	Portal	Morro do Mateus	N. Rio Formoso
Cicadellidae		X								
Derbidae		X								
Heteroptera										
Gerridae					X					
Veliidae							X			
Belostomatidae					X					
Coreidae					X					
Reduviidae		X			X					
Emesinae		X								
Hymenoptera										
Braconidae	X									
Ichneumonidae		X								
Solenopsis grupo *geminata*		X		X						
Vespidae	X	X	X							
Polybia ignobilis		X								
Isoptera	X	X			X					
Nasutiterminae *Nasutitermes* sp.		X								
Lepidoptera										
Tineidae	X	X		X	X	X				
Noctuoidea	X	X	X							
Noctuidae		X		X		X				
Psocoptera	X			X	X					
Psyllipsocidae		X								

in epigean water courses until now. Widespread taxa with troglobitic representatives in other karst areas include gastropods, and the several families of Collembola, a very ubiquitous group in caves worldwide. On the other hand, the low diversity of carabid beetles and oniscidean isopods, taxa usually abundant and diversified in most Brazilian caves, is unexpected.

When compared to other karst areas in Brazil, the Bodoquena distinguishes as a spot of high diversity of troglobites, together with the Alto Ribeira, in São Paulo state, and Serra do Ramalho, São Desidério and Chapada Diamantina, in Bahia state.

Among aquatic troglobites (also referred as stygobites), those occurring in the phreatic zone, such as spelaeogriphaceans, amphipods, planarians and *Trichomycterus* catfish, have a wide distribution across the sectors defined by the microbasins, indicating a connection across the aquifer, at least in the past. On the other hand, aquatic species living in base-level streams (for a habitat definition, see Trajano, 2001), such as *Rhamdia* n. sp. and *Ancistrus* n. sp. from Fadas system, and a new gastropod species, which may be the second reported to genus *Spiripockia*, present a locally restricted distribution. As well, terrestrial troglobites generally occur in caves in the same microbasin, probably due to fragmentation of the vadose (aerated) zone.

A higher degree of fragmentation may explain the higher diversity of terrestrial troglobites in the north plateau. Sallun-Filho & Karmann (2007) defined the Salobra River area as a canyons and alluvial plains (CAP). In fact, the Salobra base level already reached the impermeable substrates by the fluvial incision at times cutting down to the noncarbonated rocks (Sallun-Filho & Karmann 2007). This would explain the occurrence of different species of taxa as ctenid spiders, onychophorans and *Eusarcus* opilionids separated by the river valley (mostly a canyon).

As expected, many typically tropical taxa are more frequent in the north plateau, e.g., onychophorans, amblypygids, *Eusarcus* opilionids (also highly diversified in caves from Goiás and Bahia states – M.E. Bichuette, pers. comm., 2012). Troglophilic taxa widespread in Brazilian caves include *Loxosceles*, *Plato*, *Mesabolivar* and ctenid spiders, *Endecous* crickets, *Conicera* and *Megaselia* phorids.

The Serra da Bodoquena may be the southern limit of the distribution area of *Heterophynus vesanicus*, typical Cerrado species (A. Giuponni, pers. comm.). Females with eggs were observed in several caves during the dry season, which seems to correspond to the reproductive peak for the species in the Bodoquena area (Cordeiro 2008); caves are probably an important refuge for this troglophilic species during this period.

Table 7. Non-troglobitc invertebrates, except Psychodidae dipterans, recorded in caves of the Prata and Perdido river basins and intermediate areas of the Serra da Bodoquena karst area, Mato Grosso do Sul state. * Identified as *Blechroscelis* in Pinto-da-Rocha (1995); ** at least three species recognized by specialists./Tabela 7. Invertebrados não troglóbios, exceto dípteros Psychodidae, registrados em cavernas das bacias dos rios Prata e Perdido e áreas intermediárias do carste da Serra da Bodoquena, estado de Mato Grosso do Sul. * Identificado como *Blechroscelis* em Pinto-da-Rocha (1995); ** pelo menos três espécies reconhecidas pelos especialistas.

TAXA	Caves																					
	Intermediate					Prata River								Perd. R.			Indeterminate					
	Pitangueiras	Mimoso	Ametista	João Arruda	Serradinho	B. das Abelhas	Vale do Prata I	Vale do Prata II	Dona Matilde	X – Coqueiro	Poço	Santa Maria	Curê	Curvelo	Curvelinho	Harmonia	Cantagalo	T. do Tamanduá	Pindó	Serrasul	Alto Salobra	Eldorado
Nematomorpha Gordiacea				X																		
Annelida Oligochaeta																						
Hirudinea													X									
Mollusca Gastropoda																						
Pulmonata	X																					
Prosobranchia Thiaridae																						
Melanoides tuberculatus		X																				
Chelicerata Arachnida																						
Acari				X					X	X			X									
Acaroidea	X	X																				
Ixodida																						
Argasidade *Ornithodoros* sp.		X	X				X								X							
Oribata																						
Malaconothridae *Malaconothrus* sp.		X																				
Mesostigmata		X																				
Araneae																						
Araneidae	X	X												X								
Alpaida sp.		X																				
Ctenidae																			X			
Ctenus sp.	X			X							X								X			
Ochyroceratidae *Ochyrocera* sp.														X								
Speocera sp.										X												
Pholcidae	X	X					X			X									X			
Mesabolivar sp.2		X																				
Mesobolivar sp.6*	X										X		X	X	X	X						
Psilochorus sp.												X	X									
Salticidae	X																					
Scytodidae	X																					
Scytodes sp.	X																					

Continued on next page

Table 7. Continued.

TAXA	**Caves**																					
	Intermediate					**Prata River**								**Perd. R.**			**Indeterminate**					
	Pitangueiras	Mimoso	Ametista	João Arruda	Serradinho	B. das Abelhas	Vale do Prata I	Vale do Prata II	Dona Matilde	X – Coqueiro	Poço	Santa Maria	Curê	Curvelo	Curvelinho	Harmonia	Cantagalo	T. do Tamanduá	Pindó	Serrasul	Alto Salobra	Eldorado
Selenopidae	X																					
Sicariidae *Loxosceles* sp.	X	X	X	X									X			X						
Theridiidae		X								X												
Achaearanea sp.							X															
Chrosiothes sp.							X															
Theridion sp.		X																				
Theridiosomatidae																			X		X	
Plato sp.	X	X	X	X						X		X		X	X							
Thomosidae		X																				
Uloboridae *Ponella fasciata*	X																					
Zoridae		X																				
Opiliones																						
Gonyleptidae *Parabalta* sp.		X		X							X								X			
Cosmetidae	X																					
Pseudoscorpiones											X											
Lechytiidae *Lechytia chthoniiformis*													X									
Chernetidae *Spelaeochernes dubius*	X	X														X						
Spelaeochernes eleonorae		X	X	X			X		X	X	X	X	X					X				
Spelaeochernes sp.																		X				
Zaona cavicola												X										
Scorpiones	X																					
Buthidae *Tityus confluens bodoquena*	X																					
Amblypygi																						
Prhynidae *Heterophrynus* sp.			X	X						X	X	X	X									
Crustacea																						
Isopoda												X										
Armadillidae *Venezillo* sp.							X						X			X						
Sphaeroniscidae *Circoniscus* sp.												X										
Oniscidea *Trichorhina acuta*																		X				
Myriapoda																						
Chilopoda																			X			

Continued on next page

Table 7. Continued.

TAXA	**Caves**																					
	Intermediate					**Prata River**								**Perd. R.**			**Indeterminate**					
	Pitangueiras	Mimoso	Ametista	João Arruda	Serradinho	B. das Abelhas	Vale do Prata I	Vale do Prata II	Dona Matilde	X – Coqueiro	Poço	Santa Maria	Curê	Curvelo	Curvelinho	Harmonia	Cantagalo	T. do Tamanduá	Pindó	Serrasul	Alto Salobra	Eldorado
Geophilomorpha				X																		
Diplopoda				X					X													
Spirostreptida																						
Pseudonannolenidae	X			X	X		X					X			X					X		
Pseudonannolenidae gen.1 sp.1	X																					
Pseudonannolenidae gen.2 sp.1	X																					
Pseudonannolene sp.	X																					
Spirostreptidae	X			X	X		X	X									X	X				
Orthoporus sp.	X			X	X			X									X	X				
Polydesmida															X				X			
Chelodesmidae *Arthrosolaenomeris* sp.				X					X													
Leptodesmus sp.																	X					
Strongylomorpha sp.																			X			
Paradoxosomatidae *Cantharosoma* sp.	X																					
Mestosoma sp.	X																					
Fuhrmannodesmidae	X																					
Oniscodesmidae		X							X													
Crypturodesmus sp.	X		X									X			X	X	X	X				
Cryptodesmidae *Cryptodesmus* sp.									X													
Hexapoda																						
Collembola																						
Entomobryidae	X	X													X							
Paronellidae	X	X		X			X								X	X						
Archaeognatha															X							
Meinertellidae															X							
Blattaria							X		X													
Blaberidae											X				X							
Epilampra cf. *yersiniana*										X												
Blattellidae *Nyctibora* sp.				X																		
Coleoptera																						
Carabidae	X																					
cf. *Paratachys* sp.		X																				

Continued on next page

Table 7. Continued.

TAXA	Caves																					
	Intermediate				**Prata River**								**Perd. R.**			**Indeterminate**						
	Pitangueiras	Mimoso	Ametista	João Arruda	Serradinho	B. das Abelhas	Vale do Prata I	Vale do Prata II	Dona Matilde	X – Coqueiro	Poço	Santa Maria	Curê	Curvelo	Curvelinho	Harmonia	Cantagalo	T. do Tamanduá	Pindó	Serrasul	Alto Salobra	Eldorado
Polyphaga													X									
Cholevidae *Dissochaetus murray*		X		X												X						
Chrysomelidae													X						X			
Elateridae Pyrophorinae										X												
Ptiliidae Cephaloplectinae											X											
Scarabaeidae *Megasoma* sp.				X																		X
Scraptiidae																X						
Scydmaenidae												X										
Staphylinidae		X																				
Tenebrionidae Alleculinae							X					X										
Histeridae	X																					
Scarabaeidae	X																					
Noteridae	X																					
Leiodidae	X																					
Diptera																						
Drosophilidae	X																					
Fanniidae *Fannia* sp.		X														X						
Milichiidae *Pholeomyia* sp.				X			X						X			X						
Muscidae	X																					
Sciaridae	X						X															
Phoridae *Conicera* sp.3		X	X	X			X															
cf. *Apocephalus* sp.							X															
Dohrniphora sp.																X						
Streblidae				X																		
Tachinidae													X									
Orthoptera																						
Phalangopsidae			X	X																	X	
Endecous sp.	X	X									X											
Hemiptera																						
Cicadellidae																						
Cixiidae				X						X												
Fulgoridae	X			X															X			

Continued on next page

Table 7. Continued.

TAXA	**Caves**																					
	Intermediate					**Prata River**								**Perd. R.**			**Indeterminate**					
	Pitangueiras	Mimoso	Ametista	João Arruda	Serradinho	B. das Abelhas	Vale do Prata I	Vale do Prata II	Dona Matilde	X – Coqueiro	Poço	Santa Maria	Curê	Curvelo	Curvelinho	Harmonia	Cantagalo	T. do Tamanduá	Pindó	Serrasul	Alto Salobra	Eldorado
Heteroptera	X			X												X						
Cixiidae		X																				
Cydnidae													X									
Dipsocoridae														X								
Emesinae	X										X											
Reduviidae	X	X												X		X						
Hymenoptera																						
Formicidae *Camponotus* sp.												X			X							
Paratrechina sp.	X														X	X						
Acromyrmex sp.							X															
Solenopsis grupo *geminata*		X	X	X			X		X	X			X									
Labidus coecus														X								
Pachycondyla sp.							X															
Isoptera			X	X															X	X		
Nasutiterminae	X																					
Nasutitermes sp.							X								X							
Diversitermes sp.														X								
Syntermes sp.											X											
Lepidoptera																						
Tineidae	X	X	X	X			X					X	X									
Hesperiidae																			X			
Noctuoidea																				X	X	X
Noctuidae	X													X								
Neuroptera																						
Mantispidae	X	X																				
Psocoptera																						
Psyllipsocidae							X						X									

Table 8. Psychodidae dipterans recorded in caves of the Serra da Bodoquena karst area, Mato Grosso do Sul state (Modified from Galati et al. 2003). Co. – Corumbá; S.L. – Salobra River left margin; S.R. – Salobra River right margin; I. – Intermediate; F. – Formoso River; Pr. – Prata River; P. – Perdido River./ Tabela 8. Diptera Psychodidae registrados em cavernas da área cárstica da Serra da Bodoquena, Mato Grosso do Sul (Modificado de Galati et al. 2003). Co. – Corumbá; S.L. – margem esquerda do Rio Salobra; S.R. – margem direita do Rio Salobra; I – intermediário; F.R. – Rio Formoso; Pr. – Rio da Prata; P. – Rio Perdido.

TAXA	CAVES																			
	Co.	S.L.			S.R.			I.			F.					Pr.			P.	
	Forte Junqueira	José Cesário	Gaúcho	Patrimônio	Três Morrinhos	Estreito da Serra	Indúst. C. Corrêa	Pitangueiras	Mimoso	Ametista	N. Sra Aparecida	São Miguel	Jaraguá	Guaviral	Lago Azul	Buraco das Abelhas	Vale do Prata I	Santa Maria	Curvelo	Harmonia
Diptera Psychodidae									X	X			X	X	X		X	X	X	
Psychodinae																				X
Brumptomyia avellari												X			X					
Brumptomyia brumpti		X	X		X							X			X					
Brumptomyia cunhai			X	X				X				X			X					
Brumptomyia galindoi												X								
Brumptomyia sp.											X				X					
Evandromyia corumbaensis		X	X	X	X	X	X	X			X	X			X					
Lutzomyia almerioi		X	X	X	X	X	X	X			X	X			X					
Lutzomyia longipalpis		X		X	X	X	X	X			X	X			X					
Pintomyia kuscheli		X																		
Sciopemyia sordellii		X	X	X	X	X	X	X			X	X			X					
Sciopemyia sp.								X												
Martinsmyia oliveirai		X		X	X	X	X	X				X			X					
Nyssomyia whitmani		X	X	X	X	X	X	X			X	X			X	X				
Psathyromyia campograndensis															X					
Psathyromyia punctigeniculata		X			X		X					X			X					
Psathyromyia shannoni		X					X					X			X					
Micropygomyia acanthopharynx								X												
Micropygomyia peresi					X	X	X	X			X				X					
Micropygomyia quinquefer	X			X	X	X		X				X			X					

Table 9. Bats recorded in caves of the Serra da Bodoquena karst area, Mato Grosso do Sul state. S.L. – Salobra River left margin; S.R. – Salobra River right margin; I. – Intermediate; F. – Formoso River. / Tabela 9. Morcegos registrados em cavernas da área cárstica da Serra da Bodoquena, estado do Mato Grosso do Sul. S.L. – Rio Salobra margem esquerda; S.R. – Rio Salobra margem direita; I. – intermediário; F. – Rio Formoso.

TAXA	CAVES										
	S.L.			S.R.	I.			F.			
	Sr. Natal	Córrego Azul I	Córrego Azul III	Fadas	Pitangueiras	Mimoso	João Arruda	N. Sra. Aparecida	São Miguel	Anhumas	Morro do Mateus
Mammalia Chiroptera											
Emballonuridae *Peropterix macrotis*									X		X
Phyllostomidae *Chrotopterus auritus*						X		X	X		X
Desmodus rotundus			X		X	X			X	X	X
Anoura geoffroyi						X					
Anoura caudifer	X										
Glossophaga soricina		X	X	X		X			X		
Carolia perspicillata	X		X	X					X		
Plathyrrinus lineatus		X	X								
Phyllostomus hastatus			X			X					
Natalidae *Natalus espiritosantensis*				X			X				X

The largest dry cave in Serra da Bodoquena, Dente de Cão, with more than 2 km of mapped passage ways, harbors a rich troglomorphic fauna, including a new onychophoran species and arthropods such as Ctenidae spiders and *Eusarcus* (Gonyleptidae) opilionids, which, together with troglophilic populations, compose a unique terrestrial cave community. In Toca da Trilha cave, troglobitic velvet-worms are in syntopy with non-troglomorphic peripatids belonging to another new species, which occur at the cave entrance and the twilight zone of several caves; in one occasion, individuals of both species were observed at the same deposit of organic detritus in the twilight zone of Toca da Trilha.

2 Phylogenetically and biogeographically relevant taxa: Spelaeogriphacea, Megagidiella, Onychophora

The onychophorans found in caves on the Serra da Bodoquena represent a considerable extension of the distribution area for the Phyllum, and the first record of a troglobitic species for South America. There are only two other described troglobitic species of velvet worms, *Peripatopsis alba* Lawrence 1931, from Wynberg Cave, in the Table Mountains, South Africa, and *Speleoperipatus spelaeus* Peck 1975, from Pedro Great Cave, in Jamaica. Nothing was published after the original descriptions and there is no news about this material. The non-troglomorphic *Epiperipatus* sp. 1 (Table 3) may be troglophile since it was observed both in epigean and in aphotic habitats. It is relevant that, so far, these are the only cases of subterranean (cavernicoles *sensu latu*, herein defined as evolutionary units responding to subterranean selective regimens - Trajano 2012) onychophorans recorded for Brazil.

The distribution of *Megagidiella azul* Koenemann & Holsinger, 1999 and *P. brasiliensis* was extended to the northern plateau, encompassing now all the Bodoquena karst. In general, these species are syntopic, together with the troglomorphic planarians and oligochaetes, suggesting a responding to the same vicariant events by these invertebrates. On the other hand, in general spelaeogriphaceans are absent from caves with *Trichomycterus* catfish, except for Saracura (in the left margin of the Salobra river) and Abelhas (in the Prata River) caves, were the two co-occur. These patterns deserve further studies and we hypothesize that the predator effect on prey may maintain very low densities of *P. brasiliensis* when *Trichomycterus* are present.

The *Megagidiella* species from Ricardo Franco Cave has to be confirmed. On the other hand, the spelaeogriphaceans from this cave, and also from Curupira, in Mato Grosso, more than 700 km far from the type-locality (Lago Azul Cave) are morphological indistinguishable, all included in the nominal species *Potiicoara brasiliensis* (Pires-Vanin 2012). A deep phreatic connection, not demonstrated till now, could explain this exceptionally wide distribution (Pires-Vanin 2012). Other explanation is a former superficial connection, interrupted by the subsidence of the Pantanal basin, associated with a long period of evolutionary stasis, not unexpected for animals living in an exceptionally stable environment. A third hypothesis is dispersion via the hyporheic zone along the Paraguay riverbed.

3 Ecological notes

A marked characteristic of the Cerrado domain is an accentuated variation in the relative humidity of air between the rainy and dry seasons. Values as extreme as 4% of relative humidity in the dry winter to 80-90 % in the rainy summer were recorded outside the entrances of the caves studied by Cordeiro (2008). Several invertebrates concentrate at the entrance and twilight zones during the winter, as is the case with noctuid moths, neuropterans and epigean onychophorans. An increase in faunal richness and abundance was observed in the intensively studied Córrego Azul I and Córrego III caves during the dry season, when a migration towards the twilight zone, up to 20 m far from the entrance, was recorded (Cordeiro 2008). This illustrates the importance of caves as refuges for the epigean fauna during the stressful dry and cold seasons.

Exceptionally high population densities for guanophile invertebrates have been recorded at guano deposits, such as *Megagidiella* amphipods reaching densities of hundreds to thousands individuals per m^2 on submerged guano of hematophagous bats in Ricardo Franco Cave (Moracchioli 2002). Oniscodesmid diplopods were also observed in high densities on vampire bat guano in dry caves of the south plateau. However, it is important noting that the large populations of vampire bats in these areas are a response to the increased availability of prey represented by domestic animals, especially cattle, and do not correspond to the original condition. In undisturbed areas, such as Amazonian forests, the population densities of hematophagous bats are low. Consequently, even in caves with huge amounts of insectivorous bat guano, such as those in the Altamira-Itaituba sandstone area, hematophagous guano deposits are small and sparse (Trajano & Moreira 1990). In natural conditions, population booms of guanophilous invertebrates associated to vampire bats would be a rare occurrence, associated to maternity colonies.

Potiicoara brasiliensis also forms very large populations and, in view of its extremely wide distribution, these crustaceans may be among the most abundant aquatic troglobites in the world (copepods excluded), if this is indeed a single species. Although not reaching the exceptional densities shown by *Megagidiella* sp. in Ricardo Franco cave, *P. brasiliensis* may be quite abundant in caves such as Lago Azul – maximum densities of ca. 30 individuals per 400 cm^2 were recorded by Moracchioli (2002), and similar densities were observed by R.B. and L.M.C. in Cinco de Ouros and Buraco do Bicho caves. It is noteworthy that, although much more common in the typically lentic environment of submerged caves, *P. brasiliensis* has also been found in the base-level stream of Fadas cave, on the bottom of a reach with moderate water current, as also observed in the Buraco das Abelhas.

4 Conservation: Main threats to the subterranean biodiversity in the Mato Grosso do Sul karst areas

Subterranean ecosystems pose special problems for conservation due to their intrinsic fragility and the distinctive features of subterranean communities, including a high degree of endemism and morphological, ecological and behavioral specializations of troglobites (Bichuette & Trajano 2010). Subterranean ecosystems in Mato Grosso do Sul, especially in the Serra da Bodoquena karst area, highly relevant in view of its biodiversity, distinguishing as a high diversity spot for troglobites, both vertebrates and invertebrates, are seriously endangered by the inappropriate use of karst landscapes.

Habitat destruction is the main threat to natural environments throughout the globe, and subterranean habitats are not exception. Irreversible habitat loss due to quarrying for cement production is the cause for subterranean biodiversity decline in karst areas, along with building of dams and reservoirs for hydro-electric power plants and water storage for human use (Bichuette & Trajano 2010). In the Serra da Bodoquena karst area, several mining companies are prospecting or requiring licenses for mineral prospection throughout the region, mainly for carbonates and ore (BRASIL 2013). Limestone have been extensively exploited in the Serra da Bodoquena since the 1980′s, including the vicinity of the Parque Nacional da Serra da Bodoquena, without any public environmental studies (it is relevant that caves became fully protected in 1990, by Federal Decree 99.556, which made such studies mandatory for any area with speleological potential; its substitutive, Decree 6640/2008, kept such requirement). In the absence of previous studies, and because the areas where mining is in progress is interdict for speleologists, no information is available for such caves, therefore it is unknown how much diversity has been so far lost due to such activities in Bodoquena and Bonito County. In these cases, the lack of control and law enforcement by the authorities is an important factor responsible for subterranean diversity degradation. A similar situation concerning mining is observed in the Corumbá karst area, but the lack of knowledge (see below) does not allow for evaluation of the real or potential threats for subterranean ecosystems.

Land use in Serra da Bodoquena and Bonito area has intensified since the early 1980′s as relatively small rural settlements and, a decade later, as larger farms in the higher terrains for semi-intensive livestock farming; in the last decade, soybean monoculture has been replacing cattle in the lower terrains, around Bonito Co., with even more catastrophic consequences.

Karst areas are characterized by efficient flow of groundwater through conduit systems originated by the properties of soluble rocks. Therefore, the water usually drains rapidly into the subsurface at recharge zones, through a network of fractures and cave conduits. This groundwater emerges at the surface in discharge zones through springs and wells (Sallum-Filho & Karmann 2012). Removal of the surface vegetation is another global environmental threat, even more in the fragile karst landscapes, due to the predominance of groundwater drainages. This causes decrease in the nutrient input, negatively affecting the subterranean, and changes in hydrological regimes (Bichuette & Trajano 2010).

Intensification of sedimentation, caused by human interference, mainly deforestation and livestock, were recorded during our fieldwork in caves of high hydrological and/or biological relevance, mainly in the Rio Salobra headwaters, where three undescribed troglobitic catfish occur. In Fadas Cave, Campina settlement, where the cave catfish *Rhamdia* and *Ancistrus* populations, endemic to this cave system, have been monitored since 2006, a large deposit of sediment (1.5 m height) was formed after the deforestation of one important sinkhole area located about 300 m upstream this cave. In Califórnia and Beija-Flor caves (systems where *Trichomycterus* cf. *dali* occurs), located in a farm where livestock is expanding, the formation of erosional craters was identified at many upstream points. These caves are major sinkholes and their streams are in advanced stages of sedimentation (Cordeiro et al. 2012)

Pumping of subterranean waters for human and livestock use, another cause for disturbance of subterranean ecosystems due to lowering of water table (Proudlove 2011, Bichuette & Trajano 2010), is common in the Bodoquena and Bonito region as artesian and non-artesian wells; so far, no visible impact has been detected, but this potential threat must be monitored. Introduced species, recognized as a major danger for natural environments around the world, may also become a problem in some areas in the Bodoquena region, where water reservoirs (“açudes”) for cattle use have been build upstream subterranean systems. During rains, fish introduced in these “açudes” enter caves, such as traíras (Erythrinid characiforms) and cichlids, which are predators of cavefish and other troglobitic organisms.

Alien species are harmful for aquatic indigenous and endemic fauna in fresh waters and can have a deep and pervasive effect on ecosystems that they invade (Strayer 2010). The record of *Melanoides tuberculatus* in Mimoso Cave, where phreatic troglobites as *P. brasiliensis*, *M. azul* and *Girardia* sp. also occur, is the first record of an invasive species in Brazilian groundwaters. *M. tuberculatus* is originally from African continent and have been responsible for total substitution of benthonic communities in surface fresh waters in North and South America (Fernandez et al. 2003, Thiengo et al. 1998). The occurrence of this alien gastropod in Mimoso Cave is recorded ten years after studies on speleogriphaceans made by Morachiolli (2002) in the same site, proving that it is a very recent invasion in this environment. The monitoring and research on population control of *M. turberculatus* is quite urgent for conservation of the endemic phreatic fauna and to understand its potential to damage the groundwater ecosystem.

Pollution of subterranean drainages, another important threat for subterranean ecosystems throughout the world (Proudlove 2001, Bichuette & Trajano 2010), is also concern in the Serra da Bodoquena region. Use of dolines for garbage dumping has been observed in several settlements, such as the Campina. Pollution by detergents, largely used in settlements, may have a deleterious impact on subterranean fish due to interference on their natural behavior (Trajano 1997). Nitrogen derivates, bacteria such as fecal coliforms and chemical residues of drugs used in livestock accumulate in the subsurface. Due to the hydrological characteristics of the karst, pollutants spread fast and widely in groundwaters. In the Bonito County surroundings, the substitution of livestock by soybean monocultures have a great potential to deteriorate even more the quality of underground waters due to the use of large amounts of pesticides, which may contaminate springs and wells, calling for attention and serious actions by the authorities.

Tourism is a major economic activity in the region, especially in Bonito, where caves receive thousands of tourists every year. Therefore, the demand for caves with potential tourist use is increasing and visitation to caves not open to tourism is a recurrent problem. Negative consequences of visitation are: topoclimatic changes, introduction of alien species, including pathogens, and materials (such as metal, cement and treated wood), pollution by items left in caves, soil compacting and trampling of cavernicoles, destruction of specific microhabitats and direct disturbance of fauna (lights, loud noises, handling of specimens) (Trajano 2000). Dente de Cão and Urubu Rei are examples of diversified ecosystems endangered by uncontrolled human visitation.

For a discussion on environmental problems affecting subterranean habitats in Mato Grosso do Sul, see also Cordeiro et al. (2013).

In conclusion, Serra da Bodoquena, the largest and by far the best known karst area in Mato Grosso do Sul, is undoubtedly a priority area for conservation in Brazil, requiring urgent actions for effective protection of its subterranean ecosystems, many of which are endangered. The Park area must be increased, and its limits expanded in order to encompass the headwaters and recharge areas of the main subterranean systems, and, in the occupied areas, other categories of conservation units should be created to guarantee a sustainable use of the land. More attention should be paid to the settlements by the authorities, providing education, health and sanitary conditions adequate to the karst terrain. In addition to environment studies to fulfill the legal requirements in areas of economic interest, and a serious effort by the authorities to enforce such regulations and properly control cave visitation, a responsible attitude by the media is fundamental, in order to broadcast correct information, not distorted by sensationalism, always with the objective of preserving the subterranean biodiversity of Mato Grosso do Sul.

Final considerations

1 Perspectives for speleobiology in Mato Grosso do Sul

The major bottleneck for the progress of the speleobiology in Mato Grosso do Sul, as generally in Brazil, is taxonomic impediment. Therefore, the investment on the formation of taxonomists is of paramount importance.

The faunistic data reunited here allows for a first approach on the taxonomic diversity at the alfa (local) level, in a spatial scale. As a next step to describe the structure and functioning of subterranean ecosystems in the Bodoquena area, long term studies in selected caves are needed in order to detect temporal patterns. In addition, additional collections are needed for taxonomic studies of many taxa. As well, the addition of new caves, especially in the least known southern plateau, will allow to verify the relationship between local and regional species richness.

However, considering only the observed local diversity is not enough because taking into account only the number of species and their relative contribution have little predictive power about the functioning of the communities. A very promising research field is to compare functional and phylogenetic diversity of different types of caves in relation to epigean habitats geographically continuous. Widely distributed nominal species (e.g., *Potiicoara brasiliensis*) or species groups (*Megagidiella*, *Trichomycterus*) are excellent models for phylogeographic analyses associated to detailed morphological studies.

Genera with both troglobitic and troglophilic populations, such as ctenid spiders, *Eusarcus* opilionids and onychophorans, offer good opportunities for comparative studies focusing on genetics, biology, physiology and behavior. On the other hand, the relictual character of several troglobites, specially the aquatic ones, without living epigean relatives in the area, poses difficulties for such studies.

Other cave areas in Mato Grosso do Sul are barely known. Speleobiological studies are lacking in the Corumbá area and the Serra do Amolar, northwestern MS, both with exposed limestones and iron rocks in the vicinity, and in the sandstones of Serra de Maracajú.

2 Main collections:

After study, the material collected in subterranean habitats is send to official collections of institutions registered at the CNPQ – Conselho Nacional de Pesquisa and CGEN – Conselho de Gestão do Patrimônio Genético, mainly the Museu de Zoologia da Universidade de São Paulo – MZUSP, the Museu Biológico do Instituto Butantã, the Museu Nacional do Rio de Janeiro – MNRJ and Coleção do Zoológica da Universidade Federal do Mato Grosso do Sul - ZUFMS.

During studies, the specimens are kept in the institutions of the respective specialists; small reference collections are in the authors' institution for comparison and training.

Acknowledgements

We are especially indebted to the colleagues and students who made collections and preliminary identifications, providing original data on several caves (e.g., Anhumas, Mimoso, Buraco das Abelhas, Lago Azul, Nossa Senhora Aparecida, Pitangueiras, Curê, Tamanduá), especially to Edmundo Dinelli Costa-Junior, Regina Bessi, Sandro Secutti, Cristina Rheims, Marcos Ryotaro Hara, Fabiana C. Pioker, Melissa Regina Pioker and Jean-Jacques Geoffroy. Thanks are also due to the speleologists who helped us in the fieldwork, in special Bruna M. Cordeiro, the Grupo de Espeleologia da Serra da Bodoquena, César Augusto Miner (GBPE), José Guilherme (PNSB) and Suzana Escarpinati (UFGD). The authors acknowledge the specialists who helped with the identifications: Antônio Brescovit and Igor Cizauskas (Araneae), Cibele Bragagnolo, Ricardo Pinto da Rocha and Marcos Hara (Opiliones), João Paulo Barbosa (Diplopoda), Gustavo Graciolli (Diptera), Regina Bessi and Sérgio Vanin (Coleoptera), Marcio Bolfarini (Ensifera), Gilmar Neves (Copepoda) and Luiz Ricardo L. Simone (Mollusca). Gustavo Graciolli also for the constructive ideas in previous version of this manuscript. William Sallun Filho for the help with geological map and caves coordinates. The collections were authorized by IBAMA/SISBio and PNSB (E.T. and colaborators, licenses N° 051-DIFAP/IBAMA de 09/05/2006, 089-DIFAP-IBAMA de 21/07/2005, 137/2004-CGFAU/LIC, 0023/03-CECAV/DIREC, 004/02-CECAV/DIREC, 063/2001-DIFAS/DIREC, 027/2000-DIFAS/DIREC; L.M.C., R.B, ET. 22892-1 ICMBIO/SISBIO). The cave diving activities were authorized by PNSB/ICMBio (n° 02091.000005/2012-38) and guided by Tuta Barroco. This work was supported by FUNDECT/MS (L.M.C. Ms.c. Grant 41/100.082-2005), CNPQ (E.T., Grant n. 303362/2007-3; L.M.C. PhD Grant 143379/2009-7), FAPESP (R.B. Grant n. 2010/03084-2), IB/USP, CAPES-PROEX.

References

ANDRADE, R.M.G., GALATI, E.A.B. & TAMBOURGI, D.V. 2001. Presença de *Loxosceles similis* Moenkhaus, 1898 (Araneae, Sicariidae) na Serra da Bodoquena, Estado de Mato Grosso do Sul, Brasil. Rev. Soc. Bras. Med. Trop. 34: 275–277, http://dx.doi.org/10.1590/S0037-86822001000300008

BIZZI, L.A., SCHOBBENHAUS, C., GONÇALVES, J.H., BAARS, F.J., DELGADO, I.M., ABRAM, M.B., NETO, R.L., MATOS, G.M.M. & SANTOS, J.O.S. 2001. Geologia, Tectônica e Recursos Minerais do Brasil: Sistema de Informações Geográficas - SIG e Mapas na escala 1: 2.500.000, Brasília.

BOGGIANI, P.C. & CLEMENTE, J. 1999. A questão do Licenciamento Ambiental de empreendimentos turísticos no Planalto da Bodoquena - Mato Grosso do Sul. Rev. Geogr. 2: 29–40.

BORGHEZAN, R. 2008. Caracterização da ictiocenose em poções da Gruta das Fadas e aspectos biológicos de *Rhamdia* sp. (Siluriformes: Heptapteridae) e *Ancistrus* sp. (Siluriformes: Loricariidae), Serra da Bodoquena, Mato Grosso do Sul. Monografia, Universidade para o Desenvolvimento do Estado e da Região do Pantanal, Campo Grande.

BRASIL. 2013. Departamento Nacional de Produção Mineral - Sistema de informações geográficas da mineração. http://www.dnpm.gov.br (last accessed in 03/11/2013).

CAMPANHA, G.A.C., BOGGIANI, P.C., SALLUN-FILHO, W., SÁ, F.R., ZUQUIM, M.P.S. & PIACENTINI, T. 2011. A Faixa de Dobramento Paraguai na Serra da Bodoquena e Depressão do Rio Miranda, Mato Grosso do Sul. Geol. USP, Sér. Cient. 11(3):79–96, http://dx.doi.org/10.5327/Z1519-874X2011000300005

CORDEIRO, L.M.. 2008. Fauna cavernícola da Serra da Bodoquena: revisão bibliográfica e um estudo de ecologia de comunidades. Dissertação, Universidade Federal do Mato Grosso do Sul, Campo Grande.

CORDEIRO, L.M., BORGHEZAN, R, TRAJANO, E., ESCARPINATI, S., MARQUES, K. 2012. The silence degradation in the water paradise: Sedimentation in caves of Serra da Bodoquena karst area, State of Mato Grosso do Sul, NW Brazil. In: 49th Annual Meeting of the Association for Tropical Biology and Conservation - Abstracts, Bonito. http://www.atbc2012.org/ (last accessed in 10/09/2012).

CORDEIRO, L.M., Borghezan & TRAJANO, E. 2013. Distribuição, riqueza e conservação dos peixes troglóbios da Serra da Bodoquena, MS, Brasil (Teleostei: Siluriformes). Rev. Biol. 10(2): 21–27.

COSTA, JR., E. 2004. Fish from the Underwater Caves of Bodoquena Plateau, Mato Grosso do Sul, Southwestern Brazil. DIR Lifestyle & Underwater Adventure Magazine. 5:8-12.

ERIKSSON, A. & GONÇALVES, F. 2010. Síntese do conhecimento da fauna de morcegos cavernícolas na Serra da Bodoquena. O Carste. 22(2):39–42.

FERNANDEZ, M.A., THIENGO, S.C. and SIMONE, L.R.L. 2003. Distribution of the introduced freshwater snail *Melanoides tuberculatus* (Gastropoda: Thiaridae) in Brazil. Nautilus. 117: 78–82.

FORD, D. &WILLIAMS, P. 2007. Karst Hydrogeology and Geomorphology. Wiley, Chichester.

GALATI, E.B., NUNES, V.L.B., BOGGIANI, P.C., DORVAL, M.E.C., CRISTALDO, G., ROCHA, H.C., OSHIRO, E.T., ANDRADE, R.M.G. & NAUFEL, G. 2003. Phlebotomines (Diptera, Psychodidae) in caves of the Serra da Bodoquena, Mato Grosso do Sul State, Brazil. Rev. Bras. Entomol. 47:283–296, http://dx.doi.org/10.1590/S0085-56262003000200017

GNASPINI, P. & TRAJANO, E. 1994. Brazilian cave invertebrates, with a checklist of troglomorphic taxa. Rev. Bras. Entomol. 38(3/4):549–584.

GNASPINI, P., TRAJANO, E. & SANCHEZ, L.E. 1994. Província Espeleológica da Serra da Bodoquena, MS: exploração, topografia e biologia. Espeleo-tema 17:19–44.

GODOY, N.M. 1986. Nota sobre a fauna cavernícola de Bonito, MS. Espeleo-tema 15:80-92.

LABRUNA, M.B. & VENZAL, J.M. 2009. *Carios fonsecai* sp. nov. (Acari, Argasidae), a bat tick from the central-western region of Brazil. Acta parasitol. 54(4):355–363.

MAHNERT, V.. 2001. Cave-dwelling pseudoscorpions (Arachnida, Pseudoscorpiones) from Brazil. Rev. Suisse Zool. 108:95–148.

MORACCHIOLI, N.. 2002. Estudo dos Spelaeogriphacea brasileiros, crustáceos Peracarida subterrâneos. Tese de Doutoramento. Instituto de Biociências da Universidade de São Paulo, São Paulo, Brazil.

PINTO-DA-ROCHA, R.. 1995. Sinopse da fauna cavernícola do Brasil (1907-1994). Pap. Avulsos Zool. 39:61–173.

PINTO-DA-ROCHA, R. & SOSSEGOLO, G.C. 2001. Estudo da fauna da gruta de São Miguel I, Serra da Bodoquena (MS), como subsídio para o plano de manejo. In: Org. Grupo de Estudos Espeleológicos do Paraná – Açungui, Conservando cavernas: 15 anos de espeleologia, Curitiba, p.125–135.

PIRES-VANIN, A.M.S. 2012. The discovery of male *Potiicoara brasiliensis* (Crustacea, Spelaeogriphacea) with notes on biology and distribution. Zootaxa 3421:61–68.

PROUDLOVE, G S. 2001. The conservation status of hypogean fishes. Environ. Biol. Fish. 62:201–213, http://dx.doi.org/10.1023/A:1011828726038

SALLUN FILHO, W, KARMANN, I & LOBO, H.A.S. 2010. Cavernas na Serra da Bodoquena. O Carste 22(2):27-33.

SALLUN FILHO, W, KARMANN, I, PETRI, I & UTIDA, G. 2009. A deposição de tufas quaternárias no estado de Mato Grosso do Sul: Proposta de definição da Formação Serra da Bodoquena. Geol. USP, Sér. Cient. 9(3):47–60, http://dx.doi.org/10.5327/Z1519-874X2009000300003

SALLUN FILHO, W & KARMANN, I. 2012. Províncias cársticas e cavernas do Brasil. In: Geologia do Brasil. 629-641.

SALLUN FILHO, W & KARMANN, I. 2007. Geomorphological map of the Serra da Bodoquena karst, west-central Brazil. Journal of Maps 2007:282–295.

SALLUN FILHO, W, Karmann, I. & BOGGIANI, P.C. 2004. Paisagens cársticas da Serra da Bodoquena (MS). In (Mantesso Neto, V, Bartorelli, A, Carneiro, C.D.R. & Brito Neves, B.B orgs). Geologia do continente Sul-americano: evolução da obra de Fernando Flávio Marques de Almeida. 1 ed. São Paulo, 424–433.

SBE - Sociedade Brasileira de Espeleologia.Cadastro Nacional de Cavernas. http://www.sbe.com.br/cnc. (last access in 30/08/2012).

SCREMIN-DIAS, E., POTT, V.J., SOUZA, P.R. & HORA, R.C. 1999. Nos Jardins Submersos da Bodoquena: Guia para Identificação das Plantas Aquáticas de Bonito e Região de Bonito/MS. Editora da Universidade Federal de Mato Grosso do Sul, Campo Grande.

STRAYER, D.L. 2010. Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. Freshwater Biol. 55: 152–174, http://dx.doi.org/10.1111/j.1365-2427.2009.02380.x

THIENGO, S.C., FERNANDEZ, M.A., BOAVENTURA, M.F., STORTTI, M.A. 1998. A Survey of gastropods in the Microrregião Serrana of Rio de Janeiro, Brazil. Mem. I. Oswaldo Cruz. 93: 233–234, http://dx.doi.org/10.1590/S0074-02761998000700041

TRAJANO, E. 1997. Threatened fishes of the World: *Pimelodella kronei* (Ribeiro, 1907). Environ. Biol. Fish. 49:332–332, http://dx.doi.org/10.1023/A:1007353902363

TRAJANO, E. 2001. Ecology of subterranean fishes: an overview. Environ. Biol. Fish. 62:133–160, http://dx.doi.org/10.1023/A:1011841913569

TRAJANO, E. 2012. Ecological classification of subterranean organisms. In: WHITE, W.B. & CULVER D.C. (EDS.) ENCYCLOPEDIA OF, C.a.v.e.s. Ed. Elsevier Amsterdam, p.275–277.

TRAJANO, E., MOREIRA, J.R.A. 1991. Estudo da fauna de cavernas da Província Espeleológica Arenítica Altamira-Itaituba. Rev. Bras. Biol. 51(1)13–29.

TRAJANO, E., GOLOVATCH, S.I., GEOFFROY, J.J., PINTO-DA-ROCHA, R. & FONTANETTI, C.S. 2000. Synopsis of Brazilian cave-dwelling millipedes (Diplopoda). Pap. Avulsos Zool. 41:259–287.

Received 22/07/2014
Accepted 29/07/2014

Biota Neotropica 14(3): e20140055, 2014
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)

short communication

An opportunity for testing multiple paternity in a wild Jaguar (Panthera onca)

Gabriela Medeiros de Pinho[1,2,4], *Rogerio Fonseca*[3] & *Izeni Pires Farias*[1]

[1] *Departamento de Biologia, Universidade Federal do Amazonas, Manaus, AM, Brazil.*
[2] *Department of Ecology and Evolutionary Biology, University of California, Los Angeles, California, United States*
[3] *Departamento de Ciências Florestais, Universidade Federal do Amazonas, Manaus, AM, Brazil.*
[4] *Corresponding author: Gabriela Medeiros de Pinho, e-mail: gabriela.m.pinho@gmail.com*

PINHO, G.M., FONSECA, R., FARIAS, I.P. **An opportunity for testing multiple paternity in a wild Jaguar (Panthera onca).** Biota Neotropica. 14(3): e20140055. dx.doi.org/10.1590/1676-06032014005514

Abstract: We performed a paternity test for three cubs from one wild female jaguar (*Panthera onca*). The opportunity for this study was generated by an accident involving a vehicle collision with a pregnant jaguar in the central Amazon. The cubs are polyzygotic triplets and were found to have been sired by the same male. Here, we also provide an overview and discuss several aspects of jaguar reproduction.

Keywords: *Felidae, Paternity test, Multiple paternity, Road kill, Amazon.*

PINHO, G.M., FONSECA, R., FARIAS, I.P. **Uma oportunidade para testar múltipla paternidade em onça selvagem (Panthera onca).** Biota Neotropica. 14(3): e20140055. dx.doi.org/10.1590/1676-06032014005514

Resumo: Nós realizamos um teste de paternidade em três filhotes de uma onça selvagem (*Panthera onca*). A oportunidade para este estudo foi criada a partir de um acidente envolvendo a colisão entre um veículo e uma onça grávida na Amazônia central. Os filhotes são trivitelinos e foram gerados por um mesmo macho. Neste estudo nós também oferecemos uma revisão e discutimos aspectos da reprodução de onças.

Palavras-chave: *Felidae, Teste de Paternidade, Multipla paternidade, Atropelamento, Amazônia.*

Introduction

Felids frequently use roads and trails to move and to find prey (Sunquist 1981), and both male and female jaguars (*Panthera onca*) use this resource (Rabinowitz & Nottingham 1986). For researchers, this habit facilitates behavioral and diet studies due to the large number of direct observations and fecal samples found on roads (e.g., Rabinowitz & Nottingham 1986, Garla et al. 2001). In spite of these advantages, accidental mortality caused by vehicle collision is a serious threat to carnivores with large home ranges (Costa et al. 2005). This work is the result of a paternity test on a female jaguar pregnant with three cubs, that was fatally injured in one such occurrence.

Primary knowledge about reproduction in felids is derived from studies sampling a small number of individuals (*e.g.* Sadleir 1966a, Soares et al. 2006, Carrillo 2009). These reports, when accumulated, allow us to infer patterns and are particularly important in regard to the jaguar, one of the most difficult large cats to study (Harmsen et al. 2010). Research on captive animals has provided useful evidence such as describing the hormones responsible for oocyte maturation and gestation (Brown 2011), and contributes to the success of assisted reproduction in felines (Morato & Barnabe 2002, Swanson 2006, Paz et al. 2007). Jointly with the *ex situ* approach, studies on wild individuals generate precious information for estimating population growth rates and analyzing population viability (Desbiez et al. 2012, Miller 2013).

The felid mating system is often classified as polygynous or promiscuous, but there is a lack of studies on the relationships between individuals (Macdonald et al. 2010). Regarding jaguars, analyses based on the body mass percentage allocated in testes suggest that females rarely breed with more than one male in one estrus (see Azevedo et al. 2006 and Costa et al. 2006 for more details). However, ecological approaches indicate polygamy, where studies have reported females overlapping home ranges with more than one adult male (Rabinowitz & Nottingham 1986, Soares et al. 2006, Jedrzejewski et al. 2011, Palomares et al. 2012); and two to four males following one female (Hoogesteijn and Mondolfi 1992, Almeida 1976; in Sunquist and Sunquist 2002). Also, males appear not to be territorial in their use of space (Harmsen et al. 2010, Jedrzejewski et al. 2011), but are not usually found to use an area at the same time (Rabinowitz & Nottingham 1986). In conclusion, the possibility of multiple paternity is still not clear; especially due to the lack of studies on parentage in wild felids (Culver et al. 2010).

Considering the scarce information on the species' reproduction, and the rare opportunities in accessing wild individuals, we analyzed the paternity of three jaguar cubs from one litter in the central Amazon, Brazil. The female was hit by a truck on the Tronco road, close to the Geólogo Pedro Moura operation station (04° 53' 04.64"S; 65° 18' 34,61"W)—municipality of Coari, Amazonas, Brazil. The truck was loaded and at a velocity of 60 km/h. The impact killed the female instantaneously, and post-mortem examination revealed a fracture in the left front foot, displacement of the scapula and left ribs (with lung perforation), and damage to the braincase and fracture of the jaw (which caused a cerebral perforation). The female had a total body length of 163 cm, head and thorax circumferences of 48 cm and 74 cm respectively, average canine tooth length 3.2 cm, and average foot length of 6 cm (excluding the claw). Moreover, the female was approximately 3.5 years old, weighting 58.9 kg, and was at the beginning of the second month of gestation—based on the data for the domestic cat from Abreu et al. (2011)—of three cubs.

Material and methods

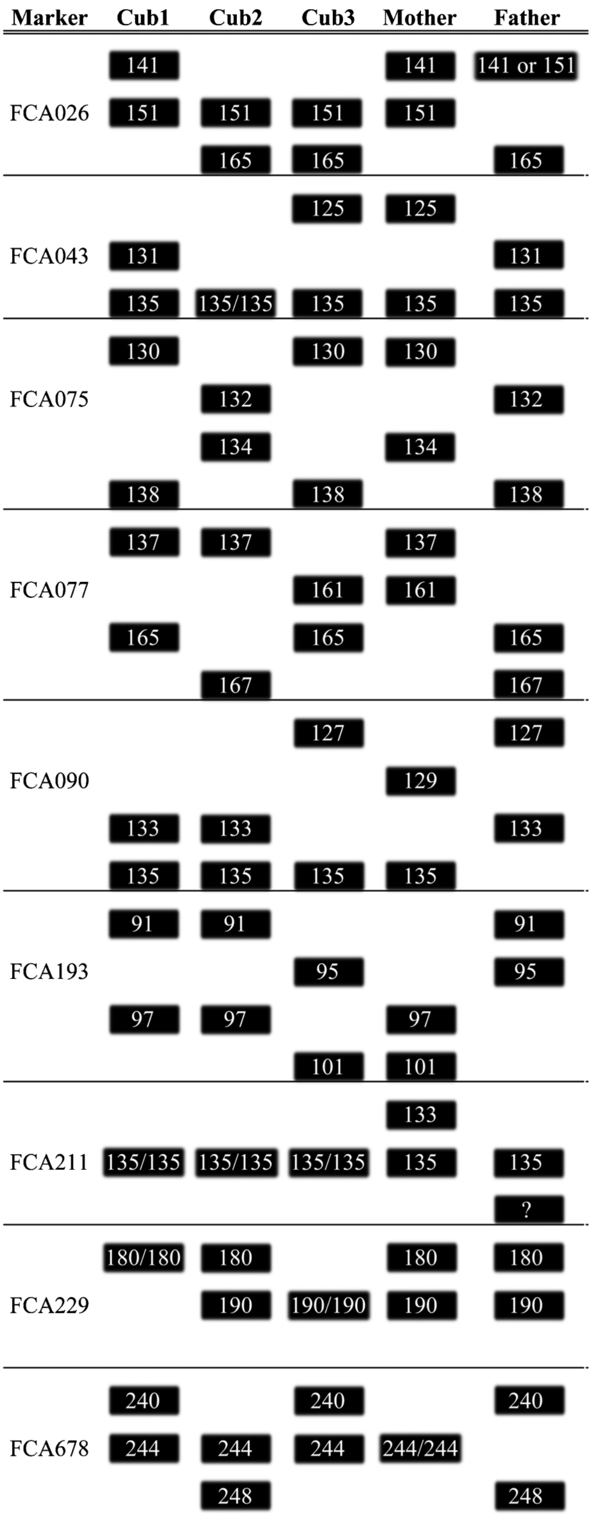
The skeleton and skin of the female are deposited in the Paulo Bürnheim Zoology Collection, at the Federal University of Amazonas (UFAM). Tissue samples are stored in Tissue Collection at the Laboratory of Evolution and Animal Genetics at UFAM (CTGA-M 5669; CTGA-M 5670; CTGA-M 5671; CTGA-M 5672). Data were added to the Global Biodiversity Information Facility (http://www.gbif.org/).

We collected tissue samples from the mother and her cubs, and these were stored in absolute ethanol at -20 °C. DNA was isolated using the QIAmp DNA extraction tissue kit (QIAGEN), following the manufacturer's protocol. We tested 20 microsatellites markers described by Menotti-Raymond et al. (1999) and, from the 12 markers that amplified well, nine were polymorphic and therefore used for the paternity analysis: FCA026, FCA043, FCA075, FCA077, FCA090, FCA193, FCA211, FCA229, FCA678. The primers were labeled with a fluorescent dye using the method described by Schuelke (2000). The polymerase chain reaction conditions were: 93 °C for 2 min; 35 cycles of 93 °C for 5 s, 55 °C for 15 s and 68 °C for 1 min; 25 cycles of 93 °C for 5 s, 50 °C for 15 s and 68 °C for 1 min; followed by 68 °C for 30 min and a final hold at 15 °C. Genotyping was performed on an ABI 3130xl sequencer (Applied Biosystems), using a mix of 1µl of the PCR product, 8µl of formamide and 1µl of the ROX size standard (DeWoody et al. 2004). Allele size was inferred in the program GENEMAPPER v4.0 (Applied Biosystems). The presence of multiple paternity was tested by manual counting of allele number, inferring the father(s) genotype from the comparison between the mother and the cubs genotypes (Figure 1). We also analyzed the data with GERUD 2.0 (Jones 2005), to check for any mistakes in our manual analyses.

Results and discussion

As shown in the figure 1, there is no evidence for multiple paternity in the litter—the number of father's inferred alleles was equal or smaller than two in all loci, probably representing one individual. The analyses in GERUD 2.0 confirmed the results from the mannual counting of alleles, estimating only one probable sire for the three cubs and two possible genotypes for the sire; these are the same genotypes as reported in Figure 1. Therefore we conclude that the three cubs have the same parents. Each cub had originated from a unique oocyte, having distinct genotypes. Moreover, the mother and father shared just five alleles across nine loci, suggesting they are non-relatives.

Our result was similar to previous research testing paternity in wild jaguars: Soares et al. (2006) found two cubs from one litter having the same father in Emas National Park (Central Brazil). Based on these two results, it is possible to hypothesize that males follow the females during the entire estrous period, guaranteeing the birth of their cubs. Studies have reported males close to females for at least two days during the mating event (Harmsen 2006, in Harmsen et al. 2010; Sunquist and Sunquist 2002). However this behavior can change in respect to other factors, such as population sex ratio and number of females on estrus at the same time (reproductive seasonality). Natoli et al.



Marker	Cub1	Cub2	Cub3	Mother	Father
FCA026	141			141	141 or 151
	151	151	151	151	
		165	165		165
FCA043			125	125	
	131				131
	135	135/135	135	135	135
FCA075	130		130	130	
		132			132
		134		134	
	138		138		138
FCA077	137	137		137	
			161	161	
	165		165		165
		167			167
FCA090			127		127
				129	
	133	133			133
	135	135	135	135	
FCA193	91	91			91
			95		95
	97	97		97	
			101	101	
FCA211				133	
	135/135	135/135	135/135	135	135
					?
FCA229	180/180	180		180	180
		190	190/190	190	190
FCA678	240		240		240
	244	244	244	244/244	
		248			248

Figure 1. Genotypes of the mother and cubs for each microsatellite locus and father's inferred genotype.

(2007) also argue that some features of female reproduction—induced ovulation, long ovulation period and delayed ovulation after copulation—can favor multiple copulations and, therefore, generate a mixing of sperm from multiple males.

Generally, female cats mate many times, and several days are necessary to stimulate ovulation (Kleiman & Eisenberg 1973, Brown 2011). But, there is also a possibility of spontaneous ovulation, and this can vary even among individuals of the same species (Brown 2011). Wildt et al. (1979) found no evidence for spontaneous ovulation in a jaguar—the vesicular follicles gradually regressed during the second half of estrous when the induction was absent. The jaguar estrous period is between 6 to 17 days, with a mean of 12-12.9 days (Sadleir, 1966; Wildt et al., 1979), when the females remain receptive. Theoretically, jaguars can breed the year round (Kleiman & Eisenberg 1973, Sunquist & Sunquist 2002), but there is evidence of peak male-fecal androgen concentrations and copulations occurring mainly in the wet season in the Brazilian Southeast and Pantanal (Quigley & Crawshaw 2002, Morato et al. 2004). Therefore, jaguars have a fairly long estrous period, generally induced ovulation, and, if they do have breeding seasons, males can try to attend to more than one female in the same season, which would expose females that already copulated to other males.

Population sex ration, as mentioned above, is another factor that may induce the presence of multiple paternity, as reported for other felid species. In African lions (*Panthera leo*), groups with lower male-to-female ratio were significantly more likely to have extra-group paternity, with an overall extra-group paternity in 41% of the cubs (Lyke et al. 2013). Also, from the 22 litters analyzed by Lyke et al. (2013), four presented multiple paternity and, for each mixed litter, at least one extra-group father was identified. Similarly, Natoli et al. (2007) observed from one multi-male multi-female group (six reproductive females and 14 reproductive males), a percentage of multiple paternity as high as 78%; they concluded that being a dominant male does not guarantee the total control of the receptive females. To the best of our knowledge, cases of multiple paternity were described only for social cats.

In jaguars, several studies found a major proportion of males in populations (varying from 4:1 to 15:1), which could enhance the proportion of multiple paternity in the litters due to the increased competition for females. However, estimated sex ratios in natural jaguar populations may be inaccurate for reasons usually related to methodological sampling biases induced by jaguar behavior, such as: males having bigger home ranges, the higher overlap between male home ranges, and the smaller rate of females captured (Harmsen et al. 2010). Palomares et al. (2012) observed more males than females in six study areas even after identifying individuals from fecal samples, and they attributed this result to the increased use of the sampled routes by males. Aware of these biases induced by sex-specific behavior, Sollmann et al. (2011) used sex-specific capture-recapture models and found a sex ratio of one male to four females, a result that, according to the authors, should be interpreted cautiously. To perform a population viability analysis, studies have used a sex ratio of 1:1 (Desbiez et al. 2012, Miller 2013). Therefore the population sex ratio for jaguars is difficult to estimate and there is no consensus among studies.

In addition to the paternity test, we observed a female jaguar attracting males and becoming pregnant at approximately 60 Kg (≈3.5 years) in the central Amazon. This observation, added to previous and future reports, is important due to the poor knowledge regarding the age of sexual maturity in the jaguar (Quigley & Crawshaw 2002). Here, we reinforce the conclusions of Carrillo et al. (2009), who observed pregnancy while radio-tracking a 60 kg female in Costa Rica, and from Wildt et al. (1979), who registered the first behavioral estrus at 29.5 months of age in a captive female. Also, Seymour (1989) and Sunquist and Sunquist (2002) state, based on few studies, that females become sexually mature at 2 years old and males at 3 years.

Moreover, we registered three cubs in one gestation, an event that is not unheard of, but is relatively rare. The female jaguar give birth to an average of two cubs *per* gestation, with a gestation of approximately four months (Sadleir 1966). In Belize, from 23 observations, 52% represented two cubs, 35% one cub and only 13% were three cubs (Rabinowitz 1986). Carrillo et al. (2009) reported the birth of one cub, Scognamillo et al. (2003) and Soares et al. (2006) two cubs, and Quigley and Crawshaw (2002) observed one female having two cubs in one gestation and one cub in other. The interval between births is 22-24 months, and in 18-24 months the juveniles leave the mother (Quigley & Crawshaw 2002, Carrillo et al. 2009).

In this study, we present an overview about several aspects of jaguar reproduction and report for the first time, to our knowledge, the pregnancy and paternity test of a wild Amazonian jaguar. Also, this work is a result of an opportunity generated by a fatality and equivalent opportunities should be taken by scientists to gather more information about the jaguar's reproduction and behavior. The accumulation of small reports can lead to increasingly accurate conclusions about this poorly studied animal.

Acknowledgments

The authors are grateful to the Wildlife Core (NUFAS) from the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) for sending the jaguar individual to be analyzed at the Universidade Federal do Amazonas. GMP was supported by a National Council for Scientific and Technological Development (CNPq) fellowship and IPF by a Bolsa de Pesquisa scholarship from CNPq during the study. GMP and IPF acknowledges the members of the Laboratory of Evolution and Animal Genetics (LEGAL), especially Julia T. Verba and Rupert A. Collins for reviewing previous versions of the manuscript.

References

ABREU, D.K., RODRIGUES, E.A.F., MONTEIRO, J.M., FRANCIOLLI, L., COSTOLA-SOUZA, C., ROBALLO, K.C.S., AMBRSIO, C.E., & MIGLINO, M.A. 2011. Estudo microscópico e macroscópico, com enfoque radiográfico e de alizarina, no desenvolvimento embrionário e fetal de gatos domésticos (Felis catus) em diferentes idades gestacionais. Pesquisa Veterinária Brasileira. 31:57–66, http://dx.doi.org/10.1590/S0100-736X2011001300010

AZEVEDO, M.H.F., PAULA, T.A.R., DA MATTA,, S.L.P, FONSECA, CC, & NEVES, M.T.D. 2006. Morfometria testicular e o túbulo seminífero da onça-pintada (Panthera onca) adulta. Ceres. 53(307):374–381.

BROWN, J.L. 2011. Female reproductive cycles of wild female felids. Animal reproduction science. 124(3)–(4):155–62 Available at http://www.ncbi.nlm.nih.gov/pubmed/20888156 (verified 19 June 2013), http://dx.doi.org/10.1016/j.anireprosci.2010.08.024

CARRILLO, E., SAENZ, J., & FULLER, T. 2009. Interbirth interval of a free-ranging jaguar. Mammalian Biology - Zeitschrift für

Säugetierkunde. 74(4):319–320 Available at http://linkinghub.elsevier.com/retrieve/pii/S1616504709000330 (verified 27 June 2013), http://dx.doi.org/10.1016/j.mambio.2009.02.005

COSTA, L.P., LEITE, Y.L.R., MENDES, S.L., & DITCHFIELD, A.D. 2005. Mammal Conservation in Brazil. Conservation Biology. 19(3):672–679, http://dx.doi.org/10.1111/j.1523-1739.2005.00666.x

COSTA, D.S., PAULA, T.A.R., & DA MATTA SLP. 2006. Cat, Cougar, and Jaguar Spermatogenesis: a Comparative Analysis. Brazilian Archives of Biology and Technology. 49(5):725–731, http://dx.doi.org/10.1590/S1516-89132006000600006

CULVER, M., DRISCOLL, C., EIZIRIK, E., & SPONG, G. 2010. Genetic applications in wild felids. *In* MACDONALD, D.W., LOVERIDGE, A.J. (eds.), Biology and Conservation of Wild Felids. Oxford University Press. p. 762.

DESBIEZ, A.L.J., TRAYLOR-HOLZER, K., LACY, B., BEISIEGEL, B.M., BREITENMOSER-WRSTEN, C., SANA, D.A., MORAES JR, E.A., CARVALHO JR, E.A.R., LIMA, F., BOULHOSA, RLP., DE PAULA RC., MORATO, RG., CAVALCANTI, SMC., & DE OLIVEIRA TG.. 2012. Population Viability Analysis of jaguar populations in Brazil. Cat News. 7:35–37.

DEWOODY, J.A., SCHUPP, J., KENEFIC, L., BUSCH, J., MURFITT, L., & KEIM, P. 2004. Universal method for producing ROX-labeled size standards suitable for automated genotyping. Biotechniques. 37:348–352.

GARLA, R.C., SETZ, E.Z.F., & GOBBI, N. 2001. Jaguar (Panthera onca) Food Habits in Atlantic Rain Forest of Southeastern Brazil. Biotropica. 33(4):691–696, http://dx.doi.org/10.1111/j.1744-7429.2001.tb00226.x

HARMSEN, B.J., FOSTER, R.J., SILVER, S.C., OSTRO, L.E.T., & DONCASTER, C.P. 2010. The ecology of jaguars in the Cockscomb Basin Wildlife Sanctuary, Belize. *In* MACDONALD, D.W., LOVERIDGE, A.J. (eds.), Biology and Conservation of Wild Felids. Oxford University Press. p. 762.

JEDRZEJEWSKI, W., ABARCA, M., VILORIA, Á., CERDA, H., LEW, D., TAKIFF, H., ABADA, E., VELOZO, P., & SCHMIDT, K. 2011. Jaguar Conservation in Venezuela against the backdrop of current knowledge on its biology and evolution. Interciencia. 36(12):954–966.

JONES, A.G. 2005. Gerud 2.0: a computer program for the reconstruction of parental genotypes from half-sib progeny arrays with known or unknown parents. Molecular Ecology Notes. 5:708–711, http://dx.doi.org/10.1111/j.1471-8286.2005.01029.x

KLEIMAN, D.G., & EISENBERG, J.F. 1973. Comparisons of canid and felid social systems from an evolutionary perspective. Animal behaviour. 21:637–659Available at http://www.ncbi.nlm.nih.gov/pubmed/4798194.

LYKE, M.M., DUBACH, J., & BRIGGS, M.B. 2013. A molecular analysis of African lion (Panthera leo) mating structure and extra-group paternity in Etosha National Park. Molecular ecology. 22:2787–2796Available at http://www.ncbi.nlm.nih.gov/pubmed/23495802 (verified 18 June 2013).

MACDONALD, D.W., MOSSER, A., & GITTLEMAN, J.L. 2010. Felid society. *In* MACDONALD, D.W., LOVERIDGE, A.J. (eds.), Biology and Conservation of Wild Felids. Oxford University Press. p. 762.

MENOTTI-RAYMOND, M., DAVID, V.A., LYONS, L.A., SCHFFER, A.A., TOMLIN, J.F., HUTTON, M.K., & OBRIEN, S.J. 1999. A genetic linkage map of microsatellites in the domestic cat (Felis catus). Genomics. 57:9–23, http://dx.doi.org/10.1006/geno.1999.5743

MILLER, P.S. 2013. Population Viability Analysis for the Jaguar (Panthera onca) in the Northwestern Range.

MORATO, R.G., & BARNABE, R.C. 2002. Potencial de técnicas reproductivas para la conservación del jaguar. *In* MEDELLN, R.A., EQUIHUA, C., CHETKIEWICZ, C.L.B., CRAWSHAW, P.G., RABINOWITZ, A., REDFORD, K.H., ROBINSON, J.G., SANDERSON, E.W., TABER, E.W. (eds.), El jaguar en el nuevo milenio. Fondo de Cultura Economica y la Universidad Nacional Autonoma de Mexico y Wildlife Conservation Society, Mexico. p. 33–43.

MORATO, R.G., VERRESCHI, I.T.N., GUIMARES, M.A.B.V, CASSARO, K., PESSUTI, C., & BARNABE, R.C. 2004. Seasonal variation in the endocrine-testicular function of captive jaguars (Panthera onca). Theriogenology. 61(7)–(8):1273–81 Available at http://www.ncbi.nlm.nih.gov/pubmed/15036961(verified 30 May 2013), http://dx.doi.org/10.1016/j.theriogenology.2003.07.011

NATOLI, E., SCHMID, M., SAY, L., & PONTIER, D. 2007. Male Reproductive Success in a Social Group of Urban Feral Cats (Felis catus L.). Ethology. 113:283–289 Available at http://doi.wiley.com/10.1111/j.1439-0310.2006.01320.x (verified 12 July 2013), http://dx.doi.org/10.1111/j.1439-0310.2006.01320.x

PALOMARES, F., ROQUES, S., CHVEZ, C., SILVEIRA, L., KELLER, C., SOLLMANN, R., DO Prado D.M., TORRES, PC., ADRADOS, B, GODOY, JA, JCOMO, A.T.A, TRRES, N.M., FURTADO, M.M., & LPEZ-BAO, J.V. 2012. High proportion of male faeces in jaguar populations. PloS one. 7(12):e52923 Available at http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3532461&tool=pmcentrez&rendertype=abstract (verified 29 May 2013), http://dx.doi.org/10.1371/journal.pone.0052923

PAZ, R.C.R., ZUGE, R.M., & BARNABE, V.H. 2007. Frozen Jaguar (Panthera onca) sperm capacitation and ability to penetrate zona free hamster oocytes. Brazilian Journal of Veterinary Research and Animal Science. 44(5):337–344.

QUIGLEY, H.B., & CRAWSHAW, P.G. 2002. Reproducción, crecimiento y dispersión del Jaguar en la región del Pantanal de Brasil. *In* Medellín, R.A., EQUIHUA, C, CHETKIEWICZ, C.L.B., CRAWSHAW, P.G., RABINOWITZ, A., REDFORD, K.H., ROBINSON, J.G., SANDERSON, E.W., TABER, E.W. (eds.), El jaguar en el nuevo milenio. Fondo de Cultura Economica y la Universidad Nacional Autonoma de Mexico y Wildlife Conservation Society, Mexico. p. 289–302.

RABINOWITZ, A.R. 1986. Jaguar. Struggle and triumph in the jungles of Belize. Arbor House, New York.

RABINOWITZ, A.R., & NOTTINGHAM, B.G. 1986. Ecology and behaviour of the Jaguar (Panthera onca) in Belize, Central America. Journal of Zoology. 210:149–159, http://dx.doi.org/10.1111/j.1469-7998.1986.tb03627.x

SADLEIR, R.M.F.S. 1966a. Notes on reproduction in the larger Felidae. International Zoo Yearbook. 6:184–187, http://dx.doi.org/10.1111/j.1748-1090.1966.tb01746.x

SADLEIR, R.M.F.S. 1966b. Notes on reproduction in the larger Felidae (CORDANI, U.G., MILANI, E.J., THOMAZ-FILHO, A. and DE, D., CAMPOS, A. Eds.). International Zoo Yearbook. 6:453–480.

SCHUELKE, M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology. 18(2):233–234, http://dx.doi.org/10.1038/72708

SCOGNAMILLO, D., MAXIT, I.E., SUNQUIST, M., & POLISAR, J. 2003. Coexistence of jaguar (Panthera onca) and puma (Puma concolor) in a mosaic landscape in the Venezuelan llanos. Journal of Zoology. 259:269–279 Available at http://doi.wiley.com/10.1017/S0952836902003230 (verified 29 May 2013), http://dx.doi.org/10.1017/S0952836902003230

SEYMOUR, K.L. 1989. Panthera onca. Mammalian Species. 340:1–9, http://dx.doi.org/10.2307/3504096

SOARES, T.N., TELLES, M.P.C., RESENDE, L.V., SILVEIRA, L., JCOMO, A.T.A., MORATO, R.G., DINIZ-FILHO, J.A.F., EIZIRIK, E., BRONDANI, R.P.V & BRONDANI, C. 2006. Paternity testing and behavioral ecology: A case study of jaguars (Panthera onca) in Emas National Park, Central Brazil. Genetics and Molecular Biology. 29(4):735–740, http://dx.doi.org/10.1590/S1415-47572006000400025

SOLLMANN, R., FURTADO, M.M., GARDNER, B., HOFER, H., JCOMO, A.T.A., TRRES, N.M., & SILVEIRA, L. 2011. Improving density estimates for elusive carnivores: Accounting for sex-specific detection and movements using spatial capture-recapture models for jaguars in central Brazil. Biological Conservation. 144:1017–1024 Available at http://linkinghub.elsevier.com/retrieve/pii/S0006320710005197 (verified 29 May 2013), http://dx.doi.org/10.1016/j.biocon.2010.12.011

SUNQUIST, M.E. 1981. The Social Organization of Tigers (Panthera tigris) in Royal Chitawan National Park, Nepal. Smithsonian Contributions to Zoology. 336:1–98Available at http://si-pddr.si.edu/dspace/handle/10088/6316.

SUNQUIST, M., & SUNQUIST, F. 2002. Wild Cats of the World. University of Chicago Press.

SWANSON, W.F. 2006. Application of assisted reproduction for population management in felids: the potential and reality for conservation of small cats. Theriogenology. 66:49–58 Available at http://www.ncbi.nlm.nih.gov/pubmed/16650889 (verified 19 July 2013), http://dx.doi.org/10.1016/j.theriogenology.2006.03.024

WILDT, D.E., PLATZ, C.C., CHAKRABORTY, P.K., & SEAGER, S.W.J. 1979. Oestrous and ovarian activity in a female jaguar (Panthera onca). Journal of Reproduction and Fertility. 56:555–558, http://dx.doi.org/10.1530/jrf.0.0560555

Received 7/08/2014
Revised 24/07/2014
Accepted 25/08/2014

Biota Neotropica 14(3): e20130064, 2014
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
article

Seasonal morphological variation of the vas deferens of scorpion mud turtle *(Kinosternon scorpioides)*

Diego Carvalho Viana[1], *Leandro Almeida Rui*[1], *Amilton Cesar dos Santos*[1], *Maria Angélica Miglino*[1], *Antônio Chaves de Assis Neto*[1], *Lianne Polliane Fernandes Araujo*[2], *Antonia Santos Oliveira*[2] & *Alana Lislea Sousa*[2,3]

[1] *Anatomy of Domestic and Wild Animals, University of Sao Paulo, CEP 05508-270, São Paulo, SP, Brazil.*
[2] *Clinical Veterinary, State University of Maranhão, São Luís, MA, Brazil.*
[3] *Corresponding author: Alana Lislea Sousa, e-mail: alana@elo.com.br*

VIANA, D.C., RUI, L.A., SANTOS, A.C. dos, MIGLINO, M.A., ASSIS NETO, A.C., ARAUJO, L.P. F., OLIVEIRA, A.S., SOUSA, A.L. **Seasonal morphological variation of the vas deferens of scorpion mud turtle *(Kinosternon scorpioides)*.** Biota Neotropica. 14(3): e20130064. http://dx.doi.org/10.1590/1676-06032014006413

Abstract: This study aimed to characterize the morphology of the vas deferens of *Kinosteron scorpioides* by macroscopic and microscopic analysis. Were used 20 adult male jurarás collected at regular intervals during the year and divided into four experimental groups in the rainy and dry seasons, being processed for light microscopy, scanning electron microscopy and transmission electron microscopy. Morphometry for tubular and luminal diameters and epithelial height were also performed. On rainy season, vas deferens presented pseudostratified epithelium with cylindrical cells, spermatozoids and milky fluid in the lumen, with cytoplasmic organelles and lipid vesicles. On dry season, epithelium was pseudostratified with cuboid cells, with cellular debris and no spermatozoids. There was significant variation ($p<0,05$) for morphometry of vas deferens, with lower values of tubular and luminal diameters on rainy season, and higher epithelial height on dry season.

Keywords: *Kinosternon, Reproduction, Vas deferens, Morphometry, Ultrastructural.*

VIANA, D.C., RUI, L.A., SANTOS, A.C. dos, MIGLINO, M.A., ASSIS NETO, A.C., ARAUJO, L.P.F., OLIVEIRA, A.S., SOUSA, A.L. **Variação morfológica sazonal dos ductos deferentes de tartaruga jurará *(Kinosternon scorpioides)*.** Biota Neotropica. 14(3): e20130064. http://dx.doi.org/10.1590/1676-06032014006413

Resumo: Este estudo teve como objetivo caracterizar a morfologia dos ductos deferentes de *Kinosteron scorpioides* por meio de análise macroscópica e microscópica. Foram utilizados 20 machos adultos, coletados em intervalos regulares durante o ano, os quais foram divididos em quatro grupos experimentais nas estações chuvosa e seca. Os ductos deferentes foram processados para análise por microscopia de luz, microscopia eletrônica de varredura e microscopia eletrônica de transmissão. Morfometria para diâmetros tubular e luminal e altura epitelial também foram realizadas. Na época das chuvas, o ducto deferente apresentou epitélio pseudoestratificado com células cilíndricas, espermatozoides e líquido leitoso no lúmen, além de com organelas citoplasmáticas e vesículas lipídicas. Na estação seca, o epitélio do ducto deferente foi do tipo pseudoestratificado com células cuboides e debris celulares, sendo que nenhum espermatozoide foi encontrado nesta estação. Houve variação significativa ($p <0,05$) para a morfometria dos ductos deferentes, com menores valores de diâmetros tubular e luminal na estação chuvosa, e maior altura do epitélio na estação seca.

Palavras-chave: *Kinosternon, Reprodução, Ducto deferente, Morfometria, Ultraestrutural.*

Introduction

Brazil has 35 species of chelonians distributed in its various terrestrial and aquatic ecosystems, of which 28 species are freshwater, two are terrestrial (land turtles), and 5 are marine turtles (SBH, 2005). The family Kinosternidae is composed of semi-aquatic species of small to medium size, being distributed from Canada to South America (Erns & Barbour 1989). It is composed of 22 species subdivided in four genus: Kinosternon, Sternotherus, Staurotypus and Claudius. In the Brazilian Amazon it is possible to found only one species of this family, *Kinosternon scorpioides*, also known as scorpion mud turtle (Molina & Rocha 1996).

The scorpion mud turtle is preferably an aquatic species, and inhabits both stagnant and flowing water, being also able to develop semi-aquatic behavior (Pritchard & Trebbau 1984). It displays a shell with three evident keels, especially the median, which runs through the shell in the longitudinal direction (Vanzolini et al. 1980). It also has a strong jaw and a structure similar to a nail at the end of the body, like a scorpion's stinger, which termed scientifically this species.

Kinosternon scorpioides is well distributed in the coast of South America, including Colombia, the Guianas and Trinidad. In Brazil it is found in the states of Pará, Maranhão, north of Goias, Ceará, Rio Grande do Norte and Pernambuco (Pritchard & Trebbau 1984). In Maranhao its presence is confirmed on the edge of rivers (Pereira 2007), and it is considered an important species, both economically and as a source of protein.

The male reproductive system consists on a pair of oval testes of variable size, between light yellow to golden yellow color, and being fixed by mesorchium and mesocolon; epididymis located along the dorsal part of medial surface of each testis, being very delicate, presented as very convoluted structures of whitish color; and the vas deferens, which are continuous to the epididymis and culminate in the region of the cloaca. The vas deferens in jurará are a pair of simple structures, with a convoluted path that extends from the epididymis (Viana 2013) to the cloaca, with the function of transporting and storing spermatozoids. The penis, in turn, is grooved, and composed by the root, body and gland, located in the ventral floor of the cloaca, in which it attaches via a retractor muscle, and is protected by the foreskin (Carvalho et al. 2010).

To our knowledge, this is the first study aimed to characterize the morphology of the vas deferens of *Kinosteron scorpioides* by macroscopic and microscopic analysis, contributing to describe the reproductive characteristics of this species and elucidate the spermatogenic cycle, strategies of sperm storage, and morphology of the reproductive tract.

Materials and methods

Were used 20 adult male Kinosternon scorpioides, from the capture ex-situ in the city of São Bento, state of Maranhão, Brazil, as authorized by IBAMA for the purpose of scientific activities with number 26136-1, and approval of the Ethics and Animal Research Committee of the Course of Veterinary Medicine (EAEC/UEMA), protocol number 011/2010.

The research was developed in the Laboratory of Veterinary Anatomy and Anatomopathology of the Course of Veterinary Medicine in the Center for Agricultural Sciences of State University of Maranhão - UEMA, Sao Luis - MA, and Laboratories of Light and Electron Microscopy, of the School of Veterinary Medicine and Animal Science of University of Sao Paulo - USP.

The animals were collected at regular intervals during the year and divided into four experimental groups in the rainy and dry seasons, the rainy season being understood by the collections of March/2011 and June/2011, and dry season being December/2010 September/2011.

The twenty animals were anesthetized with xylazine 2% (40mg/kg/IM) and ketamine hydrochloride 1% (60mg/kg/IM) and euthanized by administration of thiopental sodium 2.5% (60mg/kg/EV) by catheterization of the cervical venous sinus.

Subsequently, was held the opening of the coelomic cavity with steel handsaw, for the disarticulation of the bone bridge that connects the carapace and the plastron, and visualization and removal of the reproductive tract and isolation of the vas deferens, the processing being specific for each microscopy.

For light microscopy, the vas deferens were fixed in buffered formaldehyde 4% for about 24 hours for paraffin embedding. They were then dehydrated in increasing alcohol concentrations (70 ° - 100 °) and diaphanized in xylol, with an interval of switching between the solutions of 1 hour. After dehydration, the fragments were embedded in paraffin, sectioned at 4μm thickness, and stained with hematoxylin-eosin (HE), Masson's trichrome and periodic acid-Schiff - PAS, and examined under an optical microscope.

For scanning electron microscopy (SEM), the fragments were fixed in 2.5% glutaraldehyde, frozen for 72 hours and thereafter cryofracturated in liquid nitrogen, washed in 0.1 M phosphate buffer, post-fixed in osmium tetroxide 1% and dehydrated in series of alcohols (50 ° – 100 °). The samples were dried in a critical point apparatus Balzers CPD 020 using liquid CO_2 and mounted on metal aluminum basis (stub), using carbon paste. Subsequently, were subjected to a metallic coating ("sputting") with gold in sputter device EMITECH K550, analyzed and photographed under a scanning electron microscope LEO 435VP.

For transmission electron microscopy (TEM), the fragments were fixed in 2.5% glutaraldehyde, washed in 0.1 M phosphate buffer and post-fixed in osmium tetroxide 1%. Subsequently, were dehydrated in series of increasing alcohols (50 ° - 100 °), propylene oxide and resin. The resin mixture was replaced by pure resin and placed in molds. The ultrathin sections were collected on copper screens and contrasted with uranyl acetate solution at 2% and 0.5% lead citrate. The samples were analyzed in transmission electron microscopy apparatus MORGANI 268d.

Images for morphometric studies were obtained using a binocular microscope Olympus BH-41 equipped with a digital camera for the photographic record. Histomorphometric analyses were performed with the aid of the program GIMP 2 to obtain the average height of the epithelium and the luminal and tubular diameters of the vas deferens, obtained with the use of micrometric ocular adapted to the microscope. Were made ten slides with three serial sections; the tubular sections have been made around the tubules in the base of epithelium, by the level of the the basal membrane, to obtain the total tubular diameter, and adjacent to the apical edge to obtain the luminal diameter, using a 10x objective. Similarly, a 40x objective was used for the measurement of the height of epithelium from its base to the apical edge.

Results

Vas deferens are continuous to the epididymis, presenting as a small structure, resembling a sinuous tube in its final portion, following lateral to ureters, and inserting on the dorsolateral wall of the cloaca, expanding in a shape of a small bulb.

In *Kinosternon scorpioides*, vas deferens observed by light microscopy presents as covered by pseudostratified cylindrical epithelium with secretory cells (Figure 1). Vas deferens also has a layer of dense connective tissue with the presence of blood vessels surrounded by muscle tissue. Inside the lumen are found spermatozoids and a milky fluid on the tubular center.

Structural morphology of vas deferens of scorpion mud turtle on the rainy season was a pseudostratified epithelium with cylindrical cells, spermatozoids in the lumen and milky fluid characteristic of semen, while on dry season were observed modifications, presenting pseudostratified epithelium

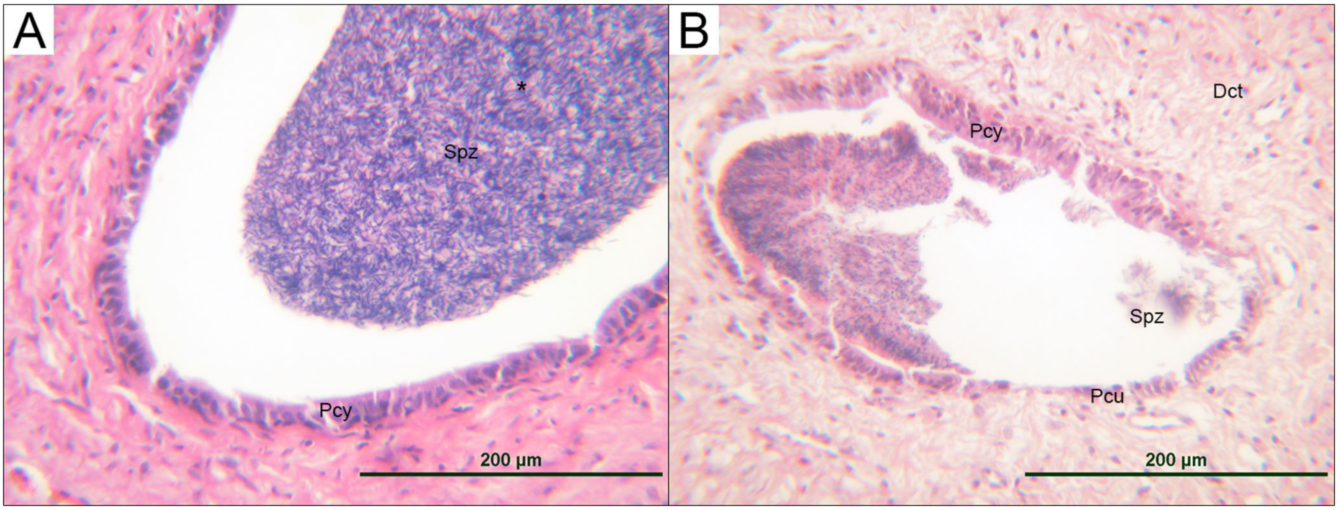


Figure 1. Histology of vas deferens of scorpion mud turtle (*Kinosternon scorpioides*) in rainy and dry seasons. A: vas deferens on rainy season with presence of spermatozoids in the lumen (Spz), pseudostratfied cylindrical epithelium (Pcy) and milky fluid (*). B: Vas deferens on dry season wish spermatozoids on luminal edges (Spz), pseudostratified cuboid epithelium (Pcu) and pseudostratified cylindrical epithelium (Pcy), with presence of dense connective tissue (Dct). Hematoxylin-eosin staining.

with cuboid cells, absence of spermatozoa in the lumen and cellular debris.

Scanning electron microscopy of vas deferens by cryofracture technique showed disposition of spermatozoid on tubular epithelium and arrangement of dense connective tissue along to a muscular layer close to the blood vessels. It is believed that these elevations favor storage of sperm in the region (Figure 2).

Transmission electron microscopy on rainy season showed spermatozoids, cytoplasmic organelles (mitochondria), indicating high metabolic activity, and lipid vesicles responsible by nutrition of spermatozoid during storage. On dry season, ultrastructural findings were disorganized and sparse cytoplasm, with endoplasmic reticulum, indicating protein production, and few spermatozoids (Figure 3).

There was significant variation ($p<0,05$) between seasons for morphometry of vas deferens. The tubular and luminal diameters had lower values on rainy season. However, epithelial height in dry season had higher averages (Table 1).

Discussion

Although in scorpion mud turtle the vas deferens expands in a shape of a small bulb, this feature was not observed for the same species by Carvalho et al. (2010), which does not report the expansion of vas deferens before its insertion on the cloaca. Chaves (2011), however, described the same characteristic, characterizing this expansion in bulb shape on vas deferens of scorpion mud turtle.

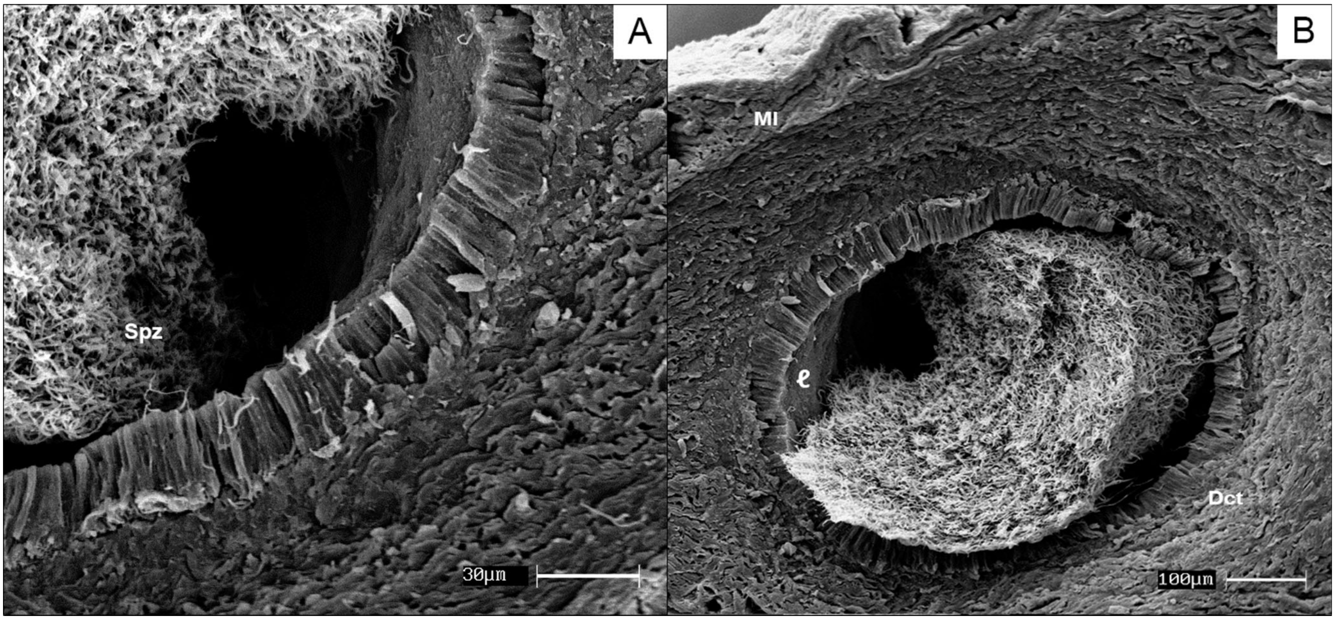


Figure 2. Fotomicrography of scanning electron microscopy (SEM) of vas deferens of scorpion mud turtle (*Kinosternon scorpioides*). A: vas deferens, with pseudostratified cylindrical epithelium and spermatozoids in lumen (Spz). B: vas deferens, with evidence of tubular lumen (ℓ), dense connective tissue (Dct) and muscular layer(Ml).

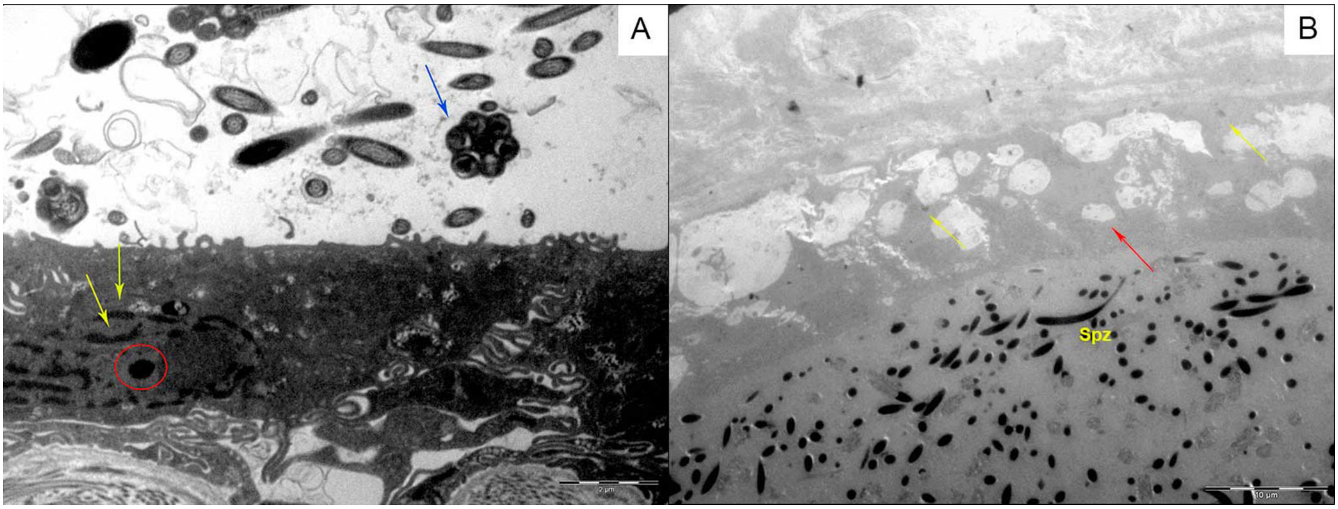


Figure 3. Fotomicrography of transmission electron microscopy (TEM) of vas deferens of scorpion mud turtle (*Kinosternon scorpioides*). A: vas deferens on dry season with disorganized cytoplasm, presenting endoplasmic reticulum (yellow arrow), vesicle (red circle) and spermatozoids (blue arrow). B: vas deferens on rainy season with spermatozoids (Spz), mitochondria (yellow arrow) and lipidic vesicles (red arrow).

By light microscopy, in snakes (*Bothrops jararaca* and *Crotalus durissus*), epithelial cells showed microvilli which were not observed in scorpion mud turtle, even though similar secretion was observed by the epithelium (Almeida-Sousa 2005). Similarity was described on close species, such as the crocodile (Guerrero et al. 2004), and birds, particularly in the rooster (Tingari 2001). In the snake *Seminatrix pygaea* of South Carolina, was observed arrangement of spermatozoids tangent to the epithelium, most being slightly separated from epithelium (Ssever 2004).

Structural morphology was also closed to that observed in snakes, presenting lots of spermatozoa in the lumen of vas deferens in rainy season, indicating copulation period, when it is used as a storage organ; and decrease of spermatozoa in the lumen on dry season, indicating a postmating stage (Rojas et al. 2013). However, in rat snakes, although vas deferens is also the main sperm storage organ, spermatozoa is present in large numbers throughout all the year, except in July (Gang et al. 2011).

Scanning electron morphology in snakes reported vas deferens as being an organ of spermatozoids storage (Almeida-Sousa 2005),. In this study of scorpion mud turtle, it is believed that vas deferens is also adapted for storage, due to its structural characteristics such as absence of cilia or stereocilia in the cells. On crocodiles (*Caiman crocodilus*), non-ciliated cells were also found, indicating storage function of vas deferens in another species of reptiles (Guerrero et al. 2004).

Transmission electron microscopy also presented lipid vesicles in snakes on the rainy season, which are responsible for nutrition of spermatozoids (Rojas 2013). On dry season, the visualization of spermatozoa in the lumen demonstrates that the organ is in reproductive activity. On domestic quails, was reported that vas deferens showed little annual variability, with a significant increase in tubular caliber, intraluminal storage of spermatozoids and occurence of mitochondria, lysomes, endoplasic reticulum and variable vesicles in the cytoplasm of principal cells. These ultrastructural features of principal cells seems to be indicative of the occurence of active processes of endocytosis, and degenerative characteristics were observed at the supranuclear cytoplasm of epididimary P cells on autumn (Orsi et al. 2007). On crocodiles, endoplasmic reticulum was also abundant, indicating protein production, despite of absence of visible secretory material (Guerrero et al. 2004).

By morphometry, the decrease of tubular and luminal diameters, along with increase of epithelial heighs on rainy season, are correlated to seasonal variations in synchrony with the spermatogenic and epididymal cycles. In the same sense, was described in snakes (*Cerastes vipera* and *Psammophis sibilans*), a larger diameter and short epithelial linings during reproductive season as a result of elongation of stored spermatozoids (Sivan et al. 2012, AMER et al. 1978).

Table 1. Mean and standard deviation of morphometry (mm) of tubular and luminal diameters and height of the vas deferens of turtle (Kinosternon scorpioides), captured in São Bento - MA, according with the season. Sao Luis - MA – 2012.

Vas deferens	Period of the year			
	Rainy season		Dry season	
	mar/11	jun/11	sep/11	dec/10
Tubular diameter	395,72 ± 60,77 a	298,96 ± 50,50 ab	401,88 ± 69,14	302,70 ± 52,87 b
Luminal diameter	292,99 ± 59,46 a	243,68 ± 75,68 ab	337,29 ± 66,14 ac	220,13 ± 52,87 bd
Epithelial height	37,24 ± 13,65 a	18,67 ± 5,52 b	42,49 ± 16,83 ac	21,70 ± 4,61 b

Means in different letters in the same line indicate statistical difference (p<0,05) for Student-Newman-Keuls (SNK), normality test t Cramer-von Mises W-Sq 0.03979, Pr > W-Sq > 0,2500. Dec - December. Mar - March. Jun - June. Sep - September

http://dx.doi.org/10.1590/1676-06032014006413

We conclude that vas deferens is the main sperm storage organ on the scorpion mud turtle, presenting a large number of spermatozoids in reproductive season, and morphological findings that represent an adaptation to its function. However, on dry season, the storage was smaller, indicating that organ is in reproductive activity, but production of spermatozoids is reduced in comparison to the rainy season. Further studies on hormonal levels and quality of spermatozoids are suggested, in order to refine the knowledge on the reproductive biology of *Kinosternon scorpioides*.

Acknowledgments

The authors would like to thank the State University of Maranhão (UEMA), the National Program of Academic Cooperation (Procad I-CAPES/UEMA/USP Amazon) and the Foundation for Research Support of the State of Maranhão (FAPEMA) for funding current research.

References

ALMEIDA-SANTOS, S.M. 2005. Modelos reprodutivos em serpentes: estocagem de esperma e placentação em *Crotalus durissus* e *Bothrops jararaca* (Serpentes: Viperidae). Tese (Doutorado)- Programa de pós-graduação em Anatomia dos animais domésticos e silvestres. Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo. 204p.

AMER, F.I. & ELSHABKA, H.A. 1978. Studies on the reproductive organs of the colubrid snakes, Psammophis sibilans and spalerosophis diadema, I. The Male Organs. Bulletin of the Faculty of Science-King Abdulaziz University, 2: 1-16.

CARVALHO, R.C., SOUSA, A., SILVA, A.L.A. & PEREIRA, J.G. 2010. Anatomia dos órgãos genitais do muçuã *Kinosternon scorpioides* macho (Chelonia, Kinosternidae). Pes. Vet. Bras. 30: 289-294, doi: http://dx.doi.org/10.1590/S0100-736X2010000400001

CHAVES, L.P.F.A. 2011. Orgãos genitais masculino e nível sérico de testosterona de jurará (*kinosternon scorpioides,* linnaeus, 1766*)* criado em cativeiro na região da baixada maranhense no estado do maranhão. Dissertação (Mestrado em Ciências Animal) - Universidade Estadual do Maranhão, São Luís, 92p.

ERNST, C.H. & BARBOUR, R.W. 1989. Turtles of the United States. Lexington: Univ. of Kentucky Press, 347p.

GANGA, L., QIAO-QIAO, L., HU-HU, Y. & QIONG, X. 2011. Histological and immunocytochemical study of deferens ducts in the Chinese rat snake (*Zaocys dhumnades*). Zool. Res. 32: 663-669.

GUERRERO, M.S., CALDERON, L.M., PEREZ, R.G. and PINILLA, M.P.R. 2004. Morphology of the male reproductive duct system of *Caiman crocodilus* (Crocodylia, Alligatoridae). Ann. Anat. 186: 235-245, doi: http://dx.doi.org/10.1016/S0940-9602(04)80009-8

MOLINA, F.B. & ROCHA, M.B. 1996. Algumas observações sobre a biologia e manejo do muçuã. Aquacultura. 2: 25-26.

ORSI, A.M., DOMENICONI, F.R., SIMÕES, K., STEFANINI, A.M. & BARALDI-ARTONI, S.M. 2007. Variabilidade sazonal no ducto epididimário de codorna doméstica: observações morfológicas. Pesq. Vet. Bras. 27: 495-500, doi: http://dx.doi.org/10.1590/S0100-736X2007001200005

PEREIRA, L.A., SOUSA, A.L., CUTRIM, M.V.J., MOREIRA, E.G. 2007. Características ecológicas do habitat de Kinosternon scorpioides scorpioides Linnaeus, 1766 (Reptila, Chelonia, Kinosternidae) no Município De São Bento – Baixada Maranhense (MARANHÃO, BRASIL). Boletim do laboratório de hidrobiologia, 20: 9-14.

PRITCHARD, P.C. & TREBBAU, P. 1984. The Turtles of Venezuela. Society the study of amphibians and reptiles. Ithaca, 403p.

ROJAS, C.A., BARROS, V.A., ALMEIDA-SANTOS, S.M. 2013. The Reproductive Cycle of the Male Sleep Snake *Sibynomorphus mikanii* (Schlegel, 1837) From Southeastern Brazil. J. Morphol. 274: 215-288, doi: http://dx.doi.org/10.1002/jmor.20099

SBH. Brazilian reptiles – List of species. 2005. Available in: http://www.sbherpetologia.org.br. Sociedade Brasileira de Herpetologia. Acessed in 05/01/2013.

SEVER, D.M. 2004. Ultrastructure of the Reproductive System of the Black Swamp Snake (*Seminatrix pygaea*). IV. Occurrence of an Ampulla Ductus Deferentis. J. Morphol. 262: 714-730.

SIVAN, J., KAM, M., HADAD, S., DEGEN, A.A., ROZENBOIM, I. & ROSENSTRAUCH, A. 2012. Reproductive cycle of free-living male Saharan sand vipers, *Cerastes vipera* (Viperidae) in the Negev desert, Israel. Gen. Comp. Endocrinol. 179: 241-247, doi: http://dx.doi.org/10.1016/j.ygcen.2012.08.021

TINGARI, M.D. 1971. On the structure of the epididymal region and ductus deferens of the domestic fowl (*Gallus domesticus*). J. Anat. 109: 423-35.

VANZOLINI, P.E.A. 1996. Contribuição zoológica dos primeiros naturalistas *viajantes* do Brasil. Revista USP, 30: 190-238.

VIANA, D.C., RUI, L.A., MIGLINO, M.A., ARAUJO, L.P.F., OLIVEIRA, A.S., SOUSA, A.L. 2013. Morphological study of epididymides in the scorpion mud turtle in natural habitat (Kinosternon scorpioides – Linnaeus, 1976). Biotemas, 26: 153-162.

Received 05/11/2013
Accepted 10/07/2014

Biota Neotropica 14(3): e20140094, 2014
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
article

Forest structure and species composition along a successional gradient of Lowland Atlantic Forest in Southern Brazil

Márcia C.M. Marques[1,4], Victor P. Zwiener[1], Fernando M. Ramos[1], Marília Borgo[2] & Renato Marques[3]

[1]Departamento de Botânica, Laboratório de Ecologia Vegetal, SCB, Universidade Federal do Paraná,
Caixa Postal 19031, 81531-980, Curitiba, PR, Brazil.
[2]The Nature Conservancy, Rua Padre Anchieta, 392, 80410-030, Curitiba, PR, Brazil.
[3]Departamento de Solos e Engenharia Agrícola, SCA, Universidade Federal do Paraná, Rua dos
Funcionários, 1540, 80035-050, Curitiba, PR, Brazil.
[4]Corresponding author: Márcia C.M. Marques, e-mail: mmarques@ufpr.br

MARQUES, C.M.M., ZWIENER, V.P., RAMOS, F.M., BORGO, M., MARQUES, R. **Forest structure and species composition along a successional gradient of Lowland Atlantic Forest in Southern Brazil.** Biota Neotropica. 14(3): e20140094. http://dx.doi.org/10.1590/1676-06032014009414

Abstract: The Lowland Forest is one of the most disturbed and fragile ecosystems in the Atlantic Forest biome, yet little is known regarding its successional trajectory and resilience. We evaluated changes in species assemblages and forest structure of the canopy and understory along a successional gradient (young 21-yrs old forest, immature 34-yrs old forest and late successional 59-yrs old forest) aiming to assess changes in species composition and successional trajectory of different strata of secondary forests. A 0.1 ha plot (ten 10x10 m sub-plots) from each forest stand was surveyed for trees and shrubs with a diameter at breast height (DBH) $\geqslant$ 4.8 cm (canopy) and for individuals with heights $\geqslant$ 1 m and DBH $<$ 4.8 cm (understory). A total of 3,619 individuals from 82 plant species were sampled. The successional gradient was marked by a unidirectional increase in species richness and a bidirectional pattern of density changes (increasing from young to immature forest and decreasing from immature to late successional forest). Community assemblages were distinct in the three forests and two strata; indicator species were only weakly shared among stands. Thus, each successional forest and stratum was observed to be a unique plant community. Our results suggest slight predictability of community assemblages in secondary forests, but a relatively fast recovery of forest structure.

Keywords: *canopy, chronosequence, Restinga, understory.*

MARQUES, C.M.M., ZWIENER, V.P., RAMOS, F.M., BORGO, M., MARQUES, R. **Estrutura e composição ao longo de um gradiente sucessional de Floresta de Terras Baixas no sul do Brasil.** Biota Neotropica. 14(3): e20140094. http://dx.doi.org/10.1590/1676-06032014009414

Resumo: As Florestas de Terras Baixas constituem um dos ecossistemas mais perturbados e frágeis no bioma Mata Atlântica, mas ainda pouco se sabe sobre sua trajetória sucessional e resiliência. Foram avaliadas alterações na composição de espécies e a estrutura florestal do dossel e sub-bosque ao longo de um gradiente sucessional (floresta jovem-21 anos, floresta imatura-34 anos, floresta madura-59 anos) com o objetivo de verificar as mudanças na composição de espécies e a trajetória sucessional de diferentes estratos destas florestas secundárias. Uma parcela de 0,1 ha (dez sub-parcelas de 10x10 m) foi estabelecida em cada floresta, amostrando-se árvores e arbustos com um diâmetro à altura do peito (DAP) $\geqslant$ 4,8 cm (dossel) e para indivíduos com altura $>$ 1 m e DAP $<$ 4,8 cm (sub-bosque). Um total de 3.619 indivíduos de 82 espécies de plantas foram amostrados. O gradiente sucessional foi marcado por um aumento unidirecional na riqueza de espécies com o tempo, e um padrão bidirecional de mudanças de densidade (aumentando da floresta jovem para a imatura e diminuindo da imatura para a madura). As assembléias de plantas eram distintas nas três florestas e nos dois estratos; espécies indicadoras foram pouco compartilhadas entre as florestas. Portanto, cada estádio da cronosequência e cada estrato representam uma comunidade única de plantas. Nossos resultados sugerem pouca previsibilidade das assembleias de plantas destas florestas secundárias, mas uma recuperação relativamente rápida da estrutura da floresta.

Palavras-chave: *cronossequência, dossel, Mata Atlântica, Restinga, sub-bosque.*

Introduction

Secondary forests are increasing worldwide as a result of shifting cultivation patterns and rural-urban migration (Aide & Grau 2004; Wright & Muller-Landau 2006). Future global land cover scenarios include forests that have resulted from multiple successional pathways (Corlett 1995). For decades, studies have focused on factors that have influenced the structure and diversity of secondary tropical forests (Denslow 1980; Brown & Lugo 1990; Finegan 1996; Guariguata & Ostertag 2001; Chazdon 2008). However, the successional trajectories of these forests still remain uncertain. Determining changes in tropical forests during secondary succession is crucial for understanding forest resilience, resistance and, ultimately, the role of secondary forests in the conservation of biodiversity and the provision of ecosystem services (Brown & Lugo 1990; Chazdon 2008; Chazdon et al. 2009).

Plant communities are affected by multiple factors during successional processes. Features of the landscape, such as the distance and quality of nearby habitats, affect rates of seed arrival and colonization (Pickett et al. 1987; Zwiener et al. 2014). The disturbance history and previous land use strongly influence soil properties (Ding et al. 2012; Larkin et al. 2012; Long et al. 2012), presence of remnant seeds, sprouts and saplings, and current species composition of the plant community (Uhl 1987; Holl et al. 2000). All of these factors determine different paths and rates of forest recovery, which can vary from decades to centuries (Liebsch et al. 2008).

The recovery of forest structure after disturbance is often assessed in terms of how density, basal area and diversity of the canopy stratum compare with those of old-growth/primary forests (Guariguata & Ostertag 2001; Letcher & Chazdon 2009). Four structural phases are generally recognized for the recovery process in tropical forests (Chazdon 2008): stand initiation, stem exclusion, understory re-initiation and the old-growth stage. Such phases are reflected in forest physiognomy and structure, but directional changes for each community vary according to the amount of time that has passed since the last disturbance. For example, species richness is relatively low in young and undisturbed communities, but the immature phase can have a higher number of species than might be found in the other phases because of the intermediate disturbance level in these sites (Connell 1978).

Whereas sufficient knowledge exists concerning the mechanisms of structural change during secondary succession (e.g., Chazdon et al. 2007), issues about successional trends in species composition still remain contentious. Changes in temperature, humidity and light availability create new habitats that are occupied by species that differ in their resource requirements (Mesquita et al. 2001). In these situations, not only the species composition but also the community guilds are distinctive from those of primary forests (Norden et al. 2009). Decreasing light availability during succession favors shade tolerant species and decreases numbers of understory species in secondary forests (Guariguata & Ostertag 2001). Thus, species composition during the succession is still one of the most difficult approaches to succession theory, but it is highly important for forest management.

The Brazilian Atlantic Forest has been systematically altered by human occupation over successive economic cycles and a large portion of the impacted areas has now been abandoned and is undergoing secondary succession. In this biome, the Lowland Atlantic Forest type is characterized by a lower floristic diversity compared to other types of Atlantic Forests occurring mostly in sandy and nutrient poor soils (Marques et al. 2011). These forests originally covered the southern and southeastern coast of Brazil, in terrains up to 100m of altitude, but its distribution was drastically reduced due to the expansion of cities, real estate market and agricultural areas (Galindo-Leal & Câmara 2003). Thus, an effective conservation and management policy for these highly threatened forests is urgently necessary, but little is known regarding their successional trajectory and resilience in order to give support to such practices.

We evaluate the floristic and structural changes across a successional gradient of secondary Lowland Atlantic forests once disturbed by slash-and-burn agriculture in southern Brazil. We compared regenerating forests at 21 (young forest), 34 (immature forest) and 59 (late successional forest) years since abandonment of agricultural practices, seeking to answer the following questions: 1) Do forest structure (basal area and stem density) and species richness, and diversity follow the same successional trajectory in the canopy and the understory? 2) Are the species assemblages in secondary forests associated to the stratum (canopy and understory) and/or successional stage? 3) Are there species that characterize the successional stages and forest strata?

Material and methods

Study site

The study was conducted in a region of the Atlantic Forest within a protected area (*Palmito* State Forest, total area of approximately 300 ha) in the municipality of Paranaguá (25°35'S, 48°32'W, at sea level) in the state of Paraná, southern Brazil. The climate (Af in Köppen's classification) is tropical and superhumid, without a pronounced dry season or frosts. The 46-year running average temperature is 21 °C; the average annual precipitation is 2,218 mm, and it is always greater than 60 mm during the driest month (Britez & Marques 2005). Soils (Spodozols) are sandy (90-98% sand) and acidic (pH 3.2-3.9; C. Wisniewski pers. comm.).

The southern coast of Brazil is in the Atlantic Forest biome, which includes a complex of vegetation types (mainly forest and scrub) on both upland and lowland areas. In lowland areas, where the soil is composed of marine (Holocene) deposits, the vegetation type is locally called "*restinga*" (Marques et al. 2011). Specifically, in the *Palmito* reserve, the vegetation type is a *restinga* forest that is very similar in floristics and structure to those typical of Lowland Atlantic forests that are not found over Holocene deposits (Marques et al. 2011). However, the vegetation in the reserve has been modified by the last 100 years of occupation. Historically, agricultural practices have been rudimentary, using subsistence cultivation based on slashing and burning. In the *Palmito* reserve, forest cutting, planting and burning for agriculture occurred successively over several years. As a result of these practices, the reserve today is a mosaic of different successional stages (including a very small area of old-growth forest).

To assess forest dynamics during succession we used a chronosequence approach, where 0.1 ha plots were established in 2001 in young forest (21 years old), immature forest (34 years) and late successional forest (59 years). Forest ages and previous land uses were based on interviews with local agricultural workers and with the *Palmito* reserve managers. Despite some uncertainty concerning the exact ages and historical conditions, this is considered an appropriate approach for estimating forest dynamics during succession (Pickett 1989; Foster & Tilman 2000). The three forest stands were relatively close to each other (~ 1000 m), in the same soil type (Spodozols) and surrounded by old growth and secondary forest fragments, mangroves, and agricultural areas. The three plots had previously been slashed and burned several times for pineapple and manioc cultivation over the course of approximately 10 years, and had then been abandoned. Although replication of sample units would be necessary to allow robust generalizations (Hurlbert 1984), the use of multiple plots in the studied region is virtually impossible, given the highly fragmented and reduced distribution of *Restinga* Forests. We dealt with this limitation by avoiding the use of analysis requiring variance homogeneity.

Data collection

We placed 10 contiguous 10x10 m subplots in each plot in the young, immature and late successional forests. In each subplot, we sampled all trees and shrubs in both the canopy and understory, selecting individuals with a diameter at breast height (DBH) $\geqslant$ 4.8 cm and individuals with a height $>$ 1 m and a DBH $<$ 4.8 cm, respectively. For each individual, we recorded the species name, the DBH (canopy) and the basal stem diameter (understory). For coppiced trees, we counted the number of stems per individual to estimate the capacity of plant re-sprouting during the succession. We classified each species in shade-tolerant or shade-intolerant, based on previous studies in this forest type (Larcher et al. 2012). Vouchers of the species collected were deposited at the UPCB Herbarium (Herbário do Departamento de Botânica da Universidade Federal do Paraná). Species names and family names followed the International Plant Names Index (http://www.ipni.org) and taxa were systematized according to APG III (APG III, 2009).

Analysis

We considered each plot (young, immature and late successional forests) and stratum (canopy and understory) separately to compare species compositions, species richness, Shannon's index (H', Log10), and forest structure. To reduce bias due to plot size, lack of replication and differences in plant size between strata, we compared the observed accumulated species richness (S obs) of the canopy and understory assemblages with two different estimators (bootstrap and ACE) using EstimateS (Colwell 2006) software. We used subplots from each plot as the sample units and randomized 1000 times the number of individuals. Successional stages and strata structures were analyzed by comparing the average, height, density and number of stems using Kruskal-Wallis test (Zar 1999).

To visualize differences in species assemblages among the three successional stages and two strata, we performed a multivariate analysis of the communities' composition using non-metric multidimensional scaling (NMDS) and using species abundance and Bray-Curtis distance; the first two axes were selected a priori (Legendre & Legendre 1998).

The indicator species value (IndVal) approach was applied to find typical species for the young, immature and late successional forests. The indicator value of a species is the product of its group specificity (Aij) and its group fidelity (Bij): $\text{IndVal}ij = Aij \times Bij \times 100$, where Aij is the abundance of species i across sites in group j divided by the abundance of species i over all groups, and Bij is the number of sites in group j where species i is present, divided by the total number of sites in group j. IndVal is maximized (100%) when all individuals of species i are found in one group and when that species occurs in all of the plots in that group. The statistical significance of the resulting IndVal was evaluated for each species by a random re-allocation procedure for sites (999 permutations) among the three groups (Dufrêne & Legendre 1997).

Results

A total of 3,619 individuals from 82 plant species and 36 families were sampled in the three successional stages and two strata (Appendix 1). The understory in the three forests had always higher density than the canopy. In the immature forest, the densest forest, the understory was six times denser than the canopy (Table 1). Species richness and diversity in the understory were higher than in the canopy, in all three forests. The late successional forest had greater understory and canopy species richness and diversity than young and immature forests. When species richness estimators were used, the richness was even higher than the observed, and differences among forests remained (Table 1).

Forest structure changed along the successional gradient. The basal area of the canopy was greater than the understory for all three forests, and peaked in the immature forest, where it was twofold higher than in the young forest and 50% higher than in the late successional forest (Table 1). The average density of canopy trees was significantly higher in young and immature forests (about 50%) than in the late successional forest, whereas young and immature forests did not significantly differ. All forests differed regarding understory density, with the highest values in the immature forest. The late successional forest had 70% and 50% lower understory density than immature and young forests, respectively (Table 1). Stem ramification, measured as the average number of stems per individual, was highest in the young forest, for both the canopy and the understory strata, whereas immature and late successinal forests did not significantly differ in both strata (Table 1). The late successional forest had the highest average canopy height, while the understory was significantly taller in the late successional and young forests (Table 1).

Community assemblages were distinct in the three forests and in the two strata. The NMDS community ordination values (Kruskal stress = 16.66) showed six well defined groups. The sub-plots of the late successional forest canopy were less clumped in the ordination (Fig. 1).

Differences in community assemblages were followed by changes in indicator species (Table 2): the canopies of the three forests were marked by a reduced number of indicator species (young=2, immature=4, late=3), while in the understories, the number of indicator species was much higher (6, 19 and 9, respectively). From the total of 36 indicator species, only 20% were shared between two forests or strata. Shade-intolerant species, such as the trees *Ilex integerrima, Ternstroemia brasiliensis, Gaylussacia brasiliensis* and *Myrcia ilheosensis* (higher IndVals) indicated a young forest composition, whereas shade-tolerant species, such as the trees *Euterpe edulis*,

Table 1. General structural characteristics of the canopy (diameter at the breast height, DBH $\geqslant$ 4.8cm) and understorey (DBH $<$ 4.8cm; h $\geqslant$ 1m) of three successional Lowland Atlantic forests in southern Brazil. Averages followed by the same letter do not differ (Kruskal-Wallis). $^{**}p<0.01$; $^{***}p<0.001$

Strata	**Forest stage**			
	Young	**Immature**	**Late successional**	***F***
CANOPY				
Density (individuals per 0.1ha)‡	200	276	121	-
S (species richness)‡	15	28	38	-
S (Bootstrap – ACE)	17.86–28.16	31.17–37.83	44.86–60.76	-
Shannon (H')‡	1.09	2.23	3.24	-
Basal area (m^2 ha^{-1})‡	14.26	34.31	23.91	-
Average density (individuas per 0.1 ha) (n=10)	20.0±2.28A	27.6±1.98A	12.1±1.68B	14.57***
Average stem number (per individual)	3.70±0.25A	1.42±0.05B	1.21±0.08B	119.00**
Average height (m)	5.14±0.06C	8.51±0.20B	9.89±0.38A	125.70***
UNDERSTOREY				
Density (individuals per 0.1ha)‡	937	1640	445	-
S (species richness)‡	29	49	54	-
S (Bootstrap – ACE)	32.98–40.65	51.68–51.21	58.09–56.26	-
Shannon (H')‡	1.97	2.76	3.32	-
Basal area (m^2 ha^{-1})‡	9.63	4.14	2.73	-
Average density (individuas per 0.1 ha) (n=10)	93.7±13.41b	164.0±14.00a	44.5±3.09c	38.68***
Average stem number (per individual)	1.89±0.06a	1.05±0.00b	1.00±0.00b	270.94**
Average height (m)	2.43±0.04a	2.14±0.07b	2.53±0.07a	34.88***

‡Estimates for trees with DBH $\geqslant$ 5cm and DBH $\geqslant$ 10cm in Appendix 2, for comparisons.

Aparisthmium cordatum, *Myrcia racemosa* and the shrub *Geonoma schottiana*, characterized the late successional forest (Table 2). The immature forest exhibited a mix of shade-intolerant species in the canopy (for example, *Myrcia ilheoensis* and *Myrsine venosa*) and shade-tolerant species in the understory (for example, *Miconia pusilliflora* and *Ocotea pulchella*) (Table 2). During succession, shade-intolerant trees and shrubs were progressively replaced by shade-tolerant trees and shrubs in the canopy and understory (Fig. 2).

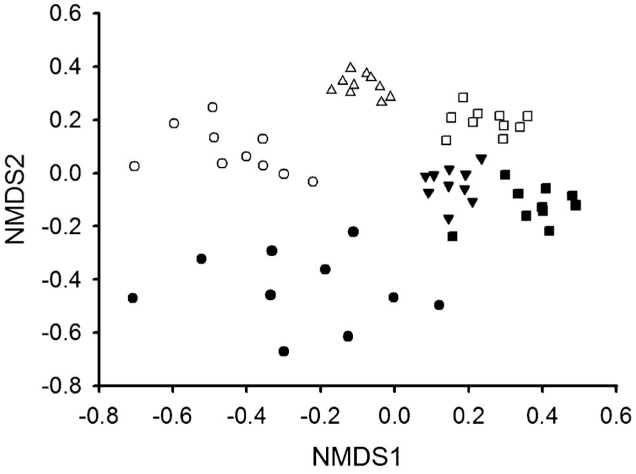


Figure 1. Non metric dimensional scale (NMDS) of species composition in 10 subplots of the canopy (filled symbols) and understorey (empty symbols) of the three successional Lowland Atlantic forests in southern Brazil. Circles: late-sucessional forest; triangles: immature forest; squares: young forest.

Discussion

Our study encompassing three Lowland Atlantic forests in southern Brazil showed differences between understory and canopy strata among forests differing in age since abandonment of impacting activities. The canopy of the forests contained a subset of different species from the respective understory, suggesting that biotic and/or abiotic filters selected the species that reached the canopy during forest development. Species richness, individual abundance, and basal area increase rapidly during the succession, indicating that forest recovery is relatively high in these Lowland Forests.

The species richness (observed and estimated) and diversity in the canopy and understory increased with forest age along the successional gradient. Patterns of changes in species richness and diversity varied across different chronosequence studies and were strongly influenced by soil fertility and land use history (Brown & Lugo 1990; Chazdon 2008). Whereas initial successional areas generally contain few tree species and low diversity (Siminski et al. 2004), the peak of tree diversity might be found in intermediate (Sheil 2001; Kalacska et al. 2004; Liebsch et al. 2007) or in late (Saldarriaga et al. 1988; Chinea 2002) phases of succession. Our results indicate an accumulation of species in the understory and the recruitment of a portion of these species to the canopy. Despite variations in methods and diversity estimates among various studies (Chazdon 2008), the unidirectional increase in species richness in our study was supported even for other stem size inclusion criteria (see Appendix 2). The diversity of the late successional forest (H'_{canopy} = 3.24) is comparable to old-growth forests (H'= 3.22-5.00) in the Atlantic Forest region (Liebsch et al. 2007; Martini et al. 2007; Siminski et al. 2011), and suggests that 59 years of abandonment is sufficient for recovering a large part of the forest diversity and composition originally found in

Table 2. Indicator species (and respective *IndVals*) of three successional Lowland Atlantic forests in southern Brazil. Species acronyms, see Appendix 1. *$p<0.05$; **$p<0.01$. In bold, species shared by two forests or strata.

	Young	Immature	Late successional
Canopy	**IINT (0.56*)** **TBRA (0.45*)**	**MILH (0.80**)** **MVEN (0.64**)** **CCRI (0.51**)** **EAMP (0.33*)**	EEDU (0.60**) **ACOR (0.50**)** MRAC (0.43*)
Understorey	GBRA (0.81**) GOPO (0.81**) **IINT (0.57**)** **MILH (0.55**)** PCAT (0.54**) **TBRA (0.52*)**	MPUS (0.94**) OPUL (0.90**) BSAL (0.86**) MMUL (0.84**) AGUI (0.82**) **EAMP (0.81**)** CPER(0.80**) **CCRI (0.68**)** **MVEN (0.66**)** MCIN (0.64**) MBRA (0.60**) ATRI (0.55**) ASER (0.45**) ABRA (0.44*) MCAB (0.41*) SYMP (0.41*) SGUI (0.37*) MCUB (0.33*) CBUB (0.14*)	GSCH (0.87**) TGUI (0.80**) ADUB (0.70**) DMON (0.60**) OACY (0.60**) AEMA (0.55**) PSEL (0.50**) AFRA (0.46*) **ACOR (0.40*)**

the study region. Despite our findings, the recovery of forest diversity is not necessarily followed by forest functionality (Díaz & Cabido 2001), which may take a longer time period and limit ecosystem processes (Liebsch et al. 2008). Therefore, forest conservation and management initiatives should consider the relative fast recovery of diversity and structure but be aware of a possible lag in the recovery of forest functionality.

The chronosequence studied was marked by a peak in basal area and tree density in the intermediate stand, for both the understory and canopy strata; the late successional forest was the least dense stand. In general, young stands are characterized by higher tree densities, lower basal areas and shorter canopy heights (Saldarriaga et al. 1988; Aide et al. 1995; Denslow & Guzman 2000) and the increase in density and basal area is either asymptotic (Saldarriaga et al. 1988; Piotto et al. 2009) or exhibits a peak in intermediate stands (Aide et al. 1995; Feldpausch et al. 2005). The immature forest is a habitat where the shade-intolerant trees and shrubs of the initial succession are still present and where the shade-tolerant species can also become established, resulting in a dense forest. The decreases in stem density and basal area from the immature forest to the late successional forest suggest a density-dependent (*sensu* Janzen 1970) mortality mechanism. Thus, the first part of the succession is marked by colonization of shade-intolerant shrubs

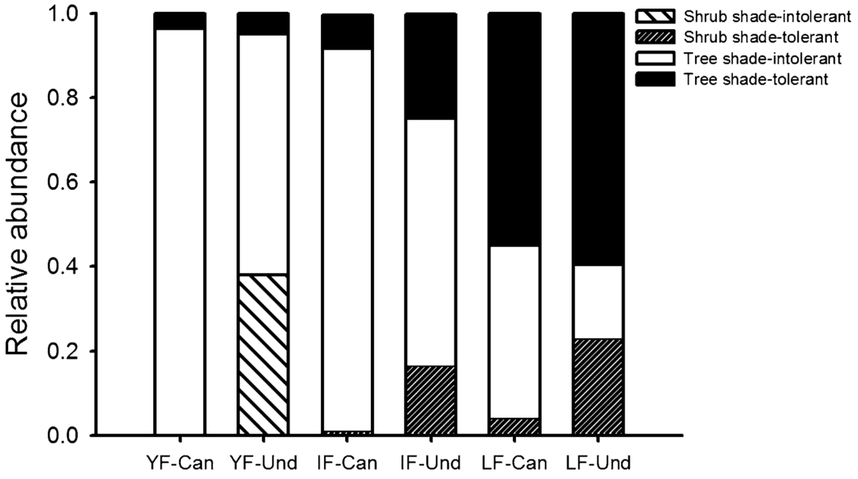


Figure 2. Relative abundance of trees and shrubs, according to shade tolerance, in three successional Lowland Atlantic forests in southern Brazil. Three non classified species (5 individuals) were excluded.

and trees; during the second part, there is an accumulation of species (both shade-tolerant and intolerant) in the limited habitat of the immature forest where intraspecific light competition may occur. During the third phase of succession, the establishment of shade-tolerant species occurs, increasing the species richness.

The three forests were marked by the occurrence of multiple stems, especially in the canopy of the young forest (3.7 stems per individual). This most likely resulted from previous land management that had included several instances of slash-and-burn agriculture. Re-sprouting has been cited as an important form of stand initiation in tropical forests (Kammesheidt 1998) and it may lead to the development of the initial cover during the first phases of forest regrowth (Schmidt-Vogt 2001). In the studied areas, after land abandonment, the stems were able to re-sprout for some period, but with the successional process, re-sprouting trees are replaced by others with single stems. In fact, this ability is restricted to the trees remaining after the first steps in the successional processes because a strong dependence on seed arrival (and not on re-sprouting) was found for the establishment of saplings at the same study sites (Simões & Marques 2007).

We found that the species assemblages for each stratum and forest stage were weakly related and that there was little species convergence among the communities of the three successional forests. Thus, each successional forest is a unique plant community. Species composition appears to vary independently of species richness in the succession and is strongly determined by differences in land use and the colonizing vegetation (Finegan 1996; Chazdon 2003). The three successional forests had similar histories and were embedded in a relatively forested landscape, which potentially allowed for the same possibilities of seed arrival in the three abandoned areas (unpublished data). One possible explanation for the differences in species composition are the age lags between the forests (young to immature forest: 13 years; immature to late successional forest: 25 years) which would be sufficient for the arrival and establishment of allochthonous species, resulting in different species compositions. In addition, it is possible that plant colonization after the abandonment of the area had been affected by the regional landscape that had changed over the 60 years. The 10-30-year-old (phase 2) forest is believed to contrast with the 30-100-year-old (phases 3 and 4) forest in species composition because short-lived pioneers are replaced by long-lived pioneer and non-pioneer species (Finegan 1996). However, our results indicate that species turnover might be more complex than previously suggested and that, even after a long time (59 years), species (shade-tolerant or intolerant) are still being replaced.

Each plant community and stratum was marked by a group of specific indicator species, suggesting a strong specificity (occurrence in a single forest/stratum) and fidelity (occurrence in a large proportion of samples from each forest/stratum) of species during succession. Despite these differences in species, almost all guilds (except shade-tolerant shrubs) were present since the young forest (but in different proportions). Tropical forest succession is characterized by changes in species composition, from herbs, shrubs, vines and fast-growing pioneer tree species during the first 30 years, to long-lived pioneer trees and shade-tolerant species during the following 70-120 years (Finegan 1996). According to the initial floristic composition hypothesis (Finegan 1996; Uhl et al. 1998; Guariguata & Ostertag 2001; Peña-Claros 2003), species representing all guilds colonize soon after disturbance but reach dominance at different times according to their growth rates and longevities (Egler 1954). Our results support this hypothesis and also agree with the consensus that species composition in forest succession is highly unpredictable (Chazdon 2008).

In the current scenario, tropical forests are being reduced at alarming rates and being replaced by secondary forests in a changing tropical landscape. Knowledge of the mechanisms of forest succession and forest recovery is necessary to guarantee ecosystem functioning in this highly diverse region (Dent & Wright 2009). Our findings showed that the structure of canopy and understorey of successional forests follow approximately the same successional trajectory, but with marked differences in species composition. Thus, the recovery of these fragile Lowland Atlantic forests is relatively fast, but slightly predictable, which should be taken in considerations in future management of these systems.

Acknowledgements

The authors are grateful to Larissa Mellinger and Marina Tadei for field assistance, to IAP (Instituto Ambiental do Paraná) for allowing research in the Palmito Reserve and to CNPq (Brazilian Research Council) for the fellowship to VP Zwiener and F Ramos (Grant 520898/99-0). MCM Marques (Grant 304650/2012-9) and R Marques received a productivity grant from CNPq.

References

Aide, T.M., Zimmermann, J.K., Herrera, L., Rosario M. & Serrano M. 1995. Forest recovery in abandoned tropical pastures in Puerto Rico. For. Ecol. Manag. 77:77–86. http://dx.doi.org/10.1016/0378-1127(95)03576-V

Aide, T.M. & Grau, H.R. 2004. Ecology – Globalization, migration, and Latin American ecosystems. Science 305:1915–1916.

APG III - Angiosperm Phylogeny Group. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Bot. J. Linn. Soc. 161:105–121. http://dx.doi.org/10.1111/j.1095-8339.2009.00996.x

Britez, R.M. & Marques, M.C.M. 2005. Caracterização geral. In: Marques, M.C.M., Britez, R.M. (eds) História Natural e Conservação da Ilha do Mel. Editora da UFPR, Curitiba, 13–17.

Brown, S. & Lugo, A.E. 1990. Tropical secondary forests. J. Trop. Ecol. 6:1–32. http://dx.doi.org/10.1017/S0266467400003989

Chazdon, R.L. 2003. Tropical forest recovery: legacies of human impact and natural disturbance. Perspect. Plant Ecol. 6:51–71. http://dx.doi.org/10.1078/1433-8319-00042

Chazdon, R.L. 2008. Chance and determinism in tropical forest succession. In: Carson WP, Schnitzer lSA (eds) Tropical forest community ecology. Wiley-Blackwell, West Sussex, 384–408.

Chazdon, R.L., Letcher, S.G., van Breugel M., M., Martínez-Ramos M., Bongers F. & Finegan, B. 2007. Rates of change in tree communities of secondary Neotropical forests following major disturbances. Phil. Trans. R. Soc.B 362:273–289. http://dx.doi.org/10.1098/rstb.2006.1990

Chazdon, R.L., Peres, C.A., Dent, D., Sheil, D., Lugo, A.E., Lamb, D., Stork N.E. & Miller, S.E.. 2009. The potential for species conservation in tropical secondary forests. Conserv. Biol. 23:1046–1417. http://dx.doi.org/10.1111/j.1523-1739.2009.01338.x

Chinea, J.D. 2002. Tropical forest succession on abandoned farms in the Humacao municipality of eastern Puerto Rico. For. Ecol. Manag. 167:195–207. http://dx.doi.org/10.1016/S0378-1127(01)00693-4

Colwell, R.K. 2006. EstimateS: statistical estimation of species richness and shared species from samples. Version 8.0. User's guide and application published at http://purl.oclc.org/estimates.

Connell, J.H. 1978. Diversity in tropical rain forests and coral reefs. Science 199:1302–1310.

Corlett, R. 1995. Tropical secondary forests. Prog. Phys. Geog. 19:159–172. http://dx.doi.org/10.1177/030913339501900201

Denslow, J.S. 1980. Patterns of plant species diversity during succession under different disturbance regimes. Oecologia 46:18–21.

Denslow, J.S. & Guzman, G.S. 2000. Variation in stand structure, light and seedling abundance across a tropical moist forest chronosequence, Panama. J. Veg. Sci. 11:201–212. http://dx.doi.org/10.2307/3236800

Dent, D.H. & Wright, S.J. 2009. The future of tropical species in secondary forests: a quantitative review. Biol. Conserv. 142:2833–2843. http://dx.doi.org/10.1016/j.biocon.2009.05.035

Días, S. & Cabido, M. 2001. Viva la différence: plant functional diversity matters to ecosystem processes. Trends Ecol. Evol. 6: 646–655.

Ding, Y., Zang, R., Liu, S., He F. & Letcher, S.G. 2012. Recovery of woody plant diversity in tropical rain forests in southern China after logging and shifting cultivation. Biol. Conserv. 145:225–233. http://dx.doi.org/10.1016/j.biocon.2011.11.009

Dufrêne, M. & Legendre, P. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol. Monogr. 67:345–366.

Egler, F.E. 1954. Vegetation science concepts: Initial floristic composition – a factor in old-field vegetation development. Vegetatio 4:412–417.

Feldpausch, T.R., Riha, S.J., Fernandes, E.C.M. & Wandelli, E. 2005. Development of forest structure and leaf area in secondary forests regenerating on abandoned pastures in Central Amazonia. Earth Interact. 9:1–22. http://dx.doi.org/10.1175/EI140.1

Finegan, B. 1996. Pattern and process in neotropical secondary rain forest: the first 100 years of succession. Tree 11:119–124.

Foster, B.L. & Tilman D. 2000. Dynamic and static views of succession: testing the descriptive power of the chronosequence approach. Plant Ecol. 146:1–10. http://dx.doi.org/10.1023/A:1009895103017

Galindo-Leal, C. & Câmara, I.G. 2003. Atlantic Forest hotspot status: an overview. In: Galindo-Leal, C., Câmara, I.G. (Eds.), The Atlantic Forest of South America: Biodiversity Status, threats and outlook. CABS and Island Press, Washington, 3–11.

Guariguata, M.R. & Ostertag, R. 2001. Neotropical secondary forest succession: changes in structural and functional characteristics. For. Ecol. Manag. 148:185–206. http://dx.doi.org/10.1016/S0378-1127(00)00535-1

Holl, K.D., Loik, M.E., Lin, E.H.V. & Samuels, I.A. 2000. Tropical Montane forest restoration in Costa Rica: overcoming barriers to dispersal and establishment. Rest. Ecol. 8:339–349. http://dx.doi.org/10.1046/j.1526-100x.2000.80049.x

Hurlbert, S H. 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 54:187–211. http://dx.doi.org/10.2307/1942661

Janzen, D.H. 1970. Herbivores and the number of tree species in tropical forests. Am. Nat. 940:501–528. http://dx.doi.org/10.1086/282687

Kalacska, M., Sanchez-Azofeifa, G.A., Rivard, B., Calvo-Alvarado, J.C., Journet, A.R.P., Arroyo-Mora, J.P. & Ortiz-Ortiz D. 2004. Leaf area index measurements in a tropical moist forest: a case study from Costa Rica. Remote Sens. Environ. 91:134–152. http://dx.doi.org/10.1016/j.rse.2004.02.011

Kammesheidt, L. 1998. The role of tree sprouts in the restoration of stand structure and species diversity in tropical moist forest after slash-and-burn agriculture in Eastern Paraguay. Plant Ecol. 139:155–165. http://dx.doi.org/10.1023/A:1009763402998

Larcher, L., Boeger M.R.T., Marques M.C.M.. 2012. Biomass allocation and shade tolerance in tree species of the Atlantic Forest. Botany 90:830-838. http://dx.doi.org/10.1139/b2012-053

Larkin, C.C., Kwit, C., Wunderle Jr, J.M, Helmer, E.H., Henry, M., Stevens, H., Roberts, H.T.K., & EwertD.N.. 2012. Disturbance type and plant successional communities in Bahamian Dry Forests. Biotropica 44:10–18. http://dx.doi.org/10.1111/j.1744-7429.2011.00771.x

Legendre, P. & Legendre L. 1998. Numerical Ecology, Elsevier, Amsterdam.

Letcher, S.G. & Chazdon, R.L. 2009. Rapid recovery of biomass, species richness, and species composition in a forest chronosequence in Northeastern Costa Rica. Biotropica 41:608–617. http://dx.doi.org/10.1111/j.1744-7429.2009.00517.x

Liebsch, D., Goldenberg R. & Marques, M.C.M. 2007. Florística e estrutura de comunidades vegetais em uma cronoseqüência de Floresta Atlântica no Estado do Paraná, Brasil. Acta Bot. Bras. 21:983–992. http://dx.doi.org/10.1590/S0102-33062007000400023

Liebsch D., Marques, M.C.M. & Goldenberg, R. 2008. How long does the Atlantic Rain Forest take to recover after disturbance? Changes in species composition and ecological features during secondary succession. Biol. Conserv. 141:1717–1725. http://dx.doi.org/10.1016/j.biocon.2008.04.013

Long, W., Yang X. & Li, D. 2012. Patterns of species diversity and soil nutrients along a chronosequence of vegetation recovery in Hainan Island, South China. Ecol. Res. 27:561–568. http://dx.doi.org/10.1007/s11284-011-0923-3

Marques, M.C.M., Swaine, M.D. & Liebsch, D. 2011. Diversity distribution and floristic differentiation of the coastal lowland vegetation: implications for the conservation of the Brazilian Atlantic Forest. Biodivers. Conserv. 20:153–168. http://dx.doi.org/10.1007/s10531-010-9952-4

Martini, A.M.Z., Fiaschi, P., Amorin A.M.A. & Paixão, J.L. 2007. A hot-point within a hot-spot: a high diversity site in Brazils Atlantic Forest. Biodivers. Conserv. 16:3111–3128. http://dx.doi.org/10.1007/s10531-007-9166-6

Mesquita, R.C.G., Ickles, K., Ganade, G. & Williamson, B. 2001. Alternative successional pathways in the Amazon Basin. J. Ecol. 89:528–537. http://dx.doi.org/10.1046/j.1365-2745.2001.00583.x

Norden, N., Chazdon, R.L., Chao, A., Jiang Y. & Vilchez-Alvarado, B. 2009. Resilience of tropical rain forests: tree community reassembly in secondary forests. Ecol. Lett. 12: 385–394. http://dx.doi.org/10.1111/j.1461-0248.2009.01292.x

Peña-Claros, M. 2003. Changes in forest structure and species composition during secondary forest succession in the Bolivian Amazon. Biotropica 35:450–461.

Pickett, S.T.A., Collins, S.L. & Armesto, J.J. 1987 Models, mechanisms and pathways of succession. Bot. Rev. 53:335–371. http://dx.doi.org/10.1007/BF02858321

Pickett, S.T.A. 1989. Space for time substitution as an alternative to long-term studies. In: Likens GE (ed) Long-term studies in Ecology. Springer-Verlag, New York. 110–135. http://dx.doi.org/10.1007/978-1-4615-7358-6_5

Piotto, D., Montagnini, F., Thomas, W., Ashton M. & Oliver C. 2009. Forest recovery after swidden cultivation across a 40-year chronosequence in the Atlantic forest of southern Bahia, Brazil. Plant Ecol. 205:261–272. http://dx.doi.org/10.1007/s11258-009-9615-2

Saldarriaga, J.G., West, D.C., Thrp M.L. & Uhl C. 1988. Long-term chronossequence of forest succession in the upper Rio Negro of Colombia and Venezuela. J. Ecol. 76:938–958. http://dx.doi.org/10.2307/2260625

Schmidt-Vogt, D. 2001. Secondary forests in swidden agriculture in highlands of Thailand. J. Trop. For. Sci. 13:748–767.

Sheil, D. 2001. Long-term observations of rain forest succession, tree diversity and responses to disturbance. Plant Ecol. 155:183–199. http://dx.doi.org/10.1023/A:1013243411819

Siminski, A., Mantovani, M., Reis M.S. & Fantini A.C. 2004. Sucessão florestal secundária no município de São Pedro de Alcântara, litoral de Santa Catarina: estrutura e diversidade. Ciênc Flor. 14:21–33.

Siminski, A., Fantini, A.C., Guries, R.P., Ruschel A.R. & Reis, M.S. 2011. Secondary forest succession in the Mata Atlantica, Brazil: floristic and phytosociological trends. ISRN Ecology, Article ID 759893, 19 pages, doi: 10.5402/2011/759893

Simões, C.G. & Marques, M.C.M. 2007. The role of sprouts in the restoration of Atlantic Rain Forest in southern Brazil. Rest. Ecol. 15:53–59. http://dx.doi.org/10.1111/j.1526-100X.2006.00189.x

Uhl, C. 1987. Factors controlling succession following slash-and-burn agriculture in Amazonia. J. Ecol. 75:377–407. http://dx.doi.org/10.2307/2260425

Uhl, C., Buschbacher, R. & Serrão, E.A.S. 1998. Abandoned pastures in eastern Amazonia: I. Patterns of plant succession. J. Ecol. 76:663–681. http://dx.doi.org/10.2307/2260566

Wright, S.J. & Muller-Landau, H.C. 2006. The future of tropical forest species. Biotropica 38:287–301.

Zar, J.H. 1999. Biostatistical analysis. Prentice-Hall, New Jersey.

Zwiener, V., Cardoso, F.C.G., Padial, A.A. & Marques, M.C.M. 2014. Disentangling the effects of facilitation on restoration of the Atlantic Forest. Bas. Appl. Ecol. 15:34–41. http://dx.doi.org/10.1016/j.baae.2013.11.005

Received 03/10/2013
Revised 17/07/2014
Accepted 08/08/2014

Appendix 1. Checklist, life forms (LF: T = tree, S = shrub) and densities (per 0.1 ha) of 82 species sampled in three successional Lowland Atlantic forests in southern Brazil. YCAN= young forest – canopy, YUND = young forest – understorey, ICAN = immature forest - canopy, IUND = immature forest - understorey, LCAN = late successional forest – canopy, LUND = late successional forest – understorey.

Acronyms	Species	Family	LF	YCAN	YUND	ICAN	IUND	LCAN	LUND
ABRA	*Abarema brachystachya* (DC.) Barneby & J.W. Grimes	Fabaceae	T	0	1	2	15	0	3
ACOR	*Aparisthmium cordatum* (Juss.) Baill.	Euphorbiaceae	T	0	0	0	0	5	22
ADUB	*Attalea dubia* (Mart.) Burret	Arecaceae	T	0	0	0	0	2	11
AEMA	*Annona emarginata* (Schltdl.) H.Rainer	Annonaceae	T	0	0	0	1	0	11
AFIR	*Aniba firmula* (Nees & Mart.) Mez	Lauraceae	T	0	0	0	0	0	2
AFRA	*Andira fraxinifolia* Benth.	Fabaceae	T	5	3	9	4	9	14
AGLA	*Alchornea glandulosa* Poepp.	Euphorbiaceae	T	0	0	0	0	1	0
AGUI	*Amaioua guianensis* Aubl.	Rubiaceae	T	0	1	0	98	2	6
ASAL	*Aiouea saligna* Meisn.	Lauraceae	T	0	0	0	0	0	3
ASER	*Annona sericea* Dunal	Annonaceae	T	0	0	0	13	1	1
ATRI	*Alchornea triplinervia* (Spreng.) Müller Arg.	Euphorbiaceae	T	0	3	0	17	0	5
BSAL	*Blepharocalyx salicifolius* (Kunth) O.Berg	Myrtaceae	T	0	7	1	43	0	0
CATR	*Cyathea atrovirens* (Langsd. & Fisch.) Domin	Cyatheaceae	T	0	0	0	0	1	0
CBRA	*Calophyllum brasiliense* Cambess.	Calophyllaceae	T	1	0	3	16	2	12
CCAN	*Cabralea canjerana* (Vell.) Mart.	Meliaceae	T	0	0	0	0	1	0
CCON	*Cordiera concolor* (Cham.) Kuntze	Rubiaceae	T	0	0	0	0	1	1
CCRI	*Clusia criuva* Cambess.	Clusiaceae	T	0	13	11	65	0	17
CMIC	*Coussapoa microcarpa* (Schott) Rizzini	Urticaceae	T	0	0	0	0	1	0
CPER	*Cybianthus peruvianus* (A.DC.) Miq.	Primulaceae	T	0	0	3	80	3	12
CRUB	*Calyptranthes rubella* (O.Berg) D.Legrand	Myrtaceae	T	0	0	0	8	0	0
CSCR	*Clethra scabra* Pers.	Clethraceae	T	0	4	2	4	1	6
DAPH	*Daphnopsis* sp.	Thymelaeaceae	T	0	0	0	0	0	1
DMON	*Dendropanax monogynum* (Vell.) Seem.	Araliaceae	S	0	0	0	0	0	15
EAMP	*Erythroxyllum amplifolium* Baill.	Erythroxyllaceae	T	0	10	3	36	0	0
EEDU	*Euterpe edulis* Mart.	Arecaceae	T	0	0	0	0	13	3
EPAN	*Endlicheria paniculata* (Spreng.) J.F.Macbr.	Lauraceae	T	0	0	0	0	0	4
FORG	Ficus organensis *Miq.*	Moraceae	T	0	1	0	0	0	0
GAUS	*Guatteria australlis* A. St. 0Hil.	Annonaceae	T	1	14	3	55	1	54
GBRA	*Gaylussacia brasiliensis* (Spreng.) Meisn.	Ericaceae	S	0	355	0	0	0	0
GGAR	*Garcinia gardneriana* (Planch. & Triana) Zappi	Clusiaceae	T	0	0	0	0	1	0
GHIR	*Guapira hirsuta* (Choisy) Lundell	Nyctaginaceae	S	0	1	0	0	0	0
GMAC	*Guarea macrophylla* Vahl	Meliaceae	T	0	0	0	0	0	2

Continued on next page

Appendix 1. Continued.

Acronyms	Species	Family	LF	YCAN	YUND	ICAN	IUND	LCAN	LUND
GOPO	*Guapira opposita* (Vell.) Reitz	Nyctaginaceae	T	5	81	3	5	0	3
GSCH	*Geonoma schottiana* Mart.	Arecaceae	S	0	0	3	2	4	64
HBRA	*Hedyosmum brasiliense* Mart.	Chloranthaceae	T	0	0	0	0	0	3
HSIL	*Heisteria silvianii* Schwacke	Olacaceae	T	0	0	0	1	0	2
HUMB	*Handroanthus umbellatus* (Sond.) Mattos	Bignoniaceae	T	0	0	0	0	0	1
IDUM	*Ilex dumosa* Reissek	Aquifoliaceae	T	0	0	1	4	1	0
IINT	*Ilex integerrima* Reissek	Aquifoliaceae	T	151	226	99	126	6	17
IPSE	*Ilex pseudobuxus* Reissek	Aquifoliaceae	T	0	1	0	0	0	0
JPUB	*Jacaranda puberula* Cham.	Bignoniaceae	T	0	0	0	0	1	1
LFRU	*Laplacea fruticosa* (Schrad.) Kobuski	Theaceae	T	0	0	0	5	0	0
MBRA	*Maprounea brasiliensis* A. St.-Hil.	Euphorbiaceae	T	0	2	1	1	1	1
MBRA	*Myrcia brasiliensis* Kiaersk.	Myrtaceae	T	0	0	0	12	0	4
MCAB	*Miconia cabussu* Hoehne	Melastomataceae	S	0	0	0	12	1	2
MCIN	*Miconia cinerascens* Miq.	Melastomataceae	S	0	0	0	62	0	14
MCUB	*Miconia cubatanensis* Hoehne	Melastomataceae	S	0	0	0	10	0	0
MDIC	*Myrcia dichrophylla* D.Legrand	Myrtaceae	T	0	0	0	5	0	3
MELA	*Melastomataceae sp.*	Melastomataceae	S	*0*	*0*	*0*	*3*	*0*	*0*
MGAR	*Myrsine gardneriana* A. DC.	Primulaceae	T	0	0	0	1	0	0
MGUI	*Matayba guianensis* Aubl.	Sapindaceae	T	*0*	*0*	*0*	*0*	*0*	*3*
MILH	*Myrcia ilheosensis* Kiaersk.	Myrtaceae	T	4	88	50	67	5	5
MMUL	*Myrcia multiflora* (Lam.) DC.	Myrtaceae	T	0	1	6	29	8	2
MPUL	*Myrcia pulchra* (O.Berg) Kiaersk.	Myrtaceae	T	0	0	0	1	0	0
MPUS	*Miconia pusilliflora* (DC.) Naudin	Melastomataceae	S	0	0	0	153	0	0
MRAC	*Myrcia racemosa* (O.Berg) Kiaersk.	Myrtaceae	T	0	0	2	14	12	12
MROB	*Maytenus robusta* Reissek	Celastraceae	T	1	0	2	0	2	1
MSCH	*Maytenus schumanniana* Loes.	Celastraceae	T	0	4	0	4	0	0
MVEN	*Myrsine venosa* A. DC.	Primulaceae	T	1	9	36	62	4	2
NOPO	*Nectandra oppositifolia* Nees & Mart.	Lauraceae	T	0	0	1	1	0	4
OACY	*Ocotea aciphylla* (Nees & Mart.) Mez	Lauraceae	T	1	0	1	3	2	20
OAMI	*Ossea amygdaloides* Triana	Melastomataceae	S	0	3	0	25	0	0
OARB	*Ormosia arborea* (Vell.) Harms.	Fabaceae	T	0	2	0	0	0	2
ODIS	*Ocotea dispersa* (Nees & Mart.) Mez	Lauraceae	T	0	0	0	0	0	2
OPUL	*Ocotea pulchella* (Nees & Mart.) Mez	Lauraceae	T	8	46	10	506	10	3
PCAT	*Psidium cattleianum* Afzel. ex Sabine	Myrtaceae	T	3	19	4	0	3	0
PGLA	*Pera glabrata* (Schott) Poepp. ex Baill.	Peraceae	T	2	1	1	9	1	6

Continued on next page

Appendix 1. Continued.

Acronyms	Species	Family	LF	YCAN	YUND	ICAN	IUND	LCAN	LUND
PHOF	*Psychotria hoffmannseggiana* (Willd. ex Schult.) Müll. Arg.	Rubiaceae	S	0	0	0	8	0	6
PMYR	*Prunus myrtifolia* (L.) Urb.	Rosaceae	T	*0*	*8*	*0*	*2*	*0*	*3*
PSEL	*Podocarpus sellowii* Klotzsch ex Endl.	Podocarpaceae	T	0	0	0	5	2	13
PVENO	*Pouteria venosa* (Mart.) Baehni	Sapotaceae	T	0	0	0	0	3	0
RUBI	Rubiaceae sp.	Rubiaceae	S	0	0	0	0	0	1
SANG	*Schefflera angustissima* (Marchal) Frodin	Araliaceae	T	0	0	1	3	3	2
SGUI	*Sloanea guianensis* (Aubl.) Benth.	Elaeocarpaceae	T	0	0	0	0	1	1
SGUI	*Siphoneugena guilfoyleiana* Proença	Myrtaceae	T	0	0	0	5	0	0
SYMP	*Symplocos* sp.	Symplocaceae	T	0	1	0	11	0	2
TBRA	*Ternstroemia brasiliensis* Cambess.	Pentaphylacaceae	T	15	31	16	23	0	1
TGUI	*Tapirira guianensis* Aubl.	Anacardiaceae	T	0	0	1	4	4	34
TIBO	*Tibouchina* sp.	Melastomataceae	T	0	1	0	0	0	0
UNKN	Unknown sp.	Unknown	T	0	0	1	0	0	0
XBRA	*Xylopia brasiliensis* Spreng.	Annonaceae	T	0	0	0	1	0	0
XLAN	*Xylopia langsdorfiana* A. St.-Hil. & Tul.	Annonaceae	T	1	0	0	0	1	0

Appendix 2. Comparative forest structure (canopy) for different tree sizes.

	Young forest		Immature forest		Late successional forest	
	DBH ⩾ 5cm	**DBH ⩾ 10cm**	**DBH ⩾ 5cm**	**DBH ⩾ 10cm**	**DBH ⩾ 5cm**	**DBH ⩾ 10cm**
Density (per 0.1ha)	165	10	248	95	118	61
S (species richness)	14	4	25	15	38	27
Shannon (H')	0.98	1.28	2.14	1.38	3.25	3.05
Basal area (m^2 ha^{-1})	12.81	3.67	33.73	22.28	23.86	20.90

Biota Neotropica 14(3): e20140074, 2014
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
inventory

Medium and large-sized mammals of the Reserva Ecológica de Guapiaçú, Cachoeiras de Macacu, RJ

Israel Dias de Carvalho[1,3], *Rildo de Oliveira*[2] & *Alexandra dos Santos Pires*[1]

[1] *Laboratório de Ecologia e Conservação de Florestas, Departamento de Ciências Ambientais, Instituto de Florestas, Universidade Federal Rural do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.*
[2] *Reserva Ecológica de Guapiaçú, Rio de Janeiro, RJ, Brazil.*
[3] *Corresponding author: Israel Dias de Carvalho, e-mail: israelcarvalho31@gmail.com*

CARVALHO, I. D., OLIVEIRA, R., PIRES, A. S. **Medium and large-sized mammals of the Reserva Ecológica de Guapiaçú, Cachoeiras de Macacu, RJ.** Biota Neotropica. 14(3): e20140074. http://dx.doi.org/10.1590/1676-06032014007414

Abstract: Medium and large-sized terrestrial mammals develop important functions in ecosystems maintenance. However, this group is the most vulnerable to local extinctions, through habitat loss or hunting. This study inventoried the community of medium and large mammals in the Reserva Ecológica de Guapiaçú (REGUA), Cachoeiras de Macacu, RJ, providing data on species composition, richness and abundance. Species were registered through the use of camera traps, sign survey and visual encounters on pre-existing trails and roads. The study was conducted from January to October 2012, with each month considered as a sample unit. An effort of 1568 cameras-day and 120 km traveled, resulted on 302 records of 22 species of medium and large-sized mammals, belonging to eight orders. This value corresponds to more than three-quarters of the richness described from this group in the Atlantic Forest of Rio de Janeiro state. Five species are under some level of regional, national or global threat. Three game species, *Pecari tajacu*, *Cuniculus paca* and *Dasyprocta leporina* were abundant in the area. The observed richness was comparable to that found in other studies conducted in rain forests. Thus, for its high number of species, including those under some degree of threat, we conclude that REGUA is an important area to mammal conservation, especially in the Atlantic Forest of Rio de Janeiro state.

Keywords: *Mammal community, endangered species, camera traps, sign survey, Atlantic Forest.*

CARVALHO, I. D., OLIVEIRA, R., PIRES, A. S. **Mamíferos de médio e grande porte da Reserva Ecológica de Guapiaçú, Cachoeiras de Macacu, RJ.** Biota Neotropica. 14(3): e20140074. http://dx.doi.org/10.1590/1676-06032014007414

Resumo: Mamíferos de médio e grande porte desempenham funções importantes para a manutenção dos ecossistemas. Porém, esse grupo é um dos mais vulneráveis a extinções locais, seja pela perda de hábitat ou pela caça. Neste estudo foi inventariada a comunidade de médios e grandes mamíferos na Reserva Ecológica de Guapiaçú (REGUA), Cachoeiras de Macacu, RJ, sendo descritos dados de composição, riqueza e abundância. As espécies foram registradas através do uso de armadilhas fotográficas, busca por vestígios e visualizações diretas em trilhas e estradas pré-existentes. O estudo foi realizado de janeiro a outubro de 2012, sendo cada mês considerado uma unidade amostral. A partir de um esforço de 1568 câmeras-dia e de 120 km caminhados, obtivemos 302 registros de 22 espécies de mamíferos de médio e grande porte, pertencentes a oito Ordens. Esse valor corresponde a mais que três quartos da riqueza de espécies descrita para esse grupo na Mata Atlântica do estado do Rio de Janeiro. Cinco espécies encontram-se sob algum nível de ameaça regional, nacional ou global. Três espécies cinegéticas, *Pecari tajacu*, *Cuniculus paca* e *Dasyprocta leporina* foram abundantes na área. A riqueza observada foi comparável à encontrada em outros estudos realizados em florestas ombrófilas densas. Assim, por seu elevado número de espécies, incluindo aquelas sob algum grau de ameaça, concluímos que a REGUA é um lugar de destaque para a conservação da biodiversidade de mamíferos, especialmente na Mata Atlântica do estado do Rio de Janeiro.

Palavras-chave: *Comunidade de mamíferos, espécies ameaçadas, armadilhas fotográficas, busca por vestígios, Mata Atlântica.*

Introduction

Medium and large-sized terrestrial mammals develop important functions in ecosystems maintenance. These animals affects plant population dynamics through herbivory and physical damage (e.g. Dirzo & Miranda 1990, Keuroghlian & Eaton 2009, Beck et al. 2013), and also through their role as seed dispersers and predators (e.g. Asquith et al. 1997, Asquith et al. 1999, Galetti et al. 2006, Donatti et al. 2009, Kuprewicz 2013) contributing to plant local diversity. Acting as animal predators they also regulate herbivore populations (e.g. Terborgh 1988, Sinclair et al. 2003) affecting prey activity patterns (Lima & Dill 1990), and reducing competition by resources favoring species co-ocorrence (Miller et al. 2001). In this way, these animals favor forest complexity and heterogeneity (e.g. Miller et al. 2001). Besides that, they contribute also to the conservation of other species acting as umbrella species (e.g. Noss et al. 1996) especially by their large area requirements. These animals are also among the main flagship species (e.g. Mittermeier 1986), due to their charismatic attributes. Due to its ability to move inside habitat mosaics, these mammals have been used also as landscape species, contributing to conservation planning in larger scales (Bani et al. 2002, Sanderson et al. 2002a, Crouzeilles et al. 2010).

In spite of their importance, these animals are among the most vulnerable to local extinction following anthropogenic disturbance, due to their naturally low population densities (e.g. Cullen et al. 2005, Soisalo & Cavalcanti 2006), their large area requirements (Sanderson et al. 2002b, Cullen et al. 2005) and also because they are preferential targets to hunters (Redford 1992, Peres 1996, Cullen et al. 2001, Travassos 2011). These factors has conducted to the loss of these animals in tropical forests, promoting profound transformations in forest dynamics and threaten the maintenance of these ecosystems (Stoner et al. 2007, Jorge et al. 2013, Kurten 2013, Harrison et al. 2013).

Due to this rugged relief and edaphic characteristics, which promotes a variety of ecological conditions, the Atlantic Forest at Rio de Janeiro state harbor a high biodiversity (e.g. Bergallo et al. 2000). The unrivalled numbers of endemic species and a relentless process of post-colonial deforestation (Dean 1996) make this forest an important region inside this Brazilian *hotspot* (Myers et al. 2000). Actually, the forest cover only 19.6% of the state area (Fundação SOS Mata Atlântica & INPE 2011) in a fragmented distribution (Ribeiro et al. 2009), contributing to biodiversity loss through the reduction of suitable areas to mammal persistence, especially for the larger species (Cardillo & Bromhan 2001, Kinnaird et al. 2003, Ewers & Didham 2006). Besides that, fragmentation facilitates the access of hunters (Peres 2000), increasing the negative pressures over the populations of these animals (Chiarello 2000, Cullen et al. 2001). In this scenario, large (> 100 ha), protected remnants are responsible to maintain the diversity of medium and large-sized mammals (Canale et al. 2012).

In spite of their importance and vulnerability, few studies had been carried out with this mammal group in the Atlantic Forest. The difficulties related to the study of these animals are due to their furtive habits (Reis et al. 2006, Barea-Azcón et al. 2007), and habitat requirements (Sanderson et al. 2002b, Barea-Azcón et al. 2007). Recently, the use of new tracking technologies as camera traps (e.g. Karanth & Nichols 1998, Santos-Filho & Silva 2002, Galetti et al. 2006, Srbek-Araújo & Chiarello 2007, Goulart et al. 2009, Carvalho et al. 2013), and their combined use with other sampling techniques as footprint traps, linear transects and sign surveys (Passamani et al. 2005, Negrão & Valladares-Pádua 2006, Spínola 2008, Modesto et al. 2008a, Silva & Passamani, 2009, Espartosa et al. 2011, Delciellos et al. 2012) have favored studies with this group. However, due to be a recent approach, several areas still represents knowledge gaps for this group, especially in the Rio de Janeiro state (Cunha 2004, Modesto et al. 2008a,b, Delciellos et al. 2012). Inventories of this group are still needed to a better understanding of the regional biodiversity and its ecological patterns. These studies are of fundamental importance to verify the conservation status of the species and to develop monitoring and management and strategies (Northon-Griffiths 1978, Boddicker et al. 2002, Gaidet-Drapier et al. 2006). From these information is possible to define priority areas for conservation, establish minimum areas for reserves and to understand the main treats, consolidating the conservation strategies in these areas.

This study aim to characterize the assemblage of medium and large sized mammals of a preserved area of the Atlantic Forest, the Reserva Ecológica de Guapiaçú (REGUA), situated at Cachoeiras de Macacu, RJ. More specifically, were assessed the species composition, richness and species abundance in the area.

Material and Methods

1. *Study area*

The Reserva Ecológica de Guapiaçú (REGUA, 22°22’12” S – 22°27’18” S and 42°42’25” O – 42°49’19” O) is a private area, covering 7300 ha. The vegetation is the rain forest, covering montane, sub-montane and lowland areas (Oliveira-Filho & Fontes 2000). In the last, most part of the vegetation is in initial successional stages due to the recent history of anthropogenic disturbance. The most representative botanical families in the area are Fabaceae, Rubiaceae, Myrtaceae, Lauraceae, Meliaceae, Euphorbiaceae, Sapindaceaea, Melastomataceae, Annonaceae and Sapotaceae (Azevedo 2012). The climate is classified as Am according to Köppen (Peel et al. 2007), with annual mean temperature of 22.4 °C and maximum temperatures occurring in January and February and minimum in July. The annual rainfall is 2095 mm; December and January are the rainy months and June and July the driest ones. The altitude ranges from 20 m to ca. 2000 m. The area overlaps with the Parque Estadual dos Três Picos which is the largest state conservation unit from Rio de Janeiro, covering 58.790 ha (INEA 2013). About 94.8% of REGUA is covered by forest and the remaining areas include swamp, pastureland, and agriculture.

2. *Data collection*

The study was carried out from January to October 2012, and each month was considered a sample event. The species was registered through the combined use of camera traps, sign surveys and visual encounters carried out evenly at pre-existing trails and roads during other field activities. To avoid biased sampling of the some species baits were not used.

Camera traps were set at 20 stations spaced at least 500 m among them, covering an area of ca. 1,400 ha. Stations were placed at pre-existing trails and abandoned dirty roads (2 to 8 m width), which are periodically managed to keep them open.

Table 1. Medium and large-sized mammals of the Reserva Ecológica de Guapiaçú, Cachoeiras de Macacu, RJ. Type of record: Ct = Camera trap; Fp = Footprints; Bw = Burrow; Fs = Feeding signs; Cr = Carcass; Vi = Visualization; Vo = Vocalization; Tm = Territorial marks; F = Faeces; H = Hair. Status of Threat: PT = presumably threatened; LC = least concern; NT = near threatened; VU = vulnerable; EN = endangered; CE = critically endangered. Species nomenclature followed Paglia et al. (2012).

TAXA (Order/Family/Species)	***Common Name***	***Type of record***	***Degree of threat***		
			RJ	**BR**	**IUCN**
Didelphimorphia					
Didelphidae					
Didelphis aurita (Wied-Newied, 1826)	Big-eared opossum	Ct	-	-	LC
Cingulata					
Dasypodidae					
Dasypus novemcinctus Linnaeus, 1758	Nine-banded armadillo	Ct, Fp, Bw, Fs, Cr	-	-	LC
Cabassous tatouay (Desmarest, 1804)	Greater naked-tailed armadillo	Ct, Fp, Bw	PT	-	LC
Euphractus sexcinctus (Linnaeus, 1758)	Six-banded armadillo	Ct, Bw, Fs	-	-	LC
Bradypodidae					
Bradypus variegatus Schinz, 1825	Brown-throated sloth	Vi	-	-	LC
Myrmecophagidae					
Tamandua tetradactyla (Linnaeus, 1758)	Southern tamandua	Cr	-	-	LC
Primata					
Atelidae					
Alouatta guariba (Humboldt, 1812)	Brown howler	Vo	PT	-	LC
Brachyteles arachnoides É. Geoffroy, 1806	Southern muriqui	Vi	CE	EN	EN
Cebidae					
Sapajus nigritus (Goldfuss, 1809)	Black capuchin	Vi	-	-	NT
Carnivora					
Canidae					
Cerdocyon thous (Linnaeus, 1766)	Crab-eating fox	Ct, Fp	-	-	LC
Procyonidae					
Nasua nasua (Linnaeus, 1766)	South american coati	Ct, Vi, Tm	-	-	LC
Procyon cancrivorus (G.Curvier, 1798)	Crab-eating raccoon	Fp	-	-	LC
Mustelidae					
Eira barbara (Linnaeus, 1758)	Tayra	Ct, Fp	PT	-	LC
Lontra longicaudis (Olfers, 1818)	Neotropical otter	Fs	-	-	LC
Galictis cuja (Molina, 1782)	Lesser grison	Fp	-	-	LC
Felidae					
Leopardus pardalis (Linnaeus, 1758)	Ocelot	Ct	VU	VU	LC
Puma concolor (Linnaeus, 1771)	Cougar	Ct, Fp, Tm, F	VU	VU	LC
Artiodactyla					
Tayassuidae					
Pecari tajacu (Linneaus, 1758)	Collared peccary	Ct, Fp, Fs	VU	-	LC
Rodentia					
Erethizontidae					
Coendou insidiosus (Lichtenstein, 1818)	Bahia porcupine	H	PT	-	LC
Cuniculidae					
Cuniculus paca (Linnaeus, 1766)	Lowland paca	Ct, Fp, Fs	VU	-	LC
Dasyproctidae					
Dasyprocta leporina (Linnaeus, 1758)	Red-rumped agouti	Ct, Fp, Fs	-	-	LC
Lagomorpha					
Leporidae					
Sylvilagus brasiliensis (Linnaeus, 1758)	Tapiti	Vi	-	-	LC

Even in the wider roads the canopy is closed on most points. Each station received a digital camera trap (Tigrinus 6.0 D or Bushnell Trophicam) for 7.8 ± 1.9 days (mean ± sd) at each month. Traps were active during 24 $h.day^{-1}$ with an interval of 1 min between shots. Each picture was considered a record of occurrence; for a same species independent records where those who had at least an hour of interval among each other (Di Bitetti et al. 2006, Tobler et al. 2008, Goulart et al. 2009, Espartosa et al. 2011).

Sign surveys were carried out at the same trails and roads were the camera traps were located. Each trail was surveyed once a month, during the day, with a constant velocity of 1.5 Km/h. Recent species signs such as footprints, burrows, feeding signs, carcass, territorial marks (scratch or stripped trees),

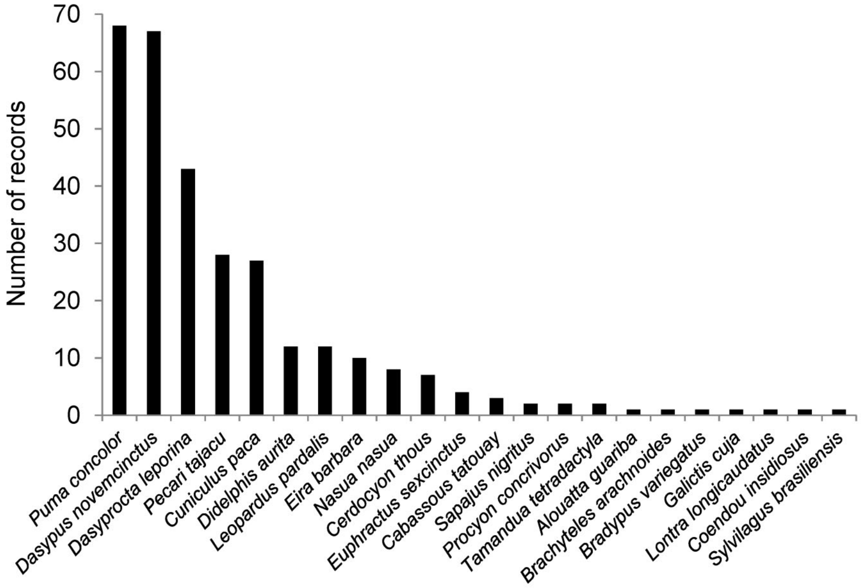


Figure 1 Number of independent records for the 22 species of medium and large-sized mammals recorded by camera traps, sign surveys and visual encounters at the Reserva Ecológica de Guapiaçú (REGUA), Cachoeiras Macacu, Rio de Janeiro.

vocalization and feces, were identified and georeferenced with a GPS. Footprints were identified based on authors knowledge and field guides (Emmons 1997, Becker and Dalponte 1999, Borges and Tomás 2008). For a same species records obtained within a same sample event were considered independent only when spaced at least 500 m among them.

Considering the independence of visual encounters, different observations of individuals or social groups of a same species in a given trail in the same day were considered as a unique record.

3. *Data analyses*

Sampling effort was evaluated using a species accumulation curve (Magurran 2004) with 1000 simulations; the curve was based on sampling events, considering each month as a sample. Expected richness was obtained through the nonparametric estimator Jackknife 2. This estimator is suitable for the analysis of communities with low evenness among species (Brose et al. 2003), which is the case of the studied group, and had a good performance in a study carried out with these mammals in the Amazon (Tobler et al. 2008). All the analyses were carried out using EstimateS 8.2.2 (Colwell 2006).

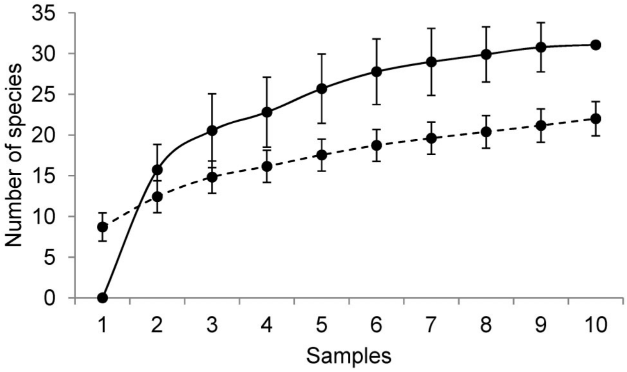


Figure 2 Species accumulation curve (dashed line) and expected richness (solid line) - obtained from the nonparametric estimator Jackknife 2 - to medium and large-sized mammals of the Reserva Ecológica de Guapiaçú, Cachoeiras de Macacu, RJ. Data obtained by camera traps, sign surveys and visual encounters. The bars indicate the standard deviation of each mean value.

For each species we recorded the degree of threat using Bergallo et al. (2000) for the state of Rio de Janeiro, Chiarello et al. (2008) for the Brazil and the International Union for Conservation of Nature (IUCN 2012) for globally threatened species.

Results

Considering all the methods used and an effort of 1589 cameras-days and 120 km of transects, we obtained 302 independent records from 22 species, belonging to eight orders and 15 families of medium and large-sized mammals (Table 1). Twelve species were detected using camera traps, 16 with the sign surveys and five were visual encounters. Only one species (*Nasua nasua*) was detected by the three methods. Camera traps and transects recorded 10 species in common. Two species were exclusively detected by camera traps, six in the sign surveys and four by visual encounters (Table 1).

Among the species recorded, eight were to order Carnivora (36.4%), three Cingulata (13.6%), Primates (13.6%) and Rodentia (13.6%), and two were Pilosa (9.1%). One species of family Didelphidae (4.5%), Tayassuidae (4.5%) and Leporidae (4.5%) were also registered (Table 1). Besides that, other two species of native mammals were observed; the brown-four-eyed-opossum *Metachirus nudicaudatus* (Desmarest, 1817) and the Ingram's squirrel *Guerlinguetus ingrami* (Thomas, 1906). In addition, the exotics *Callitrix jacchus* (Linnaeus, 1758) and *Canis lupus familiaris* (Linnaeus, 1758) (two individuals) were also recorded.

Considering all the types of records, the species with the higher number of observations were the cougar *Puma concolor* and the nine-banded-armadillo *Dasypus novemcinctus* (Figure 1).

Table 2 Inventories of medium and large-sized mammals in dense Atlantic Rainforest and their respective areas of study, richness, sampling efforts and monitoring methods. LT = linear transect; VI = visualization; SS = sign surveys; CT = camera traps; IN = interviews; SP = sand plot.

State and Local	Area (ha)	Richness	Sampling effort (months)	Methods	Reference
Rio de Janeiro Reserva Ecológica de Guapiaçú (REGUA)	7300	22	10	CT SS VI LT	This study
Alagoas Usina Serra Grande	8000	22	6	VI SS CT IN	Fernandes 2003
Rio de Janeiro Parque Nacional da Serra dos Órgãos	11800	34	7	LT VI SS CT IN	Cunha 2004
Espírito Santo Anchieta	390	11	24	VI	Passamani et al. 2005
São Paulo Reserva Florestal Morro Grande	10870	14	5	SS SP LT	Negrão & Valladares-Pádua 2006
Rio de Janeiro Parque Estadual do Desengano	22400	22	1	VI SS CT	Modesto et al. 2008b
Santa Catarina Reserva Ecológica Caraguatá	4200	16	17	CT	Goulart et al. 2009
Santa Catarina Parque Estadual da Serra do Tabuleiro	5000	13	17	CT	Goulart et al. 2009
Rio de Janeiro Parque Nacional da Serra da Bocaina	104000	18	2	LT CT	Delciellos et al. 2012
Rio de Janeiro Parque Estadual da Ilha Grande	14572	11	15	CT	Lessa, 2011

Seven species (31.8%) have only one record (*Alouatta guariba*, *Brachyteles arachnoides*, *Bradypus variegatus*, *Galictis cuja*, *Lontra longicaudatus*, *Coendou insidiosus* and *Sylvilagus brasiliensis*), obtained through the signs surveys or visual encounters.

The accumulation curve did not reaches an asymptote and the expected richness was 31.1 species (Figure 2).

Considering the degree of threat, five species (22.7%) were present in the list of the threatened fauna of the Rio de Janeiro state, while three (13.6%) are in risk at Brazil and one (4.5%), the Southern muriqui *Brachyteles arachnoides*, is globally threaten (Table 1).

The species richness found at REGUA was comparable to the registered in other areas harboring the Rain Atlantic Forest, including higher protected areas (Table 2).

Discussion

Besides the 22 species found in this study, the jaguarundi *Puma yagouaroundi* (É. Geoffroy, 1803), the margay *Leopardus wiedii* (Schinz, 1821) and the capybara *Hydrochoerus hydrochaeris* (Linnaeus, 1766) were also previously detected at REGUA (Pimentel 2005, Rocha et al. 2005). Summing these three species, the area harbor 39% of the Atlantic Forest medium and large-sized mammal species and 75.7% of those from Rio de Janeiro state (Rocha et al. 2004).

A failure in the detection of the two feline species mentioned above could be due to two non-excludent reasons, (1) their naturally low population densities (Lyra-Jorge et al. 2008, Almeida et al. 2013), and (2) the high frequency of *Puma concolor* and *Leopardus pardalis* in several trails, whose presence has been negatively related to the occurrence of these species (Oliveira et al. 2010, Oliveira 2011, Oliveira-Santos et al. 2012). In addition, for *L. wiedii*, trail characteristics - which were more open, wide and managed than the preferred by the species – could have contributed to this result (Goulart et al. 2009). For *H. hydrochaeris* the absence of favorable habitats in the trails - as swampy areas - could explain why this species was not detected in this study (Ferraz et al. 2006).

The existence of non-detected species and the higher number of species registered only once (31.8%) can be responsible to the non-establishment of the rarefaction curve. These data suggest that these species are rare in the studied area. However, this result could also be due to sampling problems. The use of camera-traps outside the trails and in other habitats (in the canopy, closer to water courses, for example), and night transects in future studies are necessary to clarify this (Harmsen et al. 2010, Melo et al. 2012).

The observed richness (22 species) represented 70.8% of the estimated one (31.1 species). As the number of species known to the region is 33 (Rocha et al 2004), the use of Jacknife 2 seems to have been adequate to the studied community, corroborating the results found by other authors (Brose et al. 2003, Tobler et al. 2008).

The species with the higher number of records were *P. concolor* and *D. novemcinctus*. Both species are habitat generalists, occurring both in preserved and anthropized areas (Dickson et al. 2005, Andrade-Nuñez & Aide 2010, Mazzoli 2010, Canale et al. 2012). The large home range sizes of *P. concolor* (100–220 Km^2, Mazzoli 2010) and the difficulty of differentiate individuals (Oliveira-Santos et al. 2010), however, can have contributed to their elevated number of records. Considering *D. novemcinctus*, the foraging behavior can have inflate the number of records as a single individual moves long distances leaving several marks (Neck 1976, Mcbee & Backer 1982).

Habitat restricted species, that occur preferentially in preserved areas were well represented at REGUA. Among them are *Pecari tajacu*, *Cuniculus paca* and *L. pardalis* that are classified as vulnerable in the state and national red lists (Table 1). The first two are game species (Peres 1996, Cullen et al. 2001) that can maintain viable populations in the area due to the protection actions carried out by the owners and their employees.

Didelphis aurita, which is considered an indicator of disturbed habitats (Bergallo 1994), was less abundant here than in others studies (e.g. Gaspar 2005, Negrão & Valladares-Pádua 2006, Srbek-Araújo & Chiarello 2007). This result enhances the degree of forest conservation, as this species is generally more abundant is areas where top predators are absent (Fonseca & Robinson 1990).

The absence of the deer species (*Mazama* Rafinesque, 1817), white-lipped peccary (*Tayassu pecari* Link, 1795) and tapir (*Tapirus terrestris* Linnaeus, 1758) were the most apparent; in the last 10 years species were not detected in the area. For *T. pecari* there is an anecdotal observation by an employee recorded over 50 years, when according to him whole herds were hunted. For *Mazama* spp. and *T. terrestris*, the last records where those carried out by naturalists that visited the region in the century XIX (Miller et al. 2006). Habitat fragmentation and principally over hunting must have contributed to the local extinction of these species at the REGUA, as found in other Atlantic Forest regions (e.g. Chiarello 2000, Canale et al. 2013).

The fact that the number of medium and large-sized mammals species found at REGUA was comparable inclusive to large protected areas could be due to the formed continuum with the Parque Estadual dos Três Picos. However, the management and protection of the area by its owners is fundamental to maintain the populations of these animals. The creation of private reserves has been an important strategy for biodiversity conservation in Brazil (e.g. Schiavetti et al. 2010, Negrões et al. 2011). At REGUA, as in other private reserves, the surveillance is done generally by locals, mostly former hunters, who know the region well and are able to keep gatherers and hunters away. The number of species, including cinegetic and endangered ones makes REGUA important to the conservation of medium and large-sized mammals in the state and a stocking area to future recolonizations of regions in their surroundings.

Acknowledgements

We thank to Nicholas and Rachel Locke for authorizing, incentive and supporting this research in your beautiful property. Many thanks for Mr. Levi and Mr. Messias to assist in the fieldwork and Dr. Jorge Bizarro for logistical support. Thanks to Dr. Nílton Cáceres, Dra. Helena Bergallo, Dra. Flávia Rocha, Dr. Diogo Loretto and anonymous reviewers for comments on previous versions of this work. Thanks to the team of Laboratório de Ecologia e Conservação de Florestas (LECF) for fellowship. To the Programa de Pós-Graduação em Ciências Ambientais e Florestais for supporting to the Master's Dissertation of the first author, which this work is part.

References

ALMEIDA, L.B., QUEIROLO, D., BEISIEGEL, B.M. & OLIVEIRA, T.G. 2013. Avaliação do estado de conservação do gato-mourisco *Puma yagouaroundi* (É. Geoffroy Saint-Hilaire, 1083) no Brasil. Biodiversidade Brasileira, 3(1), 99–106.

ANDRADE-NÚÑEZ, M.J. & AIDE, T.M. 2010. Effects of habitat and landscape characteristics on medium and large species richness and composition in northern Uruguay. Zoologia. 27(6): 909–917, http://dx.doi.org/10.1590/S1984-46702010000600012

ASQUITH, N.M., WRIGHT, S.J. & CLAUSS, M.J. 1997. Does mammal community composition control recruitment in neotropical forests? Evidence from Panama. Ecology. 78(3): 941–946.

ASQUITH, N.M., TERBORGH, J., ARNOLD, A.E. & RIVEROS, C.M. 1999. The fruits the agouti ate: *Hymenaea courbaril* seed fate when its disperser is absent. J. Trop. Ecol. 15(2): 229–235, http://dx.doi.org/10.1017/S0266467499000772

AZEVEDO, A.D. 2012. Estoque de carbono em áreas de recuperação da mata atlântica com diferentes idades na bacia do rio Guapiaçu, Cachoeiras de Macacu, Rio de Janeiro. Dissertação de Mestrado. Instituto de Florestas, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ.

BANI, L., BAIETTO, M., BOTTONI, L. & MASSA, R. 2002. The use of focal species in designing habitat network for a lowland area of Lombardy, Italy. Conserv. Biol. 16(3): 826–831, http://dx.doi.org/10.1046/j.1523-1739.2002.01082.x

BAREA-AZCÓN, J.M., VIRGÓS, E., BALLESTEROS-DUPERÓN, E.B., MOLEÓN, M. & CHIROSA, M. 2007. Surveying carnivores at large spatial scales: a comparison of four broad-applied methods. Biodivers. Conserv. 5: 387–404.

BECK, H., SNODGRASS, J.W. & THEBPANYA, P. 2013. Long-term exclosure of large terrestrial vertebrates: Implications of defaunation for seedling demographics in the Amazon rainforest. Biol. Conserv. 163: 115–121, http://dx.doi.org/10.1016/j.biocon.2013.03.012

BECKER, M. & DALPONTE, J.C. 1999. Rastros de mamíferos silvestres brasileiros: um guia de campo. Brasília. 2^a edição. Editora da Universidade de Brasília e Edições IBAMA, Brasilia.

BERGALLO, H.G. 1994. Ecology of a small mammal community in an Atlantic Forest area in southeastern Brazil. Stud. Neotrop. Fauna E. 29(4): 197–217, http://dx.doi.org/10.1080/01650529409360932

BERGALLO, H.G, ROCHA, C.F.D., ALVES, M.A.S. & SLUYS, M.V. 2000. A fauna ameaçada de extinção do estado do Rio de Janeiro. Rio de Janeiro: Editora da Universidade do Estado do Rio de Janeiro, Rio de Janeiro.

BODDICKER, M., RODRIGUEZ, J.J. & AMANZO, J. 2002. Indices for assessment and monitoring of large mammals within an adaptive management framework. Environ. Monit. Assess. 76: 105–123, http://dx.doi.org/10.1023/A:1015225022101

BORGES, P.A.L. & TOMÁS, W.M. 2008. Guia de rastros e outros vestígios de mamíferos do Pantanal. 1 ed. Embrapa Pantanal, Corumbá.

BROSE, U., MARTINEZ, N.D. & WILLIAMS, R.J. 2003. Estimating species richness: sensitivity to sample coverage and insensitivity to spatial patterns. Ecology, 84(9): 2364–2377.

CANALE, G.R., PERES, C.A., GUIDORIZZI, C.E., GATTO, C.A.F. & KIERULFF, C.M. 2012. Pervasive defaunation of forest remnants in a tropical biodiversity hotspots. PLOS One, 7(8): 1–9.

CARDILLO, M. & BROMHAN, L. 2001. Body size and risk of extinction in Australian mammals. Conserv. Biol. 15(5): 1435–1440, http://dx.doi.org/10.1046/j.1523-1739.2001.00286.x

CARVALHO, W.D., ADANIA, C.H. & ESBÉRARD, C.E.L. 2013. Comparison of two mammalian surveys made with camera traps in southeastern Brazil, focusing the abundance of wild mammals and domestic dogs. Braz. J. Biol. 73(1): 29–36, http://dx.doi.org/10.1590/S1519-69842013000100005

CHIARELLO, A.G. 2000. Influência da caça ilegal sobre mamíferos e aves das matas de tabuleiro do norte do estado do Espirito Santo. Bol. Mus. Biol. Mello Leitão 11/12: 229–247.

CHIARELLO, A.G., AGUIAR, L.M.S., CERQUEIRA, R., MELO, F.R., RODRIGUES, F.H.G. & SILVA, V.M.F. 2008. Mamíferos ameaçados de extinção no Brasil. In: MACHADO, A.B.M., DRUMMOND, G.M. & PAGLIA, A.P. (ed.) Livro vermelho da fauna brasileira ameaçada de extinção. MMA/Fundação Biodiversitas. 1420p.

COLWELL, R.K. 2006. EstimateS: statistical estimation of species richness and shared species from samples. Version 8. Persistent URL <purl.oclc.org/estimates>. (último acesso em 30/11/2012).

CROUZEILLES, R., LORINI, M.L. & GRELLE, C.E.V. 2010. Deslocamento na matriz para espécies da Mata Atlântica e a dificuldade da construção de perfis ecológicos. Oecol. Aust. 14(4): 872–900, http://dx.doi.org/10.4257/oeco.2010.1404.06

CULLEN, L. JR, BODMER, E.R. & VALLADARES-PÁDUA, C. 2001. Ecological consequences of hunting in Atlantic forest patches, São Paulo, Brazil. Oryx, 35(2): 137–144.

CULLEN, L. JR, ABREU, K.C., SANA, D.A. & NAVA, A.F.D. 2005. Jaguars as landscape detectives for the upper Parana River Corridor, Brazil. Natureza & Conservação, 3: 147–161.

CUNHA, A.A. 2004. Alterações na composição da comunidade e o status de conservação dos mamíferos de médio e grande porte da Serra dos Órgãos. In Ciência e conservação na Serra dos Órgãos (C. Cronemberger & E.B. Viveiros de Castro, orgs) IBAMA, Brasília, p. 211–224.

DEAN, W. 1996. A ferro e fogo: a história e a devastação da Mata Atlântica brasileira. Companhia das Letras, São Paulo.

DELCIELLOS, A.C., NOVAES, R.L.M., LOGUERCIO, M.F.C., GEISE, L., SANTORI, R.T., SOUZA, R.F., PAPI, B.S., RAICES, D., VIEIRA, N.R., FELIX, S., DETOGNE, N., SILVA, C.C.S., BERGALLO, H.G. & ROCHA-BARBOSA, O. 2012. Mammals of Serra da Bocaina National Park, state of Rio de Janeiro, southeastern Brazil. Check List, 8(4): 675–692.

DI BITETTI M.S, PAVIOLO, A. & DE ÂNGELO, C. 2006. Density, habitat use and activity patterns of ocelots (*Leopardus pardalis*) in the Atlantic Forest of Misiones, Argentina. J. Zool. 270: 153–163.

DICKSON, B.G., JENNESS, J.S. & BEIER, P. 2005. Influence of vegetation, topography, and roads on cougar movement in southern California. J. Wildlife Manage. 69(1): 264–276, http://dx.doi.org/10.2193/0022-541X(2005)069<0264:IOVTAR>2.0.CO;2

DIRZO, R. & MIRANDA, A. 1990. Contemporary defaunation and forest structure, function and diversity – A sequel to John Terborgh, Conserv. Biol. 4(4): 444–447.

DONATTI, C.I., GUIMARÃES JR, P.R & GALETTI, M. 2009. Seed dispersal and predation in the endemic Atlantic Forest palm *Astrocaryum aculeatissimum* across a gradient of seed disperser abundance. Ecol. Res. 24: 1187–1195, http://dx.doi.org/10.1007/s11284-009-0601-x

EMMONS, L.H. 1997. Neotropical rainforest mammals: a field guide. 2nded. Chicago University of Chicago Press.

ESPARTOSA, K.D., PINOTTI, B.T. & PARDINI, R. 2011. Performance of camera trapping and track counts for surveying large mammals in rainforest remnants. Biodivers. Conserv. 20: 2815–2829, http://dx.doi.org/10.1007/s10531-011-0110-4

EWERS, R.M. & DIDHAM, R.K. 2006. Confounding factors in the detection of species responses to habitat fragmentation. Biol. Rev. 81: 117–142, http://dx.doi.org/10.1017/S1464793105006949

FERNANDES, A.C.A. 2003. Censo de mamíferos em alguns fragmentos de Floresta Atlântica no nordeste do Brasil. Dissertação de Mestrado. Universidade Federal de Pernambuco, Recife, Pernambuco.

FERRAZ, K.M.P.M.B., FERRAZ, S.F.B., MOREIRA, J.R., COUTO, H.T.Z. & VERDADE, L.M. 2006. Capybara (*Hydrochoerus hydrochaeris*) distribution in agroecosystems: a cross scale habitat analysis. J. Biogeogr. 34: 223–230, http://dx.doi.org/10.1111/j.1365-2699.2006.01568.x

FONSECA, G.A.B. & ROBINSON, J.G. 1990. Forest size and structure: competitive and predatory effects on small mammal communities. Biol. Conserv. 53(4): 265–294, http://dx.doi.org/10.1016/0006-3207(90)90097-9

FUNDAÇÃO SOS MATA ATLÂNTICA & INPE. 2011. Atlas dos remanescentes florestais da Mata Atlântica período 2008–2010. Fundação SOS Mata Atlântica, São Paulo, e INPE, , São José dos Campos, , Brasil. 122p.

GAIDET-DRAPIER, N., FRITZ, H., BOURGAREL, M., RENAUD, P.C., POILECOT, P. CHARDONNET, P., COID, C., POULET, D. & LE BEL, S.. 2006. Cost and efficiency of large mammal census techniques: comparison of methods for a participatory approach in a communal area, Zimbabwe. Biodivers. Conserv. 15: 735–754, http://dx.doi.org/10.1007/s10531-004-1063-7

GALETTI, M., DONATTI, C.I., PIRES, A.S., GUIMARÃES JR, P.R & JORDANO, P. 2006. Seed survival and dispersal of endemic Atlantic Forest palm: the combined effects of defaunation and forest fragmentation. Bot. J. Linn. Soc. 151: 141–149, http://dx.doi.org/10.1111/j.1095-8339.2006.00529.x

GASPAR, D.A. 2005. Comunidade de mamíferos não voadores de um fragmento de Floresta Atlântica semidecídua do município de Campinas, SP. Tese de Doutorado. Universidade Estadual de Campinas, Campinas, São Paulo.

GOULART, F.V.B., CÁCERES, N., GRAIPEL, M.E., TORTATO, M.A., GHIZONI JR, I.R & OLIVEIRA-SANTOS, L.G.R. 2009. Habitat selection by large mammals in a southern Brazilian Atlantic Forest. Mamm. Biol. 74: 182–190.

HARMSEN, B.J., FOSTER, R.J., SILVER, S.C., OSTRO, L.E.T. & DONCASTER, C.P. 2010. Differential use of trails by forest mammals and implications for camera-trap studies: a case study from Belize. Biotropica, 42(1): 126–133, http://dx.doi.org/10.1111/j.1744-7429.2009.00544.x

HARRISON, R.D., TAN, S., PLOTKIN, J.B., SLIK, F, DETTO, M., BRENES, T., ITOH, A. & DAVIES, S.J. 2013. Consequences of defaunation for a tropical tree community. Ecol. Lett. 16: 687–694, http://dx.doi.org/10.1111/ele.12102

INTITUTO ESTADUAL DO AMBIENTE (INEA). 2013. Parque Estadual dos Três Picos PETP. http://www.inea.rj.gov.br/unidades/pqtrespicos.asp. (último acesso em 01/2013).

INTERNATIONAL UNION FOR CONCERVATION OF NATURE (IUCN). 2012. IUCN Red List of Threatened Species. Version 2012.2. http://www.iucnredlist.org. (último acesso em 01/2013).

JORGE, M.L.S.P., GALETTI, M., RIBEIRO, M.C. & FERRAZ, K.M.P.M.B. 2013. Mammal defaunation as surrogate of trophic cascades in a biodiversity hotspot. Biol. Conserv. 163: 49–57, http://dx.doi.org/10.1016/j.biocon.2013.04.018

KARANTH, K.U. & NICHOLS, J.D. 1998. Estimation of tiger densities in India using photographic captures and recaptures. Ecology, 79(8): 2852–2862.

KEUROGHLIAN, A. & EATON, D.P. 2009. Removal of palm fruits and ecosystem engineering in palm stands by white-lipped peccaries (*Tayassu pecari*) and other frugivores in an isolated Atlantic Forest fragment. Biodivers Conserv, 18:1733–1750.

KINNAIRD, M.F., SANDERSON, E.W., O'BIEN, T.G., WIBISONO, H.T. & WOOLMER, G. 2003. Deforestation trends in a tropical landscape and implications for endangered large mammals. Conserv. Biol. 17(1): 245–257, http://dx.doi.org/10.1046/j.1523-1739.2003.02040.x

KUPREWICZ, E.K. 2013. Mammal abundances and seed traits control the seed dispersal and predation roles of terrestrial mammals in a Costa Rican Forest. Biotropica, 45(3): 333–342.

KURTEN, E.L. 2013. Cascading effects of contemporaneous defaunation on tropical forest communities. Biol. Conserv. 163: 22–32, http://dx.doi.org/10.1016/j.biocon.2013.04.025

LESSA, I.C.M. 2012. Os mamíferos de médio e grande porte e suas respostas à fatores ambientais, físicos e antrópicos, sobre diferentes perspectivas, no Parque Estadual da Ilha Grande – RJ. Dissertação de Mestrado. Universidade Estadual do Rio de Janeiro. Rio de Janeiro.

LIMA, S.L. & DILL, L.M. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Can. J. Zoolog. 68: 619–640, http://dx.doi.org/10.1139/z90-092

LYRA-JORGE, M.C., CIOCHETI, G. & PIVELLO, V.R. 2008. Carnivore mammals in a fragmented landscape in northeast of São Paulo state, Brazil. Biodivers. Conserv. 17: 1573–1580, http://dx.doi.org/10.1007/s10531-008-9366-8

MAGURRAN, A.E. 2004. Measuring biological diversity. 1 ed. >Blackwell Publishing, Oxford, UK.

MAZZOLLI, M. 2010. Mosaics of exotic forest plantations and native forest as habitat of pumas. Environ. Manage. 46: 237–253, http://dx.doi.org/10.1007/s00267-010-9528-9

MCBEE, K. & BACKER, R.J. 1982. *Dasypus novemcinctus*. Mammalian Species, 142: 1–9.

MELO, G.L., SPONCHIADO, J. & CÁCERES, N.C. 2012. Use of camera-traps in natural trails and shelters for the mammalian survey in the Atlantic Forest. Iheringia Ser. Zool. 102(1): 88–94.

MILLER, D., WARREN, R., MILLER, I.M. & SEEHAWER, H. 2006. Serra dos Órgãos: sua história e suas orquídeas. 1 ed. Nova Friburgo, RJ, Editora Scart.

MILLER, B., DOLGEBY, B., FOREMAN, D., DEL RÍO, C.M., NOSS, R., PHILLIPS, M., READING, R., SOULÉ, M.E., TERBORGH, J. & WILLCOX, L. 2001. The importance of large carnivores to healthy ecosystems. Endangered Species Update. 18(5): 202–210.

MITTERMEIER, R.A. 1986. Primate conservation priorities in the Neotropical region. In Primates: The road to self-sustaining populations. (K. Benirschke ed). Springer-Verlag, New York, p. 221–240.

MODESTO, T.C., PESSÔA, F.S., JORDÃO-NOGUEIRA, T., ENRICI, M.C., COSTA, L.M., ATTIAS, N., ALMEIDA, J., RAICES, D.S.L., ALBUQUERQUE, H.G., PEREIRA, B.C., ESBERÁRD, C.E.L. & BERGALLO, H.G. 2008a. Mammals, Serra da Concórdia, state of Rio de Janeiro, Brazil. Check List, 4(3): 341–348.

MODESTO, T.C., PESSÔA, F.S., ENRICI, M.C., ATTIAS, N., JORDÃO-NOGUEIRA, T., COSTA, L.M., ALMEIDA, J., ALBUQUERQUE, H.G., & BERGALLO, H.G. 2008b. Mamíferos do Parque Estadual do Desengano, Rio de Janeiro, Brasil. Biota Neotrop. 8(4): 153–159, http://dx.doi.org/10.1590/S1676-06032008000400015

MYERS, N., MITTERMEIER, R.A., MITTERMEIER, C.G., DA FONSECA, G.A.B & KENT, J. 2000. Biodiversity hotspots for conservation priorities. Nature, 403(24): 853–858.

NECK, R.W. 1976. Possible adaptive significance of certain aspects of armadillo foraging behavior. Southwestern Naturalist, 21: 242–243, http://dx.doi.org/10.2307/3669965

NEGRÃO, M.F.F. & VALLADARES-PÁDUA, C. 2006. Registro de mamíferos de maior porte na Reserva Florestal de Morro Grande, São Paulo. Biota Neotrop. 6(2): 1–13, http://dx.doi.org/10.1590/S1676-06032006000200006

NEGRÕES, N., REVILLA, E., FONSECA, C., SOARES, A.M.V.M., JÁCOMO, A.T.A. & SILVEIRA, L. 2011. Private forest reserves can aid in preserving the community of medium and large-sized vertebrates in the Amazon arc of deforestation. Biodivers. Conserv. 20(3): 505–518, http://dx.doi.org/10.1007/s10531-010-9961-3

NORTHON-GRIFFITHS, M. 1978. Counting Animals. No. 1 of a series of Handbooks on techniques currently used in African wildlife Ecology. (J.J.R. Grimsdel ed). African Wildlife Foundation, Nairobi.

NOSS, R.F., QUIGLEY, H.B., HORNOCKER, M.G., MERRILL, T. & PAQUET, P.C. 1996. Conservation biology and carnivore conservation in the Rocky Montains. Conserv. Biol. 10 (4): 949–963, http://dx.doi.org/10.1046/j.1523-1739.1996.10040949.x

OLIVEIRA, T.G., TORTATO, M.A., SILVEIRA, L., KASPER, C.B., MAZIM, F.D., LUCHERINI, M., JÁCOMO, A.T., SOARES, J.B.G., ROSANE, V.M. & SUNQUIST, M. 2010. Ocelot ecology and its effects on the small-felid guild in the lowland neotropics. p. 559–580. *In:* Macdonald, D.W. & Loveridge, A.J. (eds.). Biology and conservation of wild felids. Oxford University Press.

OLIVEIRA, T.G. 2011. Ecologia e conservação de pequenos felinos no Brasil e suas implicações para o manejo. Tese de Doutorado, Universidade Federal de Minas Gerais, Minas Gerais, MG.

OLIVEIRA-FILHO, A.T. & FONTES, M.A.L. 2000 Patterns of floristic differentiation among Atlantic Forests in southeastern Brazil and the influence of climate. Biotropica, 32(4b): 793–810.

OLIVEIRA-SANTOS, L.G.R., ZUCCO, C.A., ANTUNES, P.C., & CRAWSHAW JR, P.G. 2010. Is it possible to individually identify mammals with no natural markings using camera-traps? A controlled case-study with lowland tapirs. Mamm. Biol. 75(4): 375–378.

OLIVEIRA-SANTOS, L.G.R., GRAIPEL, M.E., TORTATO, M.A., ZUCCO, C.A., CÁCERES, N.C. & GOULART, F.V.B. 2012. Abundance changes and activity flexibility of the oncilla, *Leopardus tigrinus* (Carnivora: Felidae), appear to reflect avoidance of conflict. Zoologia, 29(2): 115–120.

PAGLIA, A.P., FONSECA, G.A.B da, RYLANDS, A.B., HERRMANN, G., AGUIAR, L.M.S., CHIARELLO, A.G., LEITE, Y.L.R., COSTA, L.P., SICILIANO, S., KIERULFF, M.C.M., MENDES, S.L., TAVARES, V.da C., MITTERMEIER, R.A. & PATTON, J.L. 2012. Lista anotada dos mamíferos do Brasil. 2ª edição. Occasional Papers in Conservation Biology. No. 6. Conservation International. 76p.

PASSAMANI, M., DALMASCHIO, J. & LOPES, S.A. 2005. Mamíferos não-voadores em áreas com predomínio de Mata Atlântica da Samarco Mineração S.A., município de Anchieta, Espírito Santo. Biotemas, 18 (1): 135–149.

PEEL, M.C., FINLAYSON, B.L. & MCMAHON, T.A. 2007. Updated world map of the Koppen-Geiger climate classification. Hydrol. Earth Syst. Sc. 11: 1633–1644, http://dx.doi.org/10.5194/hess-11-1633-2007

PERES, C.A. 1996. Population status of white-lipped *Tayassu pecari* and collared peccaries *T. tajacu* in hunted and unhunted Amazonian forests. Biol. Conserv. 77: 115–123, http://dx.doi.org/10.1016/0006-3207(96)00010-9

PERES, C.A. 2000. Effects of subsistence hunting on vertebrate community structure in Amazonian Forests. Conserv. Biol. 14(1): 240–253, http://dx.doi.org/10.1046/j.1523-1739.2000.98485.x

PIMENTEL, L.M.S. 2005. Inventário de mamíferos selvagens na Reserva Ecológica de Guapiaçú. Monografia em Medicina Veterinária. Fundação Educacional Serra dos Órgãos, Teresópolis, Rio de Janeiro, RJ.

REDFORD, K.H. 1992. The empty forest. Bioscience, 42: 412–422.

RIBEIRO, M.C., METZGER, A., MARTENSEN, A.C., PONZONI, F.J. & HIROTA, M.M. 2009. The Brazilian Atlantic Forest: how much is left, and how is the remaining forest distributed? Implications for conservation. Biol. Conserv. 142: 1141–1153, http://dx.doi.org/10.1016/j.biocon.2009.02.021

ROCHA, C.F.D., BERGALLO, H.G., ALVES, M.A.S. & VAN SLUYS, M. 2005. Inventário de anfíbios, répteis, aves e mamíferos da Mata Atlântica da Reserva Ecológica de Guapiaçú, Cachoeiras de Macacu, no Estado do Rio de Janeiro. Instituto Biomas Relatório de Referência.

ROCHA, C.F.D., BERGALLO, H.G., POMBAL-JR, J.P., GEISE, L., VAN-SLUYS, M., FERNANDES, R. & CARAMASCHI, U. 2004. Fauna de anfíbios, répteis e mamíferos do estado do Rio de Janeiro, Sudeste de Brasil. Publi. Avulsas Mus. Nac. 104: 1–24.

SANDERSON, E.W., REDFORD, K.H., VEDDER, A., COPPOLILLO, P.B & WARD, S.E. 2002a. A conceptual model for conservation planning based on landscape species requirements. Landscape. Urban Plan. 58: 41–56, http://dx.doi.org/10.1016/S0169-2046(01)00231-6

SANDERSON, E.W., REDFORD, K.H., CHETKIEWICZ, C.B., MEDELLIN, R.A., RABINOWITZ, A.R., ROBINSON, J.G. & TABER, A.B. 2002b. Planning to save a species: the jaguar as a model. Conserv. Biol. 16(1): 58–72, http://dx.doi.org/10.1046/j.1523-1739.2002.00352.x

SANTOS-FILHO, M. & SILVA, M.N. 2002. Uso de habitats por mamíferos em área de Cerrado do Brasil Central: um estudo com armadilhas fotográficas. Rev. Bras. Zoociênc. 4(1): 45–56.

SCHIAVETTI, A., DE OLIVEIRA, H.T, LINS, A.S & SANTOS, P.S. 2010. Analysis of private natural heritage reserves as a conservation strategy for the biodiversity of the cocoa region of the southern state of Bahia, Brazil. Rev. Árvore. 34(4): 699–711, http://dx.doi.org/10.1590/S0100-67622010000400015

SILVA, L.D. & PASSAMANI, M. 2009. Mamíferos de médio e grande porte em fragmentos florestais no município de Lavras, MG. Rev. Bras. Zoociências, 11(2): 137–144.

SINCLAIR, A.R.E., MDUMA, S. & BRASHARES, J.S. 2003. Patterns of predation in a diverse predator-prey system. Nature. 425: 288–290, http://dx.doi.org/10.1038/nature01934

SOISALO, M.K. & CAVALCANTI, S.M.C. 2006 Estimating the density of a jaguar population in the Brazilian Pantanal using camera-traps and capture–recapture sampling in combination with GPS radio-telemetry. Biol. Conserv. 129: 487–496, http://dx.doi.org/10.1016/j.biocon.2005.11.023

SPÍNOLA, C.M. 2008. Influência dos padrões estruturais da paisagem na comunidade de mamíferos terrestres de médio e grande porte na região do Vale do Ribeira, Estado de São Paulo. Dissertação de Mestrado. Universidade de São Paulo.

SRBEK-ARAÚJO, A.C. & CHIARELLO, A.G. 2007. Armadilhas fotográficas na amostragem de mamíferos: considerações metodológicas e comparação de equipamentos. Rev. Bras. Zool. 24(3): 647–656, http://dx.doi.org/10.1590/S0101-81752007000300016

STONER, K.E., RIBA-HERNÁNDEZ, P., VULINEC, K. & LAMBERT, J.E. 2007. The role of mammals in creating and modifying seedshadows in tropical forest and some possible consequences of their elimination. Biotropica, 39(3): 316–327.

TERBORGH, J. 1988. The big things that run the world-a sequel to E. O. Wilson. Conserv. Biol. 2(4): 402–403, http://dx.doi.org/10.1111/j.1523-1739.1988.tb00207.x

TOBLER, M.W., CARRILLO-PERCASTEGUI, S.E., LEITE PITMAN, R, MARES, R & POWELL, G. 2008. An evaluation of camera traps for inventorying large- and medium-sized terrestrial rainforest mammals. Anim. Conserv. 11: 169–178, http://dx.doi.org/10.1111/j.1469-1795.2008.00169.x

TRAVASSOS, L. 2011. Impacto da sobrecaça em populações de mamíferos e suas interações ecológicas nas florestas neotropicais. Oecol. Aust. 15(2): 380–411, http://dx.doi.org/10.4257/oeco.2011.1502.14

Received 13/05/2014
Revised 22/05/2014
Accepted 01/09/2014

Biota Neotropica 14(3): e20130069, 2014
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
article

Reproduction of the silver John dory *Zenopsis conchifer* (Actinopterygii: Zeiformes) based on virgin stock condition

André Martins Vaz-dos-Santos[1,4], *Carmen Lúcia Del Bianco Rossi-Wongtschowski*[2], *Renato Massaaki Honji*[3] & *Denis Bannwart*[2]

[1] *Laboratório de Esclerocronologia, Departamento de Biodiversidade, Universidade Federal do Paraná, Rua Pioneiro, 2153, CEP 85950-000, Palotina, PR, Brazil.*
[2] *Instituto Oceanográfico, Universidade de São Paulo, São Paulo, SP, Brazil.*
[3] *Instituto de Biociências, Universidade de São Paulo, São Paulo, SP, Brazil.*
[4] *Corresponding author: André Martins Vaz-dos-Santos, e-mail: andrevaz@ufpr.br*

VAZ-DOS-SANTOS, A.M., ROSSI-WONGTSCHOWSKI, C.L.D.B., HONJI, R.M., BANNWART, D. **Reproduction of the silver John dory *Zenopsis conchifer* (Actinopterygii: Zeiformes) based on virgin stock condition.** Biota Neotropica. 14(3): e20130069. http://dx.doi.org/10.1590/1676-06032014006913

Abstract: This study describes the reproduction of the silver John dory, *Zenopsis conchifer*, based on data collected before its fishery exploitation in the southwestern Atlantic, Brazil, in the early 2000s. Biological material was collected through bottom-trawl surveys in 2001 and 2002 from depths of 100 to 600 m. A total of 1,056 individuals ranging from 57 to 504 mm Ls were analyzed. A sex ratio analysis showed that males were predominant among the shorter fishes (< 240 mm Ls) and females among the longer ones (> 320 mm Ls). Between 240 mm and 319 mm Ls sex-ratio was 1:1. The silver John dory is a multiple spawner. Spawning is protracted and more intensive during August and November, when both females and males in spawning capable actively spawning and in regression/regeneration stages were predominant. In this period average values of gonadosomatic index were also higher. For both sexes, the first maturation occurs at 220 mm Ls and at 240 mm Ls all fish can be considered adult. The recruitment apparently takes place from February to May. The length-weight relationships fitted to all specimens and for both sexes yielded *b* coefficients of ~2.7. The virgin stock of *Z. conchifer* here characterized, may serve a basis for future studies on the effects of fisheries on the reproduction of the species.
Keywords: *spawning period and area, length at first maturation, sex ratio, gonadosomatic index.*

VAZ-DOS-SANTOS, A.M., ROSSI-WONGTSCHOWSKI, C.L.D.B., HONJI, R.M., BANNWART, D. **Reprodução do peixe galo-de-profundidade *Zenopsis conchifer* (Actinopterygii, Zeiformes) baseada em um estoque virgem.** Biota Neotropica. 14(3): e20130069. http://dx.doi.org/10.1590/1676-06032014006913

Resumo: O presente estudo descreve a reprodução do peixe galo-de-profundidade, *Zenopsis conchifer,* a partir de dados pretéritos a sua explotação pesqueira no Atlântico Sudoeste, Brasil, iniciada nos anos 2000. As coletas foram realizadas durante cruzeiros de prospecção pesqueira com arrasto-de-fundo em 2001 e 2002, em profundidades entre 100 e 600 m. Um total de 1056 exemplares (comprimento padrão entre 57 mm e 504 mm) foi examinado. A proporção sexual mostrou que machos predominam em comprimentos inferiores a 240 mm, que entre 240 mm e 319 mm a proporção é de 1:1 e que fêmeas predominam a partir de 320 mm. O peixe galo-de-profundidade apresenta desova múltipla, mais intensa entre agosto e novembro, quando predominam fêmeas e machos desovantes ativos e em regressão/regeneração. Neste período foram registrados os maiores valores do índice gonadossomático. Para ambos os sexos a primeira maturação se dá aos 220 mm de comprimento padrão e com 240 mm os indivíduos são considerados adultos. O recrutamento de jovens para o estoque adulto foi mais intenso entre fevereiro e maio. Os valores dos coeficientes *b* das relações comprimento peso ajustadas para todos os peixes e por sexo ficaram em torno de 2,7. As condições do estoque virgem de *Z. conchifer* aqui caracterizadas podem servir como um referencial para futuros estudos sobre os efeitos da pesca na reprodução da espécie.
Palavras-chave: *época e área de desova, comprimento de primeira maturação, proporção sexual, índice gonadossomático.*

Introduction

The silver John dory, *Zenopsis conchifer* (Lowe 1852) (Zeiformes: Zeidae), is a benthopelagic species found in the Indian and Atlantic Oceans. In the Atlantic, this species occurs along the east coast of the Americas from Nova Scotia, Canada to Tierra del Fuego, Argentina (Froese & Pauly 2013). This zeid fish is found along the Brazilian coast (Menezes et al. 2003), with higher biomass (almost 14,000 tons) occurring on the continental shelf and slope of southeastern and southern Brazil (21-34°S) (Haimovici et al. 2008).

The first published data on the silver John dory fishery dates back to the 1990s, when FAO statistics detailed the catches of the United States and Portugal fleets (Sibeni & Calderini 2012). Between 2001 and 2010, the average annual Portuguese catch reached 26 tons, whereas the American catches averaged 46 tons. In Brazil, the species became a target of the trawl fleet in 2001, and the catches reached a peak of 147 tons in 2003 (Perez & Wahrlich 2005, Valentini & Pezzutto 2006). After this increase, the landings decreased steadily to 17 tons in 2008 (UNIVALI/CTTMar 2009), increasing again and remaining at approximately 40 tons in 2009 and 2010 (UNIVALI/CTTMar 2011). In the bottom trawl surveys conducted during the Brazilian Program for the Assessment of the Exclusive Economic Zone (REVIZEE Program, 1996-2006) (BRASIL 2006), *Z. conchifer* represented the second-ranked species in terms of weight and was considered a potential resource (Haimovici et al. 2008).

Despite the commercial importance of the silver John dory, almost all previous studies of this species biology were restricted to its distribution and occurrence (Froese & Pauly 2013). Its life cycle and habitats in the northeastern Atlantic were analyzed (Zidowitz & Fock 2004, Fock & Zidowitz 2004). In Brazilian waters, the silver John dory was studied in terms of distribution and occurrence (Vazzoler & Iwai 1971, Figueiredo & Menezes 1980) fishery potential (Yesaki et al. 1976, Haimovici et al. 1994, Perez 2006), larval development (Weiss et al. 1987), diet (Muto et al., 2005) gonad maturation (Vaz-dos-Santos 2009) and biological aspects based on harvesting (Martins & Schwingel 2012). The ecological importance of the silver John dory deserves attention. It is a predator on fishes and feeds primarily in pelagic environments and it is consumed by important species as *Trichiurus lepturus* (Muto et al. 2005) and *Thunnus albacares* (Vaske & Castello 1998). Its ecological strategy resembles that of other species of fishery importance, such as *M. hubbsi* and other demersal fishes (Gasalla et al. 2007). Changes in the species composition of the ecosystem of Southeastern-Southern Brazil due to fisheries pressure enhanced *Z. conchifer* landings (Perez et al. 2003, Perez 2006, Kolling et al. 2008).

Zenopsis conchifer occurs mainly in the upper slope and secondly at the shelf break and although it makes vertical migrations in the water column to feed on pelagic items (Muto et al. 2005), it is strongly associated with the bottom. Few small specimens were documented (n = 64; lengths 16-185 mm Lt) in the water column based on collections with midwater trawls between depths of 50 and 400 m (Figueiredo et al. 2002), whereas a larger number of fish was caught in bottom trawls (n = 14,503; 30-974 mm Lt) (Bernardes et al. 2005, Haimovici et al. 2008). The industrial fleet also uses bottom trawls (Perez & Warhlich 2005, Valentini & Pezzutto 2006, Perez & Pezzutto 2006).

An understanding of reproduction is fundamental to a better understanding of the long-term persistence and stability of a given stock (Moyle & Cech 2004, Fonteles-Filho 2011). Quinn II & Deriso (1999) emphasize the importance of knowledge on the reproductive processes of a species and the applications of this information to stock assessment and biomass estimation. The reproduction process analysis allows for a better understanding of the reproductive strategy of a species, allowing the identification of spawning areas/periods and the estimation of the length at first maturation (Wootton 1998). Several techniques are used in fish reproduction studies (Vazzoler 1996) to improve the understanding of the effect of fishing (and others) on the abundance of the species (Núñez & Duponchelle 2009). This information facilitates the implementation of management measures, such as the determination of fishing exclusion areas, minimum sizes of capture, temporal restrictions, biomass and catchability (Vincent & Sadovy 1998).

Despite the increasing importance of *Z. conchifer* as a fishery resource off southern Brazil, information about the species in the Atlantic Ocean is scarce. Haimovici et al. (2008) first pointed out that the reproductive cycle of the species in the southwestern Atlantic involves continuous maturation and spawning over the entire year. In order to understand the reproductive biology of *Z. conchifer*, this study aims to analyze the reproduction of the species (sex ratio, length-weight relationship, recruitment, spawning period and area, and length at first maturity) off southeastern Brazil.

Materials and methods

1. Study area

The study area comprises the region between Cabo Frio (23°S), Rio de Janeiro State, and Cabo de Santa Marta Grande (28°40'S), Santa Catarina State, at depths between 100 and 600, m from the edge of the continental shelf to the upper slope (Figure 1). Three water masses are present in the area and at these depths. Tropical Water (TW), with temperatures above

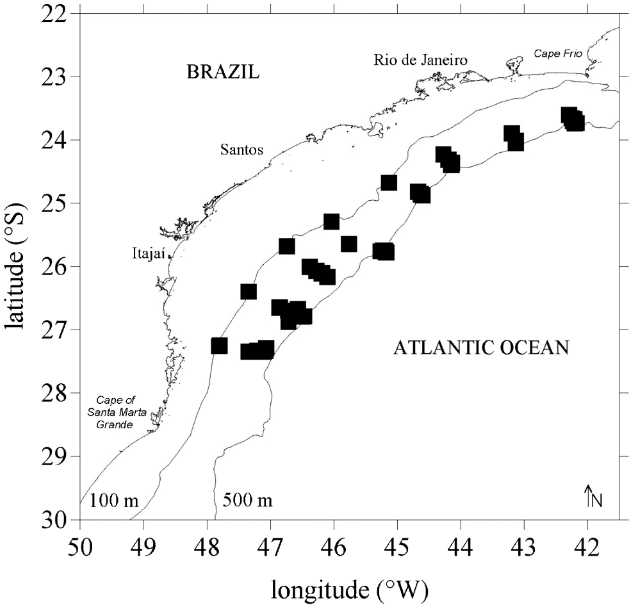


Figure 1. Capture locations for *Zenopsis conchifer* after bottom-trawl surveys conducted in 2001-2002.

20°C, salinity exceeding 36.4 and chlorophyll-a concentrations between 0.05-0.37 $\mu g.l^{-1}$ is found from the surface to depths of approximately 200 m. South Atlantic Central Water (SACW), with temperatures between 6-20°C, salinity between 34-36.4 and 0.10-2.77 $\mu g.l^{-1}$ of chlorophyll-a is found between 200-500 m (Silveira et al. 2000, Castro et al. 2006, Gaeta & Brandini 2006). Antarctic Intermediate Water (AIW), with temperatures between 3-6°C and salinity between 34.2 and 34.6, is found below 500 m (Castro et al. 2006). Sandy sediments are predominant on the continental shelf, and muddy sediments are found from the shelf break to the continental slope (Figueiredo & Madureira 2004). In terms of harvesting, the Southeastern-Southern of Brazil is the most productive area of the coast, sustaining annual catches between 200,000-300,000 tons (BRASIL 2006, Valentini & Pezzutto 2006, MPA 2011). Pelagic and demersal species have been targeted by the commercial fleet, emphasizing the growing importance that deep-sea species, such as *Z. conchifer*, have acquired from the 2000s on (Perez et al. 2009, UNIVALI/CTTMar 2011)

2. *Data source and analysis*

Samples were collected through bottom-trawl surveys conducted aboard the R/V *Soloncy Moura* (CEPSUL/IBAMA) in connection with the REVIZEE Program. The area between 23°S and 28°40'S was sampled in 2001 and 2002 (except in December/January) at depths ranging from 100 to 600 m (Figure 1). Descriptions of the fishing gear and catch operations appear in Bernardes et al. (2005), and details of the bottom trawl nets appear in Haimovici et al. (2008). The total catch included 14,000 specimens of *Z. conchifer* (Haimovici et al. 2008). A subsample of 1,056 fish was retained for biological studies.

Fish standard lengths (Ls, in millimeters), total body weight (Wt, in grams) and gonad weight (Wg, g) were obtained. Sex identification and gonadal maturation was accurately identified by Vaz-dos-Santos (2009) and, for the purposes of this study, the revision and updates on the theme of Núñez & Duponchelle (2009) and Brown-Peterson et al. (2011) were taken into consideration. Young fish showing incipient gonad development were recorded as not identified (NI). *Z. conchifer* presents asynchronous oocyte development and multiple spawning. Based on histological analysis, the female maturation data available included: immature (A), developing (B), spawning capable early development (C), spawning capable actively spawning (D) and regressing/regeneration (R) (Table 1). For the male samples, data available of gonad maturation was based on stereomicroscopic analysis and individuals were considered immature (A), developing (B), spawning capable (C) and regressing/regenerating (R) (Table 1).

The *Z. conchifer* length-frequency data (20 mm Ls) was examined in relation to depth strata of 100 m intervals. The standard lengths of the males, the females and the NI specimens were analyzed bimonthly due to the aforementioned lack of samples in the months of December and January. The sex ratio was obtained bimonthly and by standard length classes. Deviations from the expected 1:1 sex ratio were analyzed with a chi-square test (Zar 2010).

The length-weight relationships were estimated according to the standard potential model $Wt = a.Ls^{b}$ (Huxley 1993) for all

Table 1. Zenopsis conchifer: gonadal maturation stages for females (histological analysis) and males (macroscopic analysis).

Stage	Description
Females	
Immature (A) (never spawned)	Ovaries with predominance of perinucleolar oocytes and some cortical alveolar cells in the ovigeran lamellae.
Developing (B)	Maturation starts and various types of cells were present in the ovaries: perinucleolar oocytes, oocytes with cortical alveoli, with initial vitellogenesis and with complete vitellogenesis. Oocytes with cortical alveoli predominate followed by initial vitellogenesis; the others were present in low frequencies.
Spawning Capable Early Development (C)	Oocytes with initial and complete vitellogenesis cells predominate, although perinucleolar oocytes and some with cortical alveoli are present. Two situations were identified: (i) ovaries showing initial maturation in which most of the oocytes were in initial vitellogenesis, followed by oocytes with complete vitellogenesis; (ii) mature ovaries with predominance of oocytes with complete vitellogenesis and some undergoing hydration.
Spawning Capable Actively Spawning (D)	Hydrated oocytes predominate followed by oocytes with complete vitellogenesis; many ovaries contained post-ovulatory follicles as a consequence of recent spawning. Oocytes in the other phases were also observed in low frequencies.
Regressing / Regenerating (R)	Oocytes in all phases were present but perinucleolar and oocytes with cortical alveoli predominated, as in the immature ovarian, but differing from it by the presence of some oocytes with completed vitellogenesis, hydrated oocytes and post-ovulatory follicles.
Males	
Immature (A) (never spawned)	Testicles were thin and cylindrical, occupying little portions of the coelom cavity, internally translucent yellowish/albescent.
Developing (B)	Maturation starts and testicles were larger and triangular (sectioned), whitish and firm, with a milky aspect.
Spawning Capable (C)	Testicles were large and developed, with various lobules, white.
Regressing / Regenerating (R)	Testicles still remain large, but presenting diverse degrees of flaccidity, sometimes almost empties. In some cases, regeneration leading to developing stage was observed.

the fish and for the males and females separately using the iterative least squares method. A covariance analysis was used to compare the regressions between sexes. The *b* coefficients of the model were compared with a standard value of 3 to assess isometry. The statistical procedures adopted were based on Sokal & Rohlf (1995) and Zar (2010). A 95% confidence level was applied to all statistical procedures.

The reproductive cycle of *Z. conchifer* was characterized based on the frequency of gonadal maturation stages analyzed (i) per period (bimonthly) and (ii) per area (degree of latitude) (Fonteles-Filho 2011). In both analyses, the frequencies were compared with a Kolmogorov-Smirnov test (Zar 2010). For the females, once that staging was based on histological analysis, we used the reproductive phases available. For the males, once that staging was based on stereomicroscopic analysis, we classified the fish as young (immature) or adult (B + C + R stages together). In addition, the average gonadosomatic index values (GSI = Wg / Wt × 100) (Wootton 1998) were calculated for the sexes separately, using only adults (excluding immature and young fish with unidentified sex) and then compared with a two-way ANOVA (period and area) for the females and a one-way ANOVA (period) for the males. The ANOVA was followed by a Tukey test (Zar 2010).

The average length (L_{50}) at first maturity and the length at which 100% fish reach the maturity (L_{100}) were calculated by adjusting a logistic curve (King 2007) using the iterative least squares method to adult length frequencies grouped by 20 mm length classes for females and males. The immature ones were considered young while the other stages of maturation were considered adults. The formulae used was: $Pi = 1/\{1+e^{[-r(Li-L_{50})]}\}$ where Pi is the proportion of adults at length class Li, *r* is the slope of the curve and Li is the inferior limit of length class (King 2007).

In order to allow comparisons among the present results with other ones, the relationship Lt = 2.094 + 1.214 Ls (r^2 = 0.995; 57-504 mm Ls) can be used to convert lengths (Vaz-dos-Santos 2009).

Results

A total of 1,056 individuals of *Z. conchifer*, with standard lengths ranging from 57 to 504 mm Ls (mean value and standard deviation 228.27 ± 116.14 mm Ls), were sampled. The silver John dory was collected primarily on the upper slope, occupying depths from 200 m until 600 m. Young and smaller fish (< 200 mm Ls) were common from inshore to the 200 m isobath (mostly around the 100 m strata). The silver John dory became rare at depths greater than 500 m, where only one fish was captured (it was not plotted) (Figure 2). A total of 136 specimens comprised young fish with unidentified sex (these specimens were considered immature) ($Ls_{average}$ = 85.88 mm); 425 specimens were male ($Ls_{average}$ = 212.17 mm) and 495 female ($Ls_{average}$ = 282.50 mm). Young fish with unidentified sex were collected mainly between February and May (n = 131) and only five specimens in August/September. Males and females were collected during all samplings, except in December/January, due to the absence of sampling (Figure 3).

The sex ratio analysis by length classes showed that: males were predominant (2:1) at small lengths (< 240 mm Ls); females were predominant (2:1) at lengths greater than 320 mm Ls and; equal proportions of both sexes occurred between 240 mm and 319 mm Ls (χ^2 = 110.42, P < 0.001). Females predominated over the year (χ^2 = 38.40, P < 0.001) except in February/March. From February through May, young fish (immatures) were abundant (Figure 3).

The length-weight relationships were $Wt = 1.12 \times 10^{-4} Ls^{2.702}$ (r^2 = 0.992; n = 1,056) for all fish, $Wt = 1.31 \times 10^{-4} Ls^{2.678}$ (r^2 = 0.987; n = 495) for females and $Wt = 1.29 \times 10^{-4} Ls^{2.673}$ (r^2 = 0.991; n = 425) for males. The slope of the regression showed negative allometry in all cases (P < 0.001). There were significant differences between models of females and males (F = 0.034, P = 0.854 for coefficient *b* and F = 17.459, P < 0.001 for coefficient *a*).

Fish at different stages of maturation were collected during all months (Figure 4). Females and males predominated in the samples between August and November when spawning was

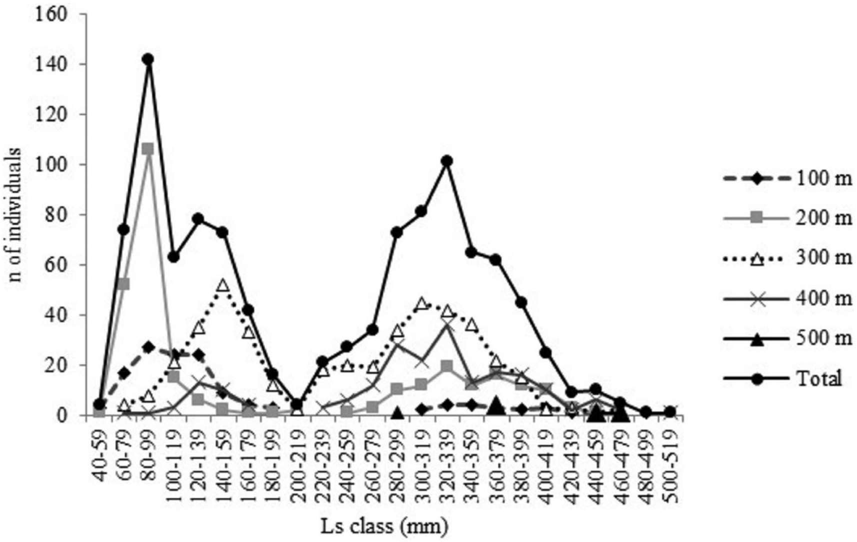


Figure 2. *Zenospsis conchifer*: length frequency distribution by depth of fish sampled for biological studies (n = 1,056).

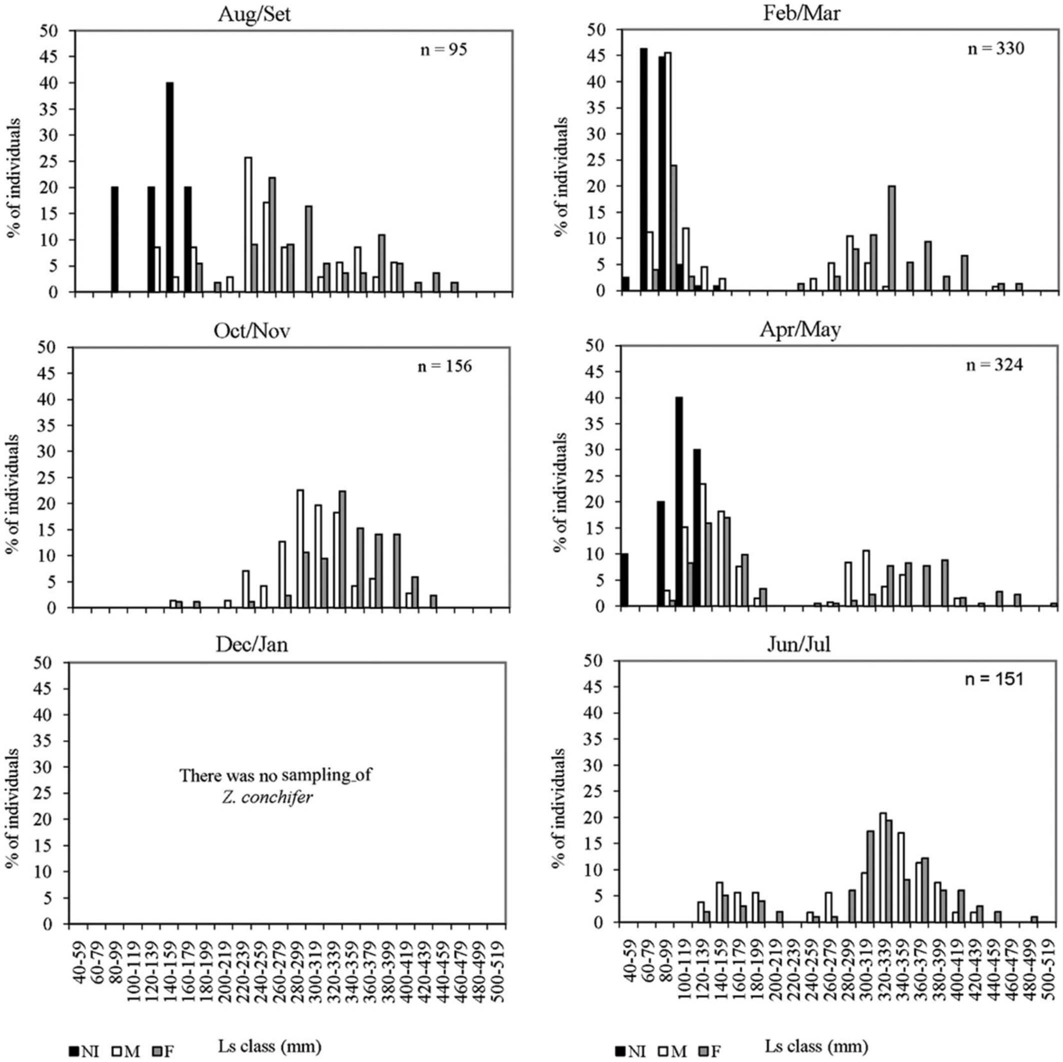


Figure 3. *Zenopsis conchifer*: bimonthly frequencies distribution of fishes by standard length classes by sex (NI – not identified; M – males; F – females).

more intensive. During that period high frequencies of spawning capable actively spawning (D) and regressing/regeneration (R) females (together) were observed (Figure 4a). A significant increase in their proportion was also observed in August/September and October/November (Table 2). High frequencies of adult males, dominating the samples, were also recorded (Figure 4b). Between February and July, the presence of fish of unidentified sex (younglings) (Figure 3) and immature females and males in larger proportions (Figure 4) indicates that recruitment occurs during this period. The increase of immature females in April/May (Figure 4a) and the decrease in the proportion of immature males in June/July (Figure 4b) were significant (P-value < 0.05, Table 2).

Both young (immature) and adult (in all maturation stages) females and males were found at all latitudes (Figure 5), suggesting that spawning does not occur at one specific site. Immature females and males were highly abundant at 25°S, and immature males also occurred at 27°S (Table 3).

The GSI ranged between 0.1% and 14.3% of the Wt in females (5% on average) and between 0.04% and 6.9% of the Wt in males (0.5% on average). Females and males had a similar pattern of GSI variation (Fig. 6). For both sexes, GSI was higher in the second half of the year (June/July for females and October/November for males). The lowest GSI values were found between February and May (during the first half of the year) for females. For males, lowest values extended until August/September. The ANOVA of the female mean GSI values showed an interaction between area and period ($F = 12.47$, $P < 0.001$). The corresponding ANOVA for males showed only an effect of period ($F = 4.38$, $P < 0.001$).

The length at first maturity for both females and males was $L_{50} = 220.00$ mm Ls (slope value of $r = 0.5$). The values of $L_{100} = 240.00$ mm Ls, for males and females. The bimodal frequency distributions showed a clear division between the modal values of young adults and immature fishes (Figure 3).

Discussion

The current study is the first to investigate the reproductive biology of a silver John dory virgin stock. As mentioned earlier, prior to the current study, there are few references (Haimovici

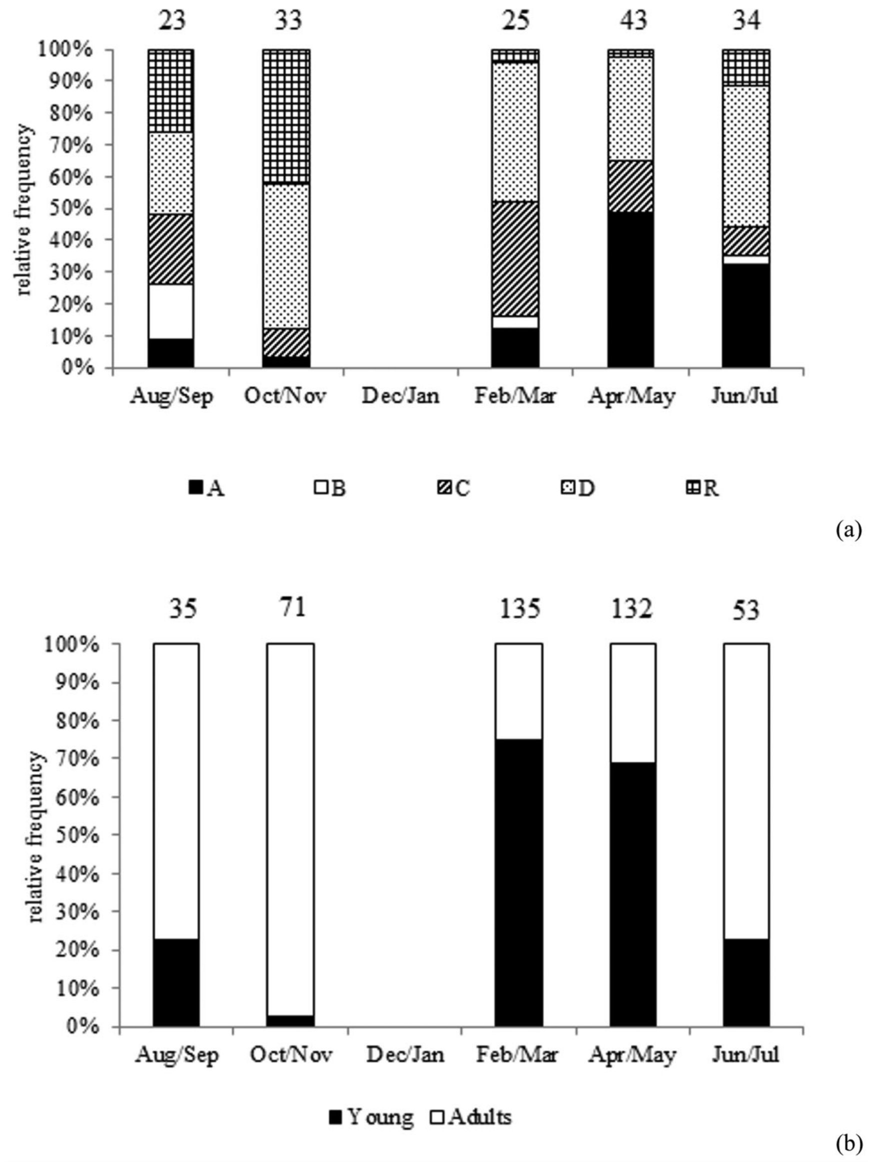


Figure 4. *Zenopsis conchifer:* females (a) and males (b) bimonthly frequency of maturation stages (the numbers above the columns indicate the number of individuals analyzed; for females: A – immature, B – developing, C – spawning capable early development, D – spawning capable actively spwaning, R – regression/regeneration).

et al., 2008; Vaz-dos-Santos, 2009; Martins and Schwingel, 2012) about the theme, which restricts comparisons.

The results of sex ratio revealed the intrinsic pattern of *Z. conchifer* in the area, which constitutes an essential element for understanding the structure of these schools structure (Walters & Martell 2004). The higher proportion of females observed in larger length classes is probably due to a differential growth rate, because there was neither evidence of aggregations during the spawning period nor spatial segregation according to length or to stages of maturation as suggested by Vazzoler (1996). Similarly, at the beginning of the 2000s, when harvesting over the species started, specimens caught had lengths of 81-525 mm Ls and females also predominated in the majority of length classes (Martins & Schwingel 2012). Parity of sexes occurred at

Table 2. *Zenopsis conchifer*: Kolmogorov-Smirnov test results for the frequency distribution of fish by period (asterisks indicates significant differences).

Sex	Statistics	Aug/Sep *vs.* Oct/Nov	Feb/Mar *vs.* Apr/May	Apr/May *vs.* Jun/Jul
Females	χ^2_{KS}	69.114	85.809	33.489
	P-value	0.0316*	0.0137*	0.1874
Males	χ^2_{KS}	37.661	0.9216	324.235
	P-value	0.1521	0.6308	< 0.0001*

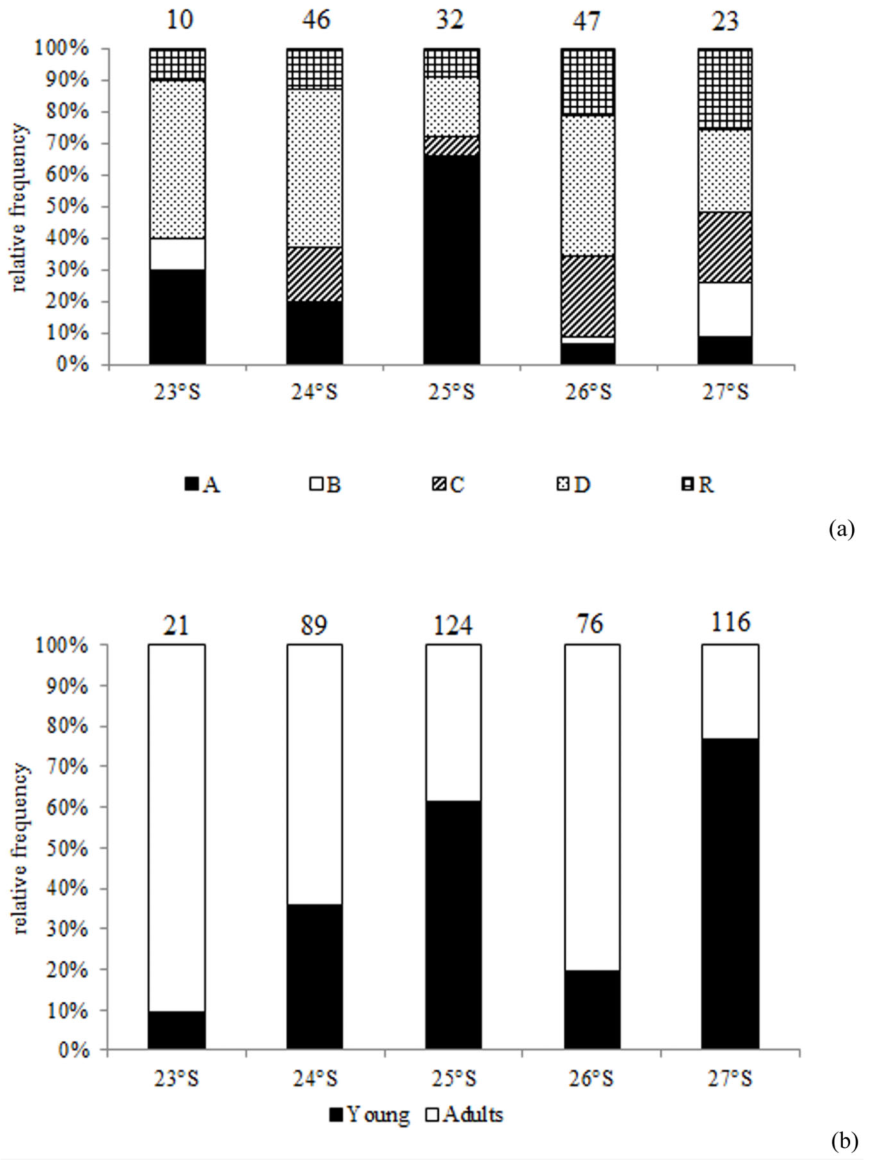


Figure 5. *Zenopsis conchifer:* females (a) and males (b) frequency of maturation stages by latitude (the numbers above the columns indicate the number of individuals analyzed; for females: A – immature, B – developing, C – spawning capable early development, D – spawning capable actively spawning, R – regression/regeneration).

the same length classes both in the virgin stock (present results) and those in the initial exploitation (Martins & Schwingel, 2012).

The sex ratio and the sex-specific length-frequencies pattern shown by *Z. conchifer* were similar to those of other species that inhabit deep regions of the area surveyed and are targeted by double rigged trawlers (Perez & Pezzutto 2006). Females of greater length are predominant in *Lophius gastrophysus* (Lopes & Schwingel 2006), *Merluccius hubbsi* (Vaz-dos-Santos & Rossi-Wongtschowski 2005, 2007) and *Urophycis mystacea* (Haimovici et al. 2006). This pattern is known as the bigger-deeper and smaller-shallower paradigm (*sensu* Macpherson & Duarte 1991) and Martins & Schwingel (2012) discussed it. These authors have argued favoring this paradigm (reduction in the competition, food availability and optimization of metabolism) and others adding new elements to explain differential

Table 3. *Zenopsis conchifer*: Kolmogorov-Smirnov test results for the frequency distribution of fish by area (degree of latitude) (asterisks indicates significant differences).

Sex	Statistics	23°S *vs.* 24°S	24°S *vs.* 25°S	25°S *vs.* 26°S	26°S *vs.* 27°S
Females	χ^2_{KS}	13.720	160.146	267.264	19.083
	P-value	0.50	0.0003*	< 0.0001*	0.38
Males	χ^2_{KS}	47.480	133.028	325.447	596.467
	P-value	0.0931	0.0013*	< 0.0001*	< 0.0001*

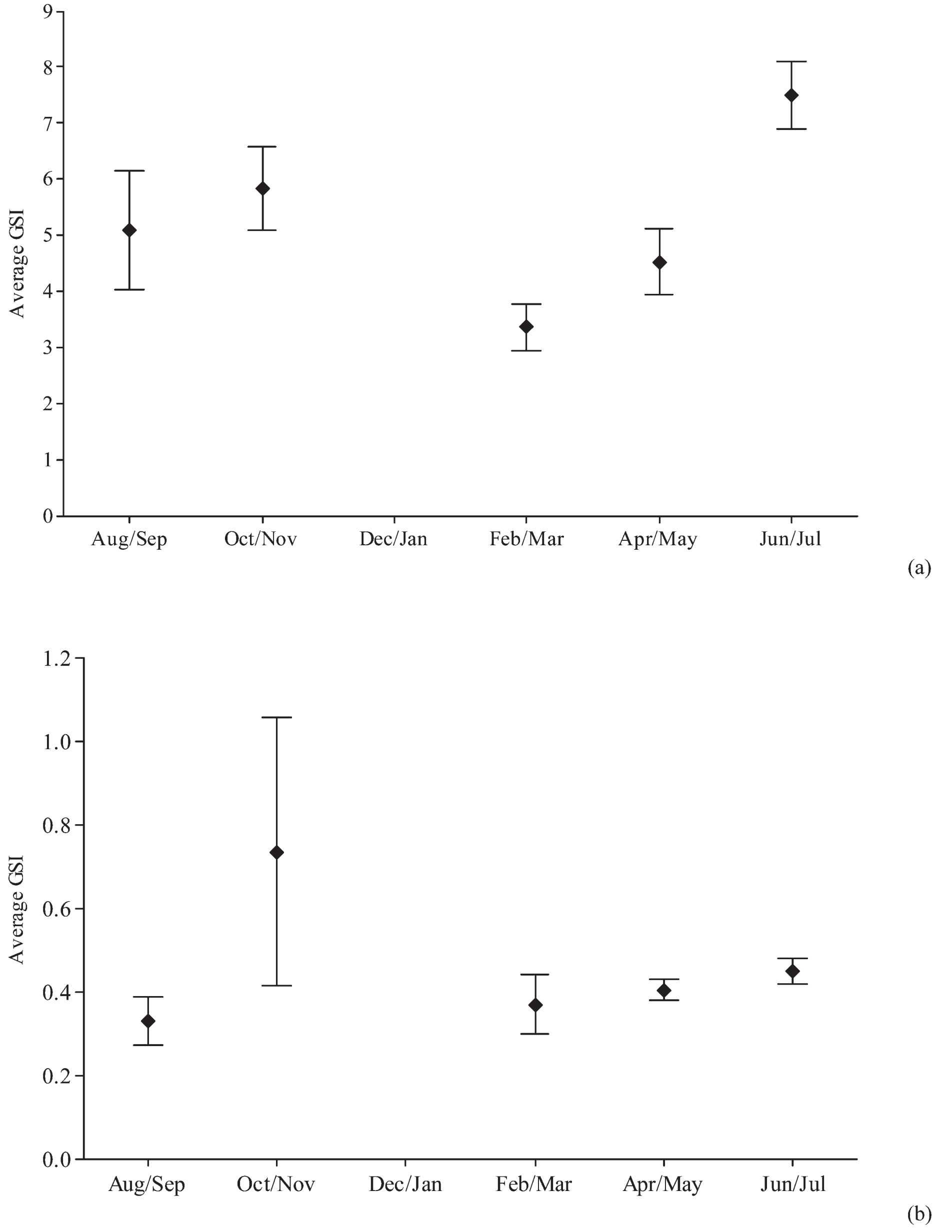


Figure 6. *Zenopsis conchifer*: bimonthly average GSI and confidence interval for (a) females (n = 331) and (b) males (n = 202).

distribution (free movements along water column, optimal environmental conditions and restriction of depth due to fishery). Our results reinforce the observations of Martins & Schwingel (2012), showing large adults of *Z. conchifer* also occurring at 100-200 m strata of depth.

The length-weight relationships data analysis yielded a value of approximately 2.7 for the allometric coefficient (*b*). This value reflects the laterally compressed body shape of the silver John dory, a schooling species with a low body volume and a carangid swimming pattern (Pough et al. 2008). Length-weight relationship values for *Z. conchifer* are available in Haimovici & Velasco (2000) for southern Brazil ($a = 1.42 \times 10^{-5}$ and $b = 2.9549$). For southeastern and southern Brazilian waters, Madureira & Rossi-Wongtschowski (2005) presented estimates for sexes pooled ($a = 1.17 \times 10^{-4}$ and $b = 2.627$) and Martins & Schwingel (2012) for sexes separated ($a = 5 \times 10^{-5}$ and $b = 2.758$ for females and $a = 5 \times 10^{-5}$ and $b = 2.696$ for males). These values are consistent with the pattern found by this study in terms of length-weight relative growth.

The silver John dory is a multiple spawner fish (Vaz-dos-Santos 2009). The recovery of the gonads (especially the ovaries) led to the long spawning period observed, extending that observed by Martins & Schwingel (2012). The use of two techniques combined (maturation + GSI) allow to identify the breeding season in multiple spawners (Núñez & Duponchelle 2009) and the results of females, whose ovaries were staged by histology (Vaz-dos-Santos 2009), were considered more refined than males. Most studies on fish reproduction have been still detailing only females (Núñez & Duponchelle 2009, Lowerre-Barbieri et al. 2011) due to their more significant contribution to the recruitment (see Palumbi 2004). In this regard, Lowerre-Barbieri et al. (2011) call attention to the effects of an extended spawning season that leads to a wide range of hatchings and increases individual and population reproductive success.

Larger females also ensure better oocytes and then eggs (Palumbi 2004). Our results do not allow to evaluate that, but it seems that these considerations apply to *Z. conchifer*.

Longhurst & Pauly (2007) cite other species with an extended reproductive cycle, a usual pattern common to species with asynchronous oocyte development and multiple spawning (West 1990, Rocha & Rocha 2006), such as *Z. conchifer*. The occurrence of *Z. conchifer* larvae in the oceanic area from October through December (Katsuragawa 2007) supports our findings about the period of relatively intensive spawning of the silver John dory in the area studied. Katsuragawa's (2007) results suggest that the spawning of the species in southern Brazilian waters depends on the intrusion of warm waters, which become more constant in springtime (Castro et al. 2006). Weiss et al. (1987) investigated the larval development of *Z. conchifer* in southern Brazil. They found larvae at water temperatures between 17.5°C and 23.4°C and salinities between 32.2 and 36.6 (Tropical Water and some mixture of water masses) from July through November on the shelf break and upper slope. The authors of that study suggested that the reproduction of *Z. conchifer* in southern Brazilian waters was related to the warm and saline conditions of the Tropical Water, contrary to the environmental conditions at which the adults inhabits, once that our results showed the silver John dory occurring mainly associated with the cold nutrient-rich waters of the South Atlantic Central Water (SACW).

In relation to spawning period, in the southwestern Atlantic other teleost species living on the shelf break reproduce at the same time as *Z. conchifer*. *Ariomma bondi* usually spawn between March and October (Vaz-dos-Santos et al. 2013). *Lopholatilus villarii* spawns from September to December (Ávila-da-Silva & Haimovici 2005). *Lophius gastrophysus* shows a spawning peak between July and October (Lopes & Schwingel 2006). *Urophycis mystacea* spawns in July and August (Haimovici et al. 2006). All of the authors quoted above found difficulties to relate spawning to environmental characteristics. However, Cury & Pauly (2000) remark that periods of better productivity favor egg and larval survival during the austral spring and summer. In this way, the Brazil Currents water flow and the higher intensity of the upwelling during spring and summer could create more favorable conditions for larval survival (Braga & Niencheski 2006).

The use of a precise scale to staging ovaries (Vaz-dos-Santos 2009) was essential to assess the first maturity (Núñez & Duponchelle 2009). The first maturity affects generation time, influences the intrinsic rate of population growth, constitutes a biological reference for harvesting and is used to estimate spawning stock biomass (Longhurst & Pauly 2007, Lowerre-Barbieri et al. 2011). It occurs when there are metabolic conditions to somatic and reproductive growth, represented by a ratio (from 0.4 to 0.9) between the L_{50} and the L_{∞} (Longhurst & Pauly 2007). Although there is no available information about *Z. conchifer* growth, a gross estimate of 0.44 ($L_{50}/Ls_{maximum}$) follows the general patterns stated by Longhurst & Pauly (2007), with the lowest values associated to the largest fishes.

The values of first maturity obtained here came from the virgin stock and constitute a referential, but they may change due to harvesting and they must not be used as a statical reference (Lowerre-Barbieri 2009). For example, in the same fishery of *Z. conchifer* this fact occurred with *Merluccius hubbsi*, whose first maturity suffered an alarming decrease due to overexploitation (Vaz-dos-Santos et al. 2005, 2009). Martins & Schwingel (2012) estimated the L_{50} value for females (245 mm Ls corresponding to 311 mm Lt), higher than the value presented. This difference was probably methodological, once authors did not included maturing (similar to our developing stage) in their calculations.

This study provides the first information about the reproductive biology of a virgin stock of the silver John dory. The results of the study show that the species exhibits a strategy similar to that of other teleost fishes in the area. These patterns consist of the occupation of deeper regions by older and larger fish, particularly females, a long period of spawning and the alternation of intense periods of recruitment and spawning. These results must be taken into consideration for evaluating the status of the *Z. conchifer* population and the effects of fisheries on the stock.

Acknowledgments

The authors express their gratitude to FAPESP for financial support (Process number 2007/00805-8), to an anonymous FAPESP and all journals referees, whose recommendations were invaluable, and to Dr. Maria Inês Borella and Dr. June Ferraz Dias for teaching us and granting us permission to use their equipment.

Literature cited

ÁVILA-DA-SILVA, A.O. & HAIMOVICI, M. 2005. *Lopholatilus villarii* Miranda Ribeiro, 1915. In Análise das principais pescarias comerciais da região Sudeste-Sul do Brasil: dinâmica populacional das espécies em explotação (Cergole, M.C., AVila-Da-Silva., A.O., & Rossi-Wongtschowski, C.L.D.B. eds.). Instituto Oceanográfico-USP/Série Documentos REVIZEE, São Paulo, p.74-80.

BERNARDES, R.A., FIGUEIREDO, J.L., RODRIGUES, A.R., FISCHER, L.G., VOOREN, C.M., HAIMOVICI, M. & ROSSI-WONGTSCHOWSKI, C.L.D.B. 2005. Peixes da Zona Exclusiva da região Sudeste e Sul do Brasil: levantamento com armadilhas pargueiras e rede de arrasto de fundo. Instituto Oceanográfico-USP/Série Documentos REVIZEE, São Paulo.

BRAGA, E.S. & NIENCHESKI, L.F.H. 2006. Composição das massas de água e seus potenciais produtivos na área entre o Cabo de São Tomé (RJ) e o Chuí (RS). In O ambiente oceanográfico da plataforma continental e do talude na região Sudeste-Sul do Brasil (Rossi-Wongtschowski, C.L.D.B., & Madureira, L.S.P. eds.). EDUSP, São Paulo, p.161-218.

BRASIL. Ministério do Meio Ambiente - MMA. 2006. Programa REVIZEE: avaliação do potencial sustentável de recursos vivos da Zona Econômica Exclusiva do Brasil - relatório executivo. MMA, Brasília.

BROWN-PETERSON, N.J., WYANSKI, D.M., SABORIDO-REY, F., MACEWICZ, B.J. & LOWERRE-BARBIERI, S.K. 2011. A standardized terminology for describing reproductive development in fishes. Mar. Coast. Fish. 3(1):52–70, http://dx.doi.org/10.1080/19425120.2011.555724

CASTRO, B.M., LORENZETTI, J.A., SILVEIRA, I.C.A. & MIRANDA, L.B. 2006. Estrutura termohalina e circulação na região entre o Cabo de São Tomé (RJ) e o Chuí (RS). In O ambiente oceanográfico da plataforma continental e do talude na região Sudeste-Sul do Brasil (Rossi-Wongtschowski, C.L.D.B. & Madureira, L.S.P. eds.). EDUSP, São Paulo, p. 11–120.

CURY, P. & PAULY, D. 2000. Patterns and propensities in reproduction and growth of marine fishes. Ecol. Res. 15:101–106, http://dx.doi.org/10.1046/j.1440-1703.2000.00321.x

FIGUEIREDO, A.G. & MADUREIRA, L.S.P. 2004. Topografia, composição, refletividade do substrato marinho e identificação de províncias sedimentares na região Sudeste-Sul do Brasil. Instituto Oceanográfico-USP/Série Documentos REVIZEE, São Paulo.

FIGUEIREDO, J.L. & MENEZES, N.A. 1980. Manual de peixes marinhos do Sudeste do Brasil vol. III: Teleostei (2). Museu de Zoologia da Universidade de São Paulo, São Paulo.

FIGUEIREDO, J.L., SANTOS, A.P., YAMAGUTI, N., BERNARDES, R.A. & ROSSI-WONGTSCHOWSKI, C.L.D.B. 2002. Peixes da Zona Econômica Exclusiva da região Sudeste-Sul do Brasil: levantamento com rede de meia água. EDUSP, São Paulo.

FOCK, H.O. & ZIDOWITZ, H. 2004. Episodic recruitment to seamount populations: evidence from *Zenopsis conchifer* (Lowe, 1852) at the Great Meteor Seamount (Subtropical North-east Atlantic). Arch. Fish. Mar. Res. 51:287–293.

FONTELES-FILHO, A.A. 2011. Oceanografia, biologia e dinâmica populacional de recursos pesqueiros. Expressão Gráfica, Fortaleza.

FROESE, R. & PAULY, D. 2013. Fishbase: World Wide Web electronic publication. Available from http://www.fishbase.org. Acessed in 20/May/2013.

GAETA, S.A. & BRANDINI, F.P. 2006. Produção primária do fitoplâncton na região entre o Cabo de São Tomé (RJ) e o Chuí (RS). In O ambiente oceanográfico da plataforma continental e do talude na região Sudeste-Sul do Brasil (Rossi-Wongtschowski, C.L.D.B. & Madureira, L.S.P. eds.). EDUSP, São Paulo, p. 219–264.

GASALLA, M.A., VELASCO, G., ROSSI-WONGSTCHOWSKI, C.L.D.B., HAIMOVICI, M & MADUREIRA, L.S.P. 2007. Modelo de equilíbrio de biomassas do ecossistema marinho da região Sudeste-Sul do Brasil entre 100-1000m de profundidade. Instituto Oceanográfico-USP/Série Documentos REVIZEE, São Paulo.

HAIMOVICI, M. & VELASCO, G. 2000. Length-weight relationship of marine fishes from Southern Brazil. Fishbyte 23(1):19–23.

HAIMOVICI, M., ÁVILA-DA-SILVA, A.O. & FISCHER, L.G. 2006. Diagnóstico do estoque e orientações para o ordenamento da pesca de *Urophycis mystacea* (Ribeiro, 1903). In Análise das principais pescarias comerciais da região Sudeste-Sul do Brasil: dinâmica populacional das espécies em explotação – II (Rossi-Wongtschowski, C.L.D.B., Cergole, M.C & ÁVila-Da-Silva, A.O eds.). Instituto Oceanográfico-USP/Série Documentos REVIZEE, São Paulo, p.86–94.

HAIMOVICI, M., MARTINS, A.S., FIGUEIREDO, J.L. & VIEIRA, P.C. 1994. Demersal bony fish of the outer shelf and upper slope of the southern Brazil Subtropical Convergence Ecosystem. Mar. Ecol. Prog. Ser. 108:59–77, http://dx.doi.org/10.3354/meps108059

HAIMOVICI, M., ROSSI-WONGTSCHOWSKI, C.L.D.B., BERNARDES, R.A., FISCHER, L.G., VOOREN, C.M., SANTOS, R.A., RODRIGUES, A.R. & SANTOS, S. 2008. Prospecção pesqueira de espécies demersais com rede de arrasto de fundo na região Sudeste-Sul do Brasil. Instituto Oceanográfico-USP/Série Documentos REVIZEE, São Paulo.

HUXLEY, J.S. 1993. Problems of relative growth, with a new introduction by Frederick B. Churchill and an essay by Richard E. Strauss. The John Hopkins University Press, Baltimore.

KATSURAGAWA, M. 2007. Ictioplâncton na plataforma continental externa e talude superior do sudeste brasileiro. Tese de Livre Docência, Universidade de São Paulo, São Paulo.

KING, M. 2007. Fisheries biology, assessment and management. 2 ed. Blackwell Publishing, Oxford.

KOLLING, J.A., BATISTA, P.A., ÁVILA-DA-SILVA, A.O. & CARNEIRO, M.H. 2008. A utilização do ambiente marinho e de seus recursos vivos pela frota pesqueira paulista: o ambiente demersal. Sér. Relat. Téc. 32:1–62.

LONGHURST, A.R. & PAULY, D. 2007. Ecologia dos oceanos tropicais. EDUSP, São Paulo.

LOPES, F.A.R. & SCHWINGEL, P.R. 2006. Reprodução, idade e crescimento do peixe-sapo *Lophius gastrophysus* (Ribeiro, 1915) na região Sudeste e Sul do Brasil. CEPSUL DOC 16 SCC CPG 04:1-5.

LOWERRE-BARBIERI, S.K. 2009. Reproduction in relation to conservation and exploitation of marine fishes. Reproductive biology and phylogeny of fishes (agnathans and bony fishes) Vol 9 Part B(Jamieson, B.G.M, ed.)Science Publishers, Enfield, p.371-394.

LOWERRE-BARBIERI, S.K., GANIAS, K., SABORIDO-REY, F., MURUA, H. & HUNTER, J.R. 2011. Reproductive timing in marine fishes: variability, temporal scales and methods. Mar. Coast. Fish. 3(1):71–91, http://dx.doi.org/10.1080/19425120.2011.556932

MACPHERSON, E. & DUARTE, C.M. 1991. Bathymetric trends in demersal fish size: is there a general relationship? Mar. Ecol. Prog. Ser. 71:103–112, http://dx.doi.org/10.3354/meps071103

MADUREIRA, L.S.P & ROSSI-WONGTSCHOWSKI, C.L.D.B. 2005. Prospecção de recursos pesqueiros pelágicos na Zona Econômica Exclusiva da região Sudeste-Sul do Brasil: hidroacústica e biomassas. Instituto Oceanográfico-USP/Série Documentos REVIZEE, São Paulo.

MARTINS, R.S. & SCHWINGEL, P.R. 2012. Biological aspects of the sailfin dory *Zenopsis conchifer* (Lowe, 1852) caught by deep-sea trawling fishery off southern Brazil. Braz. J Oceanogr. 60(2):171–179, http://dx.doi.org/10.1590/S1679-87592012000200007

MENEZES, N.A., BUCKUP, P.A., FIGUEIREDO, J.L. & MOURA, R.L. 2003. Catálogo das espécies de peixes marinhos do Brasil. Museu de Zoologia da Universidade de São Paulo, São Paulo.

MPA. Ministério da Pesca eAquicultura. 2011. Boletim estatístico da pesca e aquicultura 2011. Ministério da Pesca e Aquicultura, Brasília.

MOYLE, P.B. & CECH, J.J. 2004. Fishes: an introduction to ichthyology. 5 ed. Prentice Hall, Upper River Saddle.

MUTO, E.Y, SILVA, M.H.C., VERA, G.R., LEITE, S.S.M., NAVARRO, D.G. & ROSSI-WONGTSCHOWSKI, C.L.D.B. 2005. Alimentação e relações tróficas de peixes demersais da plataforma continental externa e talude superior da região Sudeste-Sul do Brasil. Instituto Oceanográfico-USP/Série Documentos REVIZEE, São Paulo.

NÚÑEZ, J. & DUPONCHELLE, F. 2009. Towards a universal scale to assess sexual maturation and related life history traits in oviparous teleost fishes. Fish Physiol. Biochem. 35(1):167–80, http://dx.doi.org/10.1007/s10695-008-9241-2

PALUMBI, S.R. 2004. Why mothers matter. Nature 430:621–622, http://dx.doi.org/10.1038/430621a

PEREZ, J.A.A. & PEZZUTO, P.R. 2006. A pesca de arrasto de talude do Sudeste e Sul do Brasil: tendências da frota nacional entre 2001–2003. Bol. Inst. Pesca 32(2):127–150.

PEREZ, J.A.A. & WAHRLICH, R. 2005. A bycatch assessment of the gillnet monkfish *Lophius gastrophysus* fishery off southern Brazil. Fish. Res. 72:81–95, http://dx.doi.org/10.1016/j.fishres.2004.10.011

PEREZ, J.A.A. 2006. Potenciais de rendimento dos alvos da pesca de arrasto de talude do sudeste e sul do Brasil estimados a partir de parâmetros do ciclo de vida. Braz. J. Aquat. Sci. Technol. 10(2):1–11, http://dx.doi.org/10.14210/bjast.v10n2.p1-11

PEREZ, J.A.A., WAHRLICH, R., PEZZUTTO, P.R., SCHWINGEL, P.R., LOPES, F.R.A. & RODRIGUES-RIBEIRO, M. 2003. Deep-sea fishery off southern Brazil: recent trends of the Brazilian fishing industry. J. Northwest Atl. Fish. Soc. 31:1–18.

PEREZ, J.A.A., PEZZUTO, P.R., WAHRLICH, R. & SOARES, A.L.S. 2009. Deep water fisheries in Brazil: history, status and perspectives. Lat. Am. J. Aquat. Res. 37(3):513–542, http://dx.doi.org/10.3856/vol37-issue3-fulltext-18

POUGH, F.H., JANIS, C.M. & HEISER, J. 2008. A vida dos vertebrados. 4ª ed. Atheneu, São Paulo.

QUERO, J.C. 1998. Changes in the Euro-Atlantic fish species composition resulting from fishing and ocean warming. Ital. J. Zool. 65:493–499, http://dx.doi.org/10.1080/11250009809386873

QUINN II, T.J & DERISO, R.B. 1999. Quantitative fish dynamics. Oxford University Press, New York.

ROCHA, M.J. & ROCHA, E. 2006. Morphofunctional aspects of reproduction from synchronous to asynchronous fishes – an overview. In: Fish endocrinology (Reinecke, M., Zaccone, G & Kappor, B.G.eds.). Science Publishers, New Hampshire, p.570–624.

SIBENI, F. & CALDERINI, F. 2012. FishStatJ, a tool for fishery statistics analysis. Release: 2.0.0. http://www.fao.org/fishery/statistics/software/fishstatj/en Accessed in 20/May/2013.

SILVEIRA, I.C.A., SCHMIDT, A.C.K., CAMPOS, E.J.D., GODOI, S.S. & IKEDA, I. 2000. Corrente do Brasil ao largo da costa leste brasileira. Braz. J Oceanogr. 48:171–183, http://dx.doi.org/10.1590/S1679-87592000000200008

SOKAL, R.R. & ROHLF, F.J. 1995. Biometry. 3 ed. W. H. Freeman and Company, New York.

UNIVALI/CTTMar. 2009. Boletim estatístico da pesca industrial de Santa Catarina – Ano 2008: programa de apoio técnico ao desenvolvimento da pesca no Sudeste e Sul do Brasil. UNIVALI, Itajaí.

UNIVALI/CTTMar. 2011. Boletim estatístico da pesca industrial de Santa Catarina – Ano 2010: programa de apoio técnico ao desenvolvimento da pesca no Sudeste e Sul do Brasil. UNIVALI, Itajaí.

VALENTINI, H. & PEZZUTTO, P.R. 2006. Análise das principais pescarias comerciais da região Sudeste/Sul do Brasil com base na produção controlada do período 1986 – 2004. Instituto Oceanográfico-USP/Série Documentos REVIZEE, São Paulo.

VASKE JR., T & CASTELLO, J.P. 1998. Conteúdo estomacal da albacora-laje, *Thunnus albacares*, durante o inverno e primavera no sul do Brasil. Rev. Bras. Biol. 58(4):639–647, http://dx.doi.org/10.1590/S0034-71081998000400011

VAZ-DOS-SANTOS, A.M. 2009. Caracterização do desenvolvimento ovariano e da reprodução do peixe galo-de-profundidade *Zenopsis conchifera* na região Sudeste-Sul do Brasil. Relatório de Pesquisa, Universidade de São Paulo, São Paulo.

VAZ-DOS-SANTOS, A.M. & ROSSI-WONGTSCHOWSKI, C.L.D.B. 2005. *Merluccius hubbsi* Marini, 1933. In : Análise das principais pescarias comerciais da região Sudeste-Sul do Brasil: dinâmica populacional das espécies em explotação (Cergole, M.C., AVila-Da-Silva, A.O., Rossi-Wongtschowski, C.L.D.B. & eds.). Instituto OceanogrÃfico-USP/SÕrie Documentos REVIZEE, SÐo Paulo, p.88–93.

VAZ-DOS-SANTOS, A.M. & ROSSI-WONGTSCHOWSKI, C.L.D.B. 2007. Age and growth of the Argentine hake *Merluccius hubbsi* Marini, 1933 in the Brazilian South-Southeast Region during 1996-2001. Neotrop. Ichthyol. 5(3):375-386, http://dx.doi.org/10.1590/S1679-62252007000300017

VAZ-DOS-SANTOS, A.M., HONJI, R.M., & ROSSI-WONGTSCHOWSKI, C.L.D.B. 2005. Comprimento e idade de primeira maturação gonadal de fêmeas de merluza *Merluccius hubbsi* Marini, 1933 na região Sudeste-Sul do Brasil. Bol. Inst. Pesca 31(2):109–117.

VAZ-DOS-SANTOS, A.M., ROSSI-WONGTSCHOWSKI, C.L.D.B. & FIGUEIREDO, J.L. 2009. Parâmetros da reprodução e relação comprimento-peso da merluza *Merluccius hubbsi* (Teleostei: Merlucciidae), estoque sudeste brasileiro (21°S–29°S), ano 2004. Bol. Inst. Pesca 35(1):1–16.

VAZ-DOS-SANTOS, A.M., ROSSI-WONGTSCHOWSKI, C.L.D.B., PEREIRA, N.B., KUCHINSKI, F.B. & FERNANDES, J. 2013. Biologia reprodutiva do rombudo *Ariomma bondi* (Teleostei: Ariommatidae) na bacia do Sudeste do Brasil. Bol. Inst. Pesca 39(1):27–36.

VAZZOLER, A.E.A. DE, M. 1996. Biologia da reprodução de peixes teleósteos: teoria e prática. EDUEM, Maringá.

VAZZOLER, G. & IWAI, M. 1971. Relatório sobre prospecção e pesca exploratória na plataforma continental do Rio Grande do Sul. Contrib. Avulsas Inst. Oceanogr. Oceanogr. Biol. 25:1–79, http://dx.doi.org/10.1590/S0373-55241971000200001

VINCENT, A. & SADOVY, A. 1998. Reproductive ecology in the management and conservation of fishes. In :Behavioral Behavioral ecology and conservation biology (Caro, T.M ed.). Oxford Univsertiy Press, New York, p.209–245.

WALTERS, C.J. & MARTELL, S.J.D. 2004. Fisheries ecology and management. Princeton University Press, Princeton.

WEISS, G., HUBOLD, G & BAINY, A.C.D. 1987. Larval development of the Zeiform fishes *Antigonia capros* Lowe, 1843 and *Zenopsis conchifer* (Lowe, 1852) from the South West Atlantic. Cybium 11(1):79–91.

WEST, G. 1990. Methods of assessing ovarian development in fishes: a review. Aust. J. Mar. Fresh. Res. 41:199-222, http://dx.doi.org/10.1071/MF9900199

WOOTTON, R.J. 1998. Ecology of teleost fishes. 2 ed. Chapman and Hall, London.

YESAKI, M., RAHN, E. & SILVA, G. 1976. Sumário das explorações de peixes de arrasto de fundo ao largo da costa Sul do Brasil. Ser. Doc. Tec. / Inst. Pesqui. Desenvolv. Pesq. 19:1–37.

ZAR, J.H. 2010. Biostatistical analysis. 5 ed. Prentice Hall, New Jersey.

ZIDOWITZ, H. & FOCK, H.O. 2004. Trophic ecology of *Zenopsis conchifer* (Lowe, 1852) at the Great Meteor Seamount (subtropical North-east Atlantic). Arch. Fish. Mar. Res. 51: 279–286.

Received 11/11/2013
Revised 25/08/2014
Accepted 01/09/2014

Biota Neotropica 14(3): e20140034, 2014
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)

article

Epiphytic *Eunotia* (Bacillariophyceae) on *Podostemum* from Santa Catarina, southern Brazil, including new observations on morphology and taxonomy of some rare recorded species

Kaoli Pereira Cavalcante[1,2], *Priscila Izabel Tremarin*[1], *Emanuela Cristina de Castro*[1], *Carlos Eduardo Junqueira de Azevedo Tibiriçá*[1], *Juliana Wojciechowski*[1] & *Thelma Alvim Veiga Ludwig*[1]

[1] *Laboratório de Ficologia, Departamento de Botânica, Universidade Federal do Paraná,*
Caixa Postal 19031, Jardim das Américas, CEP 81531-980, Curitiba, PR, Brazil.
[2] *Corresponding author: Kaoli Pereira Cavalcante, e-mail: kaolicavalcante@gmail.com*

CAVALCANTE, K.P., TREMARIN, P.I., CASTRO, E.C., TIBIRIÇÁ, C.E.J.A., WOJCIECHOWSKI, J., LUDWIG, T.A.V. **Epiphytic *Eunotia* (Bacillariophyceae) on *Podostemum* from Santa Catarina, southern Brazil, including new observations on morphology and taxonomy of some rare recorded species.** Biota Neotropica. 14(3): e20140034. http://dx.doi.org/10.1590/1676-06032014003414

Abstract: *Eunotia* is a highly diverse diatom genus in the Neotropics. Considerable morphological variability of the valve showed in several species of the genus resulted in a quite confused taxonomy. Twelve *Eunotia* taxa attached to *Podostemum* spp. were investigated using the light microscopy (LM). Additionally, scanning electron microscopy (SEM) was used in order to investigate the fine morphology of most abundant taxa. Samples were collected from four rivers from the State of Santa Catarina between March 2009 and July 2010. The freshwater diatom flora from this south Brazilian state is poorly known in relation to its neighboring states. *Eunotia biseriatoides* has intermediate features between *Eunotia* and *Perinotia*, and the circumscription of these genera is discussed here. *Eunotia odebrechtiana* is very similar in morphology to *E. biseriatoides*, and the synonymy is proposed, as well as the transfer of the variety *E. odebrechtiana* var. *essequiboensis* to the species *E. biseriatoides*. The fine structure of *E. epithemioides* is showed for the first time in SEM, revealing the unusual position of the rimoportula in this genus. A new variety, *E. pseudosudetica* var. *rotundata* var. nov., was proposed and compared with the typical one. Except by *E. veneris*, all other eleven taxa presented here are new records for the State of Santa Catarina, Brazil.

Keywords: *Diatom, Eunotiaceae, lotic systems, periphyton, taxonomy.*

CAVALCANTE, K.P., TREMARIN, P.I., CASTRO, E.C., TIBIRIÇÁ, C.E.J.A., WOJCIECHOWSKI, J., LUDWIG, T.A.V. ***Eunotia* (Bacillariophyceae) epífitas sobre *Podostemum* em Santa Catarina, sul do Brasil, incluindo novas observações sobre a morfologia e taxonomia de espécies raramente registradas.** Biota Neotropica. 14(3): e20140034. http://dx.doi.org/10.1590/1676-06032014003414

Resumo: *Eunotia* é um gênero de diatomáceas altamente diverso na região neotropical. A considerável variabilidade morfológica encontrada nas valvas de várias espécies deste gênero resultam em uma taxonomia muito confusa. Neste estudo, 12 táxons de *Eunotia* aderidos a *Podostemum* spp. foram investigados usando microscopia óptica (MO). Adicionalmente, microscopia eletrônica de varredura (MEV) foi utilizada para investigar a morfologia ultraestrutural de táxons mais abundantes. Amostras foram coletadas em quatro rios do Estado de Santa Catarina entre março de 2009 e julho de 2010. A diatomoflórula deste estado é pobremente conhecida em relação aos estados vizinhos no sul do país. *Eunotia biseriatoides* é um taxon com características intermediárias entre *Eunotia* e *Perinotia* e a circunscrição destes gêneros é discutida aqui. *Eunotia odebrechtiana* é muito similar morfologicamente a *E. biseriatoides* e a sinonimização é proposta, bem como a transferência da variedade *E. odebrechtiana* var. *essequiboensis* à espécie *E. biseriatoides*. A ultraestrutura de *E. epithemioides* é apresentada pela primeira vez em MEV, revelando a posição atípica da rimoportula dentro deste gênero. Uma nova variedade, *E. pseudosudetica* var. *rotundata* var. nov., foi proposta e comparada com a variedade típica. Com exceção de *E. veneris*, todos os outros 11 táxons apresentados aqui são novos registros para Santa Catarina.

Palavras-chave: *Diatomácea, Eunotiaceae, perifíton, sistemas lóticos, taxonomia.*

Introduction

Eunotia Ehrenberg is a large periphytic diatom genus common in freshwater systems worldwide (Round et al. 1990). Diacritical features include dorsiventral valves, uniseriate striae, composed by small round poroids, raphe slits shortened lying at each pole and at least one rimoportula present near to the valve ends (Round et al. 1990). The species richness of this genus is high, and at least 1,500 species are estimated to exist throughout the world (Lange-Bertalot et al. 2011).

The diatom diversity in the Neotropics is considered rather high compared to the well-studied temperate regions of Europe and North America. Every new published flora results in dozens of new taxa proposed, some particularly endemics (e.g. Metzeltin & Lange-Bertalot 1998, 2007, Kociolek et al. 2001, Metzeltin et al. 2005). With regard to *Eunotia*, there are about 245 taxa identified to Brazilian environments (Metzeltin & Lange-Bertalot 1998, 2007, Torgan et al. 1999, Ferrari et al. 2007, Tremarin et al. 2008, 2009, Bicca & Torgan 2009, Bicca et al. 2011, Silva et al. 2011, Burliga & Kociolek 2012), of which 81 taxa were proposed as new to science (e.g. Torgan & Becker 1997, Burliga et al. 2007, Wetzel et al. 2010, 2011, Metzeltin & Tremarin 2011, Burliga & Kociolek 2012, Fuhrmann et al. 2013).

Recent studies on periphytic algae suggested that architecture of *Podostemum rutifolium* Warm. provides microhabitats for many algal species (mainly diatoms) and, therefore, is a key species in the structure of those communities. Aquatic plants are important structuring of algal communities and directly affect diversity and richness of the attached or associated microorganisms, especially in the lotic systems (Ferreira et al. 2011).

In the State of Santa Catarina, southern Brazil, there are few researches about diatoms (Souza-Mosimann 1980, Rodrigues 1984). The most of them are concentrated in littoral marine/estuarine communities, some of which recording freshwater species (Fernandes et al. 1990, Saupe & Souza-Mosimann 2003, Souza-Mosimann & Laudares-Silva 2005, Souza-Mosimann et al. 2011). Epiphytic freshwater diatoms are poorly studied in this region.

Here we described 12 *Eunotia* taxa from several rheophilic environments located in Santa Catarina, based on light and scanning electron microscopies analysis. These findings contribute to knowledge of the diatom flora from Santa Catarina, as well as from Brazil.

Material and methods

Three *Podostemum* Michaux species were collected in waterfalls of four distinct lotic systems of Santa Catarina: *P. distichum* (Cham.) Wedd from river Pelotas, *P. muelleri* Warm. from rivers Jacutinga and Irani, and *P. rutifolium* Warm. from river Correas, between March 2009 and July 2010 (Table 1).

Algal biofilm attached to the macrophytes was removed using toothbrushes and preserved in a 4% formaldehyde solution. Subsamples were cleaned following the procedure described in Simonsen (1974) and modified by Moreira-Filho & Valente-Moreira (1981). Permanent slides were mounted with Naphax resin (R.I.=1.74) for taxonomic analysis on light microscope (LM) Olympus BX-40 equipped with Olympus DP71 image capture equipment. For scanning electron microscopy (SEM), subsamples were dried directly on aluminum stubs and sputter-coated with gold. Samples were observed using JEOL JSM 6360LV microscope, operated at 15 kV and 8 mm working distance, which is housed in the Electron Microscopy Center of the Federal University of Paraná. Samples and permanent slides are stored in the herbarium of the Federal University of Paraná (UPCB, Table 1).

Results and discussion

Eleven *Eunotia* species and one non typical variety were identified from our samples. Eleven taxa (except by *E. veneris*) represent new records for Santa Catarina. Morphological descriptions and taxonomic observations are presented below.

Eunotia biseriatoides Kobayasi, Ando et Nagumo **var. *biseriatoides***, 1981, Proc. 6th Diat. Symp., p. 98, pl. 4, figs. 27–34.

Figs. 1-14

Synonyms: *Eunotia odebrechtiana* Metzeltin et Lange-Bertalot var. *odebrechtiana*, 1998, Iconogr. Diatomol. 5, p. 71, pl. 56, figs. 1-6, 13, 14

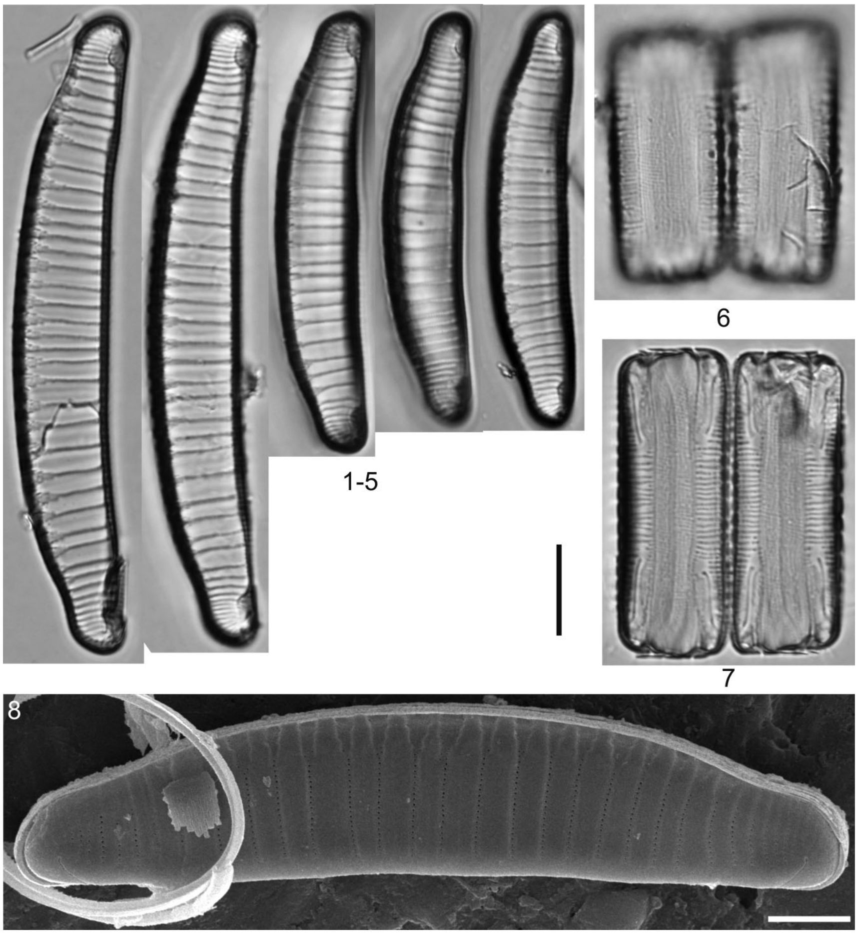
Eunotia monodon var. *constricta* Hustedt (excl. typus) sensu Simonsen, 1987, Atlas and Catalogue of the Diatom Types of Friedrich Hustedt, pl. 31 figs. 2, 3 (non fig. 1).

Rectangular frustules in lateral view, with longest sides undulate, caused by interfascicular thickening in the valve surface; dorsiventral valves, isopolar; dorsal margin convex, ventral margin concave, almost straight in median region; ends rounded, detached from the valve; radiate striae, denser in the ends, unisseriate, with short striae irregularly inserted around each striae at dorsal margin; delicate round areolae; terminal nodules thickened located on the ventral margin and somewhat distant from the ends; one rimoportulae per valve, located at valve extremity (Figs. 2, 5). Length 44.3-69.7 µm, width 7.6-10.7 µm, 5-7 striae (valve face) in 10 µm, 14-16 striae (ventral mantle) in 10 µm.

In SEM, areolae are simple round poroids, 33-35 in 10 µm (Figs. 8–10). Dorsal shortened striae are depressed compared to interstriae (Fig. 9). Striae are interrupted in the ventral valve-mantle junction by a hyaline edge (Figs. 9, 11, 13). Ventral valve mantle is more densely striated than valve surface (Fig. 13). External distal endings of raphe are simple, not reaching the valve center (Fig. 11). Externally, the raphe extends in oblique and slightly sinuous fissure at the ventral mantle, with simple

Table 1. Sampling data, with reference to local and date, host collected and register on herbarium of Federal University of Paraná (UPCB).

Herbarium Code UPCB (diatoms)	Sampling date	River	Geographic coordinates	*Podostemum* species
76020	10/07/2010	Pelotas	28°08'S; 49°37'W	*P. distichum*
76021	09/03/2010	Jacutinga	27°3'13.61"S; 51°41'54.28"W	*P. muelleri*
76022	17/03/2009	Correas	26°25'6.28"S 49°19'39.11"W	*P. rutifolium*
76024	09/03/2010	Irani	26°55'55.18"S 51°52'22.37"W	*P. muelleri*



Figures 1-8. *Eunotia biseriatoides* var. *biseriatoides*. **Figs 1-5.** Specimens in valve view, LM. **Fig. 6.** Frustules in dorsal view, LM. **Fig. 7.** Frustules in ventral view, LM. **Fig. 8.** Valve overview in SEM. Scale bars: 10 µm (Figs 1-7), 5 µm (Fig. 8).

proximal ends (Fig. 13). Internally, the raphe ends in coarse helictoglossa (Fig. 12). Internally, the rimoportula is small, sessile (Fig. 12), often hidden by septum, when valvocopula was not detached from valve (Fig. 14). Valvocopula has delicate scalloped projections towards the internal valve surface (pars interior) on each interstriae (Fig. 10, arrowheads).

Occurrence in the samples: UPCB 76024

Comments: this species, described by Kobayasi et al. (1981), is characterized by broadly raised interstriae and short striae inserted on the dorsal side of each striae. These authors have distinguished *E. biseriatoides* from *E. biseriata* Hustedt by the "undulate dorsal margin, absence of ventral striae and more slender shape of the valve" of the former. In the light microscope, undulate dorsal margins are only detected when valves lie in girdle view (figs. 6, 7). Ventral striae are probably a reflection of ventral mantle striae, rather than projections of mantle striae reaching the valve face. This feature can be observed in the Hustedt's iconotype of *E. biseriata*, but undiscernible in the Simonsen's illustration of holotype (Simonsen 1987, pl. 567, figs. 9-11). Mantle striae can be observed also in *E. biseriatoides* from present study (Figs. 5, 7) and synonyms *E. monodon* var. *constricta* (Simonsen 1987, pl. 31, fig. 2) and *E. odebrechtiana* var. *odebrechtiana* (Metzeltin & Lange-Bertalot 1998, pl. 56, figs. 1, 4). These two characters do not seem to be strong criteria for differentiation. The best distinguishing feature between *E. biseriata* and *E. biseriatoides* is the valve shape, which is slender (length/width ratio 5.1-6.9) in the latter and more robust (length/width ratio 3.4) with broadly round apices, detached from the valve, in the former (Table 2). *E. biseriata* was described in Hustedt (1952) based on a single specimen. A population-based morphometric study as well as SEM analysis of this taxon are necessary to clarify the boundaries between these taxa.

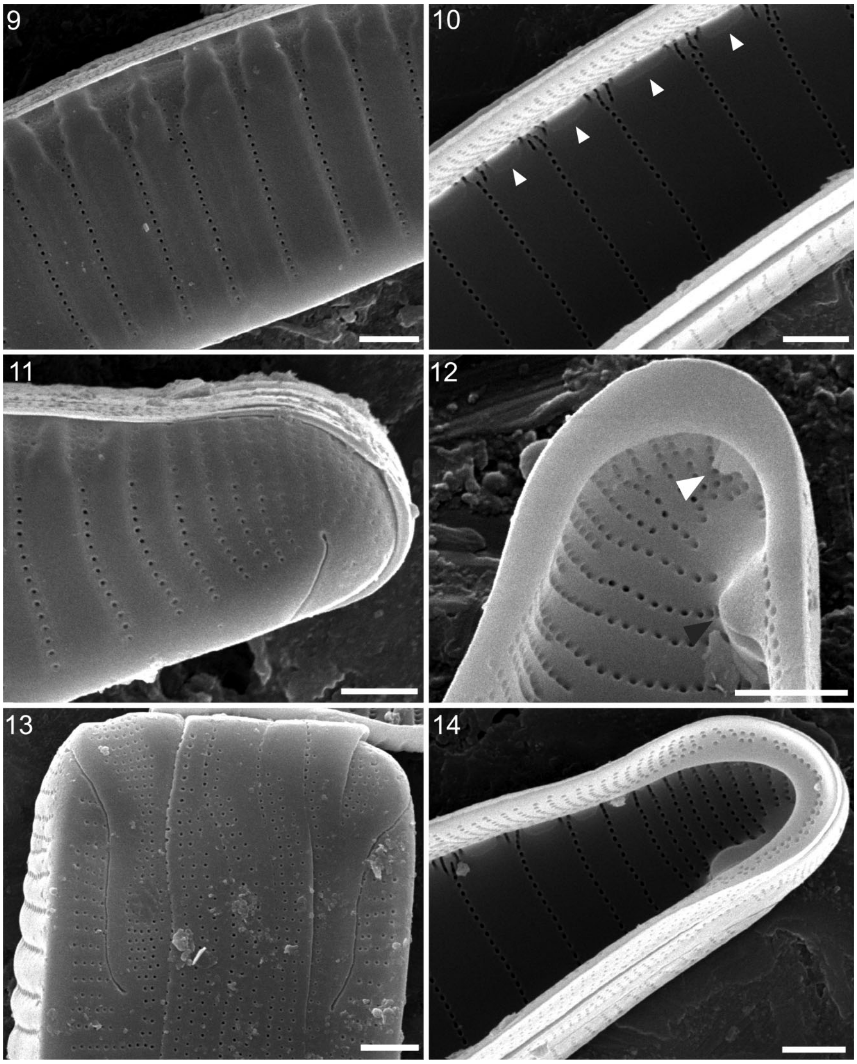
The species proposed by Metzeltin & Lange-Bertalot (1998) as *Eunotia odebrechtiana* can not be accurately distinguished of *E. biseriatoides* and, therefore, is a younger synonym from the latter. As stated by these authors, the figures 2 and 3 in plate 31 from Simonsen (1987), misidentified as isolectotype of *E. monodon* var. *constricta*, also correspond to *E. biseriatoides*.

Eunotia odebrechtiana var. *essequiboensis* was also proposed by Metzeltin & Lange-Bertalot (1998) based on valves with structure similar to the typical variety, but with narrower valves (6.5-8.5 µm) resulting in the length/width ratio of 7.8-10.2 (Table 2). Because of the typical variety is here synonymized with *E. biseriatoides*, a new combination of this variety is needed:

Eunotia biseriatoides* var. *essequiboensis (Metzeltin et Lange-Bertalot) Cavalcante, Tremarin et T. Ludwig comb. nov.

Basionym: *Eunotia odebrechtiana* var. *essequiboensis* Metzeltin et Lange-Bertalot, 1998, Icon. Diat. 5, p. 72, pl. 57, figs. 1, 2.

The striation pattern present in *Eunotia biseriatoides* and *E. biseriatoides* var. *essequiboensis* is very similar to that found in *Perinotia* Metzeltin et Lange-Bertalot. The genus *Perinotia* was



Figures 9-14. *Eunotia biseriatoides* var. *biseriatoides* in SEM. **Fig. 9.** Detail of valve center, external view. **Fig. 10.** Internal view of valve center, showing valvocopula projections (arrowheads). **Fig. 11.** External view of valve end. **Fig. 12.** Internal view of valve end , showing helictoglossa (black arrowhead) and small rimoportula (white arrowhead). **Fig. 13.** Detail of ends in ventral view of an entire frustule, showing girdle bands and raphe fissure. **Fig. 14.** Internal view of valve end, with valvocopula and septum. Scale bars: 2 µm.

described to accommodate eunotioid taxa that shows: uniseriate striae becoming multiseriate towards the dorsal side of valve, in which smaller poroids lie in deltoid depressions; internally, presence of thickened costae between each two striae; valvocopula projections on the internal dorsal valve surface. Rimoportula has not been observed in this genus. Currently, there are only two *Perinotia* species recognized (Metzeltin & Lange-Bertalot 2007, Ferrari et al. 2009).

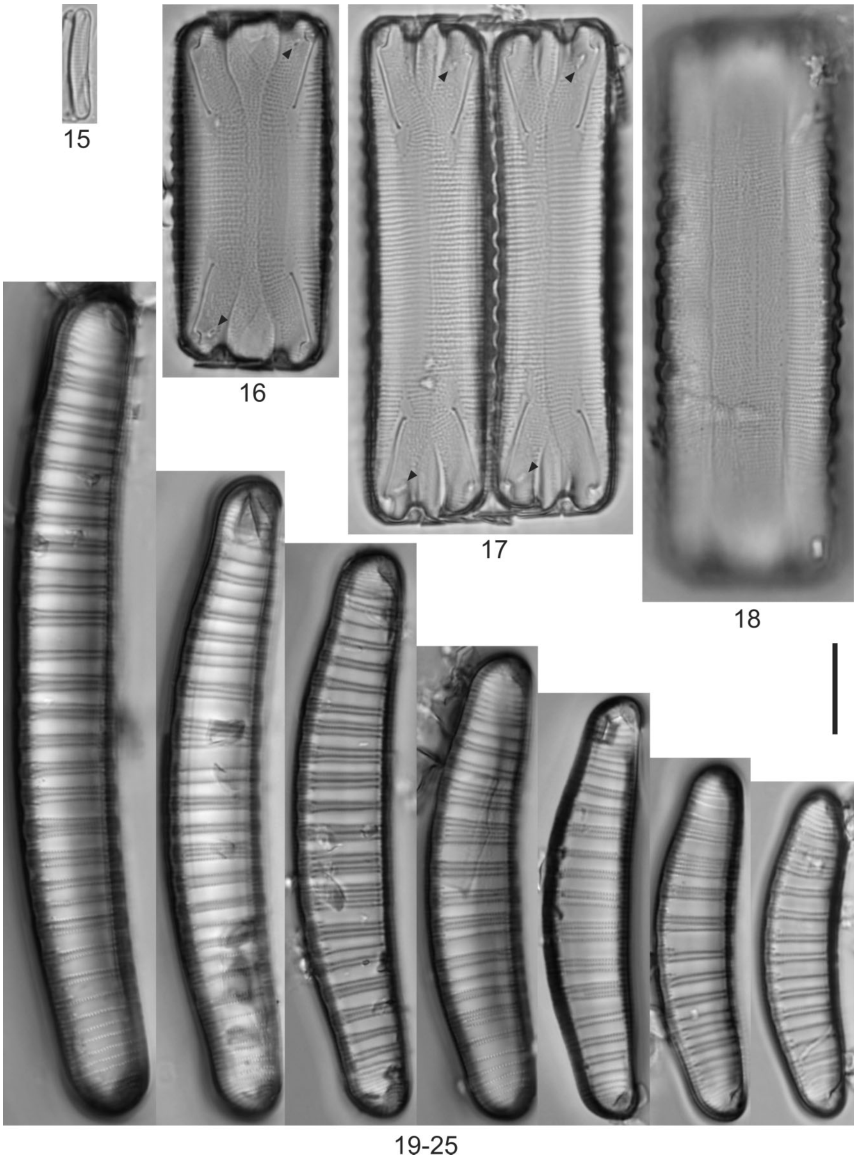
Considering the set of diagnostic features above mentioned for *Perinotia*, *Eunotia biseriatoides* deserves to be highlighted as a species that shares features of both genera: this species possess rimoportula, like in *Eunotia*, and possess striae pattern and valvocopula projections like in *Perinotia*. However, there is no internal costa between dorsal striae.

Rimoportula is frequent in all the Eunotiaceae genera, except in *Semiorbis* Patrick. It has never been observed in *Perinotia* yet. But, this genus has a well-developed septum and a thick mantle edge, which can easily hide a small sessile labiate process. In all SEM pictures of *Perinotia* taxa recorded so far (Metzeltin & Lange-Bertalot 2007, Ferrari et al. 2009), none of them accurately allow us to assert the lack of rimoportula. Therefore, presence of rimoportula in *Perinotia* remains to be confirmed. Moreover, the typical striae pattern and valvocopula projections of *Perinotia* plus the absence of costae in *Eunotia biseriatoides*, still allow us to raise questions concerning the consistency of *Perinotia* as currently diagnosed. Unusual striae pattern and presence of costae are presented as weak diagnostic characters to distinguish generic level in other diatom groups (eg. Reichardt 2007, 2008, Tremarin et al. 2011).

Perinotia taxa are poorly known, besides the frustule morphology. Plastids and chain formation, for example, have not been studied yet. Transferring *E. biseriatoides* to *Perinotia* is still premature, and requires more detailed analysis. Combined ultrastructural, molecular, and ecological investigations may provide a better elucidation about the circumscription of this taxon.

Eunotia* cf. *botuliformis Wild, Nörpel et Lange-Bertalot in Lange-Bertalot, 1993, Biblioth. Diat. 27, p. 29, pl. 33, figs. 2–15.
Fig. 15

Valve slightly dorsiventral, isopolar; dorsal margin convex, ventral margin slightly concave; ends rounded, not detached



Figures 15-25. *Eunotia* spp. in LM. **Fig. 15.** *E.* cf. *botuliformis* in valve view. **Figs. 16-25.** *E. epithemioides*. **Fig. 16, 17.** Ventral view. **Fig. 18.** Dorsal view. **Figs 19-25.** Valve view. Scale bar: 10 µm.

from the valve; striae parallel, equidistant; areolae not discernible in LM; terminal nodules near to poles; rimoportula not observed. Length 12.1 µm, width 2.2 µm, 26 striae in 10 µm.

Occurrence in the samples: UPCB 76024

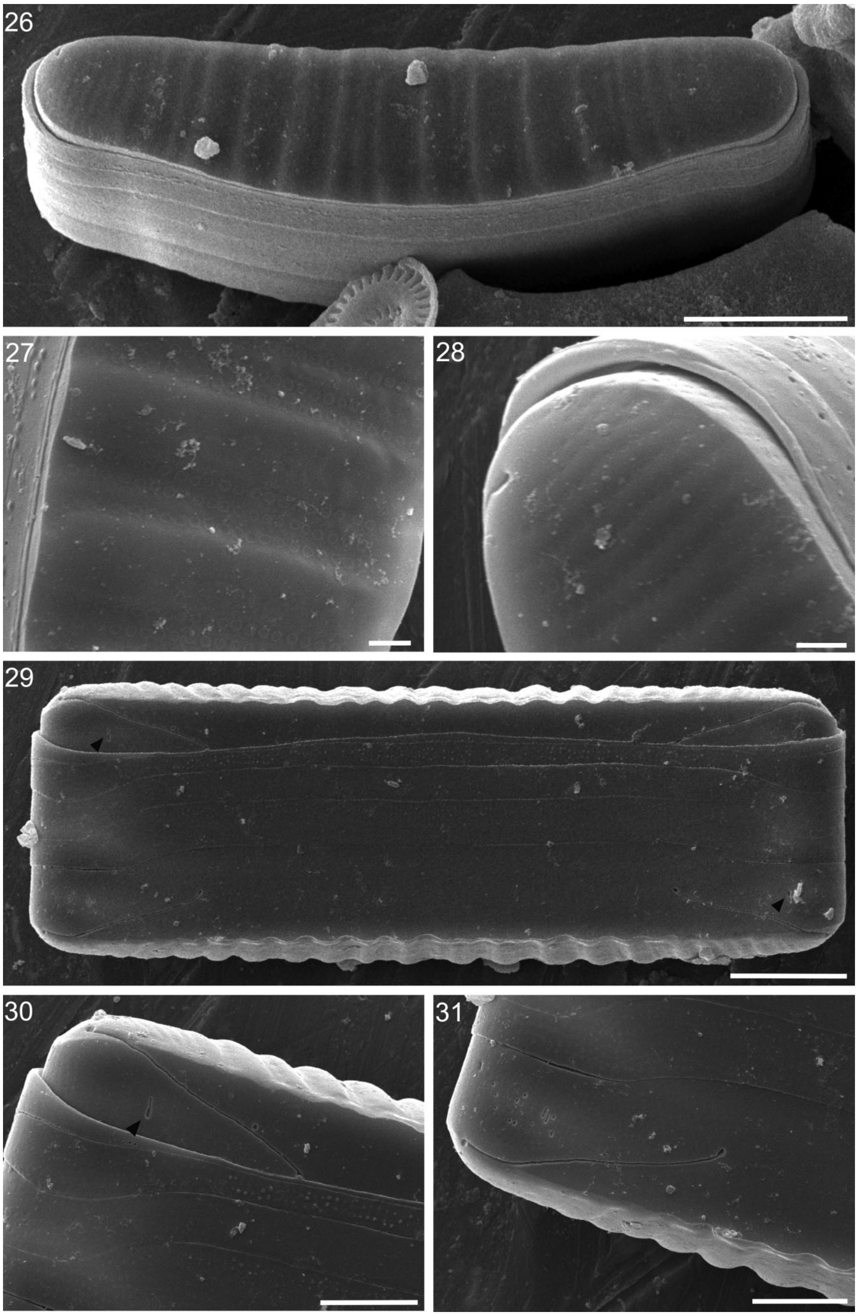
Comments: the single specimen observed in this study shows lower width and striae density than that found in the literature (width 2.7-3.8 µm, 15-22 striae in 10 µm) (Lange-Bertalot 1993; Lange-Bertalot et al. 2011). Due to the morphometric discrepancies and the rarity of this taxon in the samples, its identity cannot be accurately determined. Further studies of this population may provide clues about the precise identity of this taxon.

Eunotia epithemioides Hustedt in Schmidt 1913, Atlas Diat., pl. 287, figs. 16-19.

Figs. 16-35

Rectangular frustules in lateral view, with longest sides strongly undulate, caused by interfascicular thickening in the valve surface; dorsiventral valves, isopolar; dorsal margin convex, ventral margin concave; ends rounded, detached from the valve; parallel to radiate striae, arranged in irregular fascicles with one to five striae; intercalated by short striae at dorsal margin; delicate round areolae; terminal nodules thickened near to poles; rimoportulae located at ventral mantle, one per valve, diagonally opposed in the frustule, such that can only be observed in lateral ventral view (Figs. 2, 3). Length 35.9-89.4 µm, width 7.5-10.0 µm, 9-14 striae (valve face) in 10 µm, 16-19 striae (ventral mantle) in 10 µm, 26-30 areolae in 10 µm.

In SEM, external areolae occlusions are difficult to observe (Figs. 26, 27). External distal endings of raphe are simple, not reaching the valve center (Fig. 28). Externally, the raphe extends in oblique fissure at the ventral mantle, with proximal ends dilated in pore (Fig. 31). Externally, the rimoportula aperture is slit-like, perpendicular to the apical axis of the frustule (Figs. 29, 30), and internally sessile (Figs. 34, 35). Areolaeare simple poroids, striae were interrupted in ventral margin, at junction between valve surface and mantle, but not at dorsal margin (Figs. 32, 33). Mantle striae are equidistant and denser (Fig. 33) than those of valve surface. Short striae in valve surface are composed by slight projection of some mantle striae at dorsal



Figures 26-31. *Eunotia epithemioides* in SEM. **Fig. 26.** External valve overview. **Fig. 27.** Detail of valve center, external view. **Fig. 28.** External view of valve end. **Figs 29-31.** Frustule in ventral view, showing the slit-like external opening of the rimoportulae (arrowheads). Scale bars: 10 µm (26, 29), 5 µm (30, 31), 2 µm (27, 28).

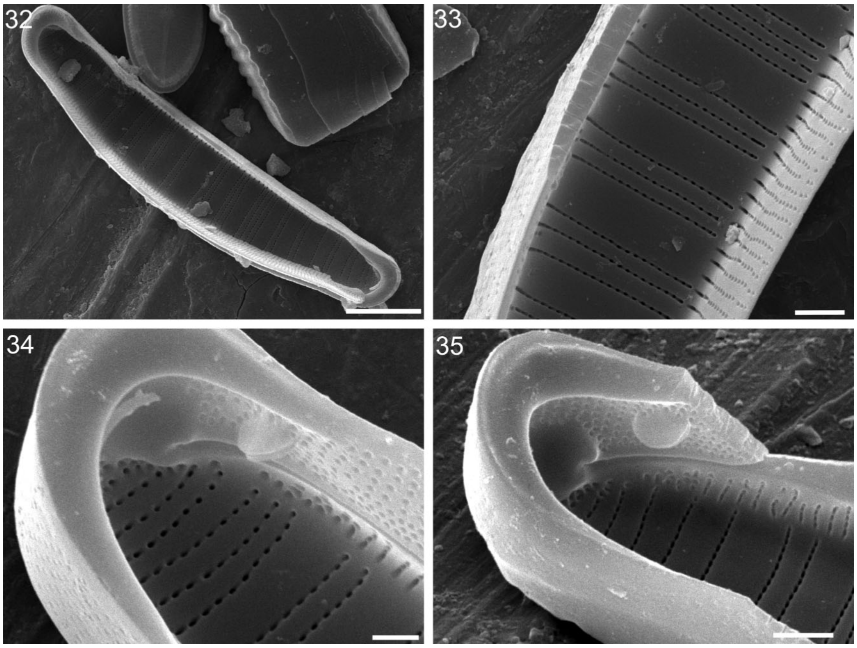
margin (Fig. 33). Pseudosepta present in the valve apices (Figs 34, 35).

Occurrence in the samples: UPCB 76022

Comments: the analyzed population is compatible with morphometric data from the iconotypes showed by Schmidt (1913, pl. 287, length 55.5-77.8 µm, width 9.4-11.7 µm, 13-19 striae in 10 µm, 27 areolae in 10 µm) and with the lectotype found in Simonsen (1987, pl. 34, length 54-72 µm, width 11-12 µm, 15-16 striae in 10µm, 24 areolae in 10µm). However, we are expanding the ranges of valve width and striae density. We found smaller individuals than those selected as type material (we could compare only to specimens illustrated as type and we do not know, however, the morphological range of the type population). Additionally, our specimens show fascicles with 1-5 striae, while the type material had 2-8 striae in each fascicle. This variation obviously results in lower striae density. This difference in striae density may be probably a factor affected by environmental conditions, further than a species-specific feature.

This species has not been previously documented in SEM, so morphological features as raphe and rimoportulae position were hitherto unknown. Rimoportula located in ventral mantle is not typical in *Eunotia* spp. Generally they have a single and sessile rimoportula, one per valve and lying in the valve apex (Round et al. 1990).

Eunotia epithemioides was originally described from a brackish lagoon of Republic of Cameroon, East Africa. The



Figures 32-35. *Eunotia epithemioides* in SEM. **Fig. 32.** Internal valve overview. **Fig. 33.** Detail of valve center in internal view. **Figs 34, 35.** Internal view of ends showing helictoglossa and rimoportula located in ventral margin. Scale bars: 10 µm (32), 2 µm (33, 35), 1 µm (34).

species has few world records from tropical Asia and Africa (Hustedt 1949). In Brazil, it has been found only in southern region, in the state of Paraná by Lozovei & Shirata (1990), Ludwig & Flôres (1995) and Bartozek et al. (2013).

Eunotia groenlandica (Grunow) Nörpel-Schempp et Lange-Bertalot in Lange-Bertalot & Metzeltin 1996, Icon. Diat. 2, p. 51, pl. 17, figs. 25–27.

Fig. 36

Dorsiventral, isopolar valve; dorsal margin slightly convex; ventral margin slightly concave; subcapitate ends, dorsally flexed, detached from the valve; striae parallel, equidistant; areolae inconspicuous on LM; terminal nodules distinct, near to poles; rimoportula not observed. Length 22.1 µm, width 2.2 µm, 19 striae in 10 µm.

Occurrence in the samples: UPCB 76021

Comments: the analyzed specimen is consistent with individuals from lectotype slide showed by Lange-Bertalot et al. (2011) except for the narrower valvae and greater density of striae (pl. 137, figs. 1-3: 2.7-4 µm wide, 12-16 striae in 10 µm). However the synonymized *E. fallax* var. *gracilima* Krasske shows similar width (2.0 µm) (Lange-Bertalot et al 1996).

Eunotia meridiana Metzeltin et Lange-Bertalot, 1998, Icon. Diat. 5, p. 67, pl. 59, figs. 7–10.

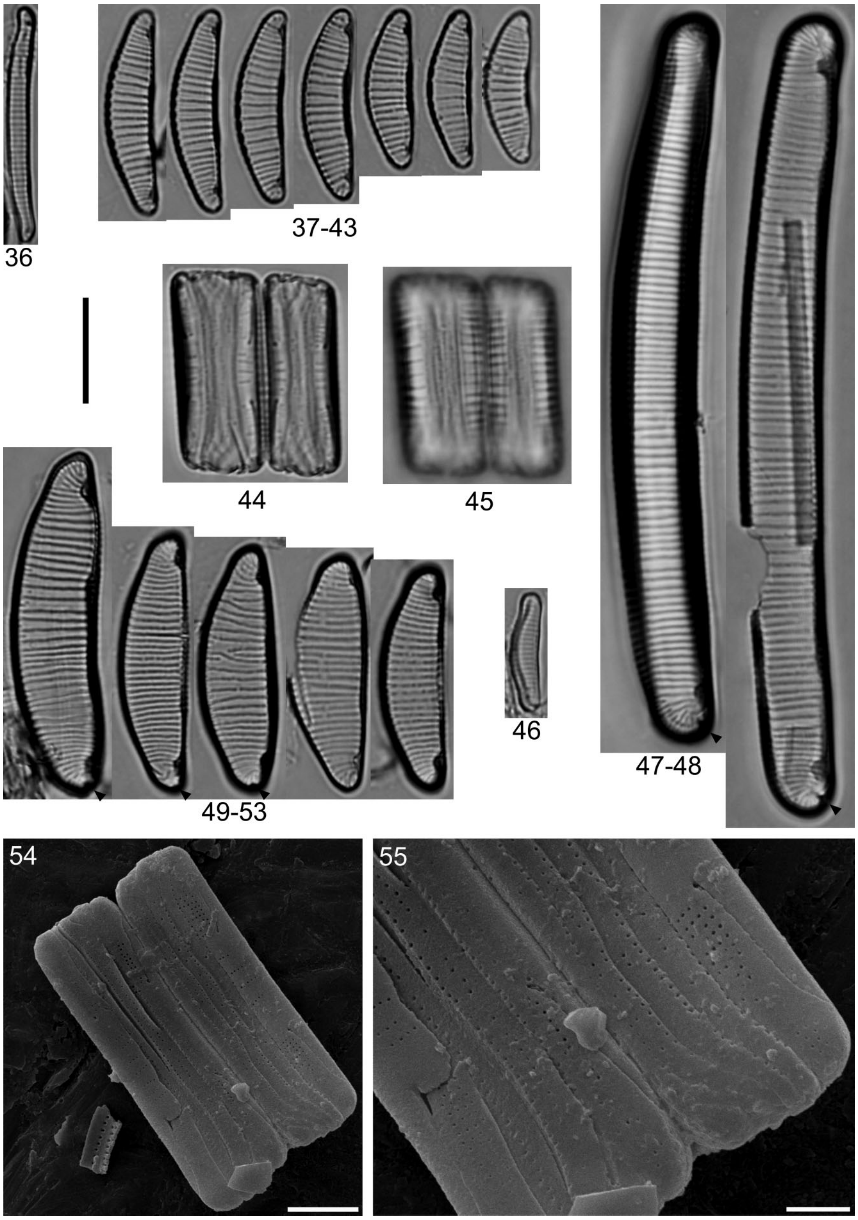
Figs. 37-45, 54, 55

Rectangular frustules in lateral view, joined in linear chains by overlapping of valve surfaces from adjacent cells; valve surface flat to slightly concave; dorsiventral, isopolar valves; dorsal margin convex, ventral margin straight to slightly concave in the middle; ends rounded to attenuated-rounded, not detached from the valve; radiate striae, not equidistant, with more spaced median striae; mantle striae more clearly spaced and separated from the others in lateral view (Figs. 44, 45); areolae inconspicuous on LM; terminal nodules distinct, rimoportula not observed. Length 14.4-19.3 µm, width 4.1-4.6 µm, 12-16 striae in 10 µm.

In SEM, delicate areolae, punctuated. About three girdle bands per valve, ornated by one or two irregular rows of poroids. Proximal raphe endings as a simple pore.

Table 2. Main morphometric and distributional features of *Eunotia biseriatoides*, its synonyms, varieties and allied taxa.*Protologue.**Measured from the type illustration.

Taxon	Reference	Length (µm)	Width (µm)	Striae in 10 µm	Length/width ratio	Distribution
E. biseriatoides	Present study	44.3-69.7	7.6-10.7	5-7	5.1-6.9	Santa Catarina, S Brazil
	Kobayasi et al. (1981)*	37.5-57	6.5-9	4-9	7.5	Various regions of Japan
E. odebrechtiana var. *odebrechtiana*	Metzeltin & Lange-Bertalot (1998)*	30-85	8.5-13	4-7.5	3.2-6.5	Santos, SE Brazil.
E. odebrechtiana var. *essequiboensis*	Metzeltin & Lange-Bertalot (1998)*	68-73	6.5-8.5	6-7 **	7.8-10.2	Guyana
Eunotia monodon var. *constricta* sensu Simonsen (1987)	Simonsen (1987), pl. 31 figs 2,3	66.5	11	6	6.0	Guyana
E. biseriata	Hustedt (1952)*	38	11	6-10	3.4	Amazon, N Brazil



Figures 36-55. *Eunotia* spp. **Fig. 36.** *E. groenlandica*, LM. **Figs 37-45.** *E. meridiana*, LM. **Figs 37-43.** Valve view. **Fig. 44.** Ventral view. **Fig. 45.** Dorsal view. **Fig. 46.** *E. paludosa*, LM. **Figs 47, 48.** *E. parallela*, LM. Arrowheads indicate rimoportulae position. **Figs. 49-53.** *E. pirla*, LM. Arrowheads indicate rimoportulae position. **Figs 54, 55.** *E. meridiana* in SEM, ventral view. Scale bars: 10 µm (36-53), 5 µm (54), 2 µm (55).

Occurrence in the samples: UPCB 76024

Comments: morphometric data agree with those recorded from original population (Metzeltin & Lange-Bertalot 1998) and another brazilian record (Tremarin et al. 2008). It is similar to *E. incisa* Gregory, which is usually narrower and has lower striae density and more elongated ends (Metzeltin & Lange-Bertalot 1998). Also differs from *E. pirla* Carter et Flower that shows more convex ventral margin, ends detached from the valve and denser striae (Carter & Flower 1988, Tremarin et al. 2008). Whole frustules have never been illustrated in lateral view and the striae morphology in the ventral mantle seems to be a regular criterium for identifying *E. meridiana* in this view. This is the first SEM documentation of the species**.**

Eunotia paludosa Grunow, 1862, Ver. Kais.-Königl. Zool.-Bot. Ges. Wien 12, p. 336, pl. 6, fig. 10.

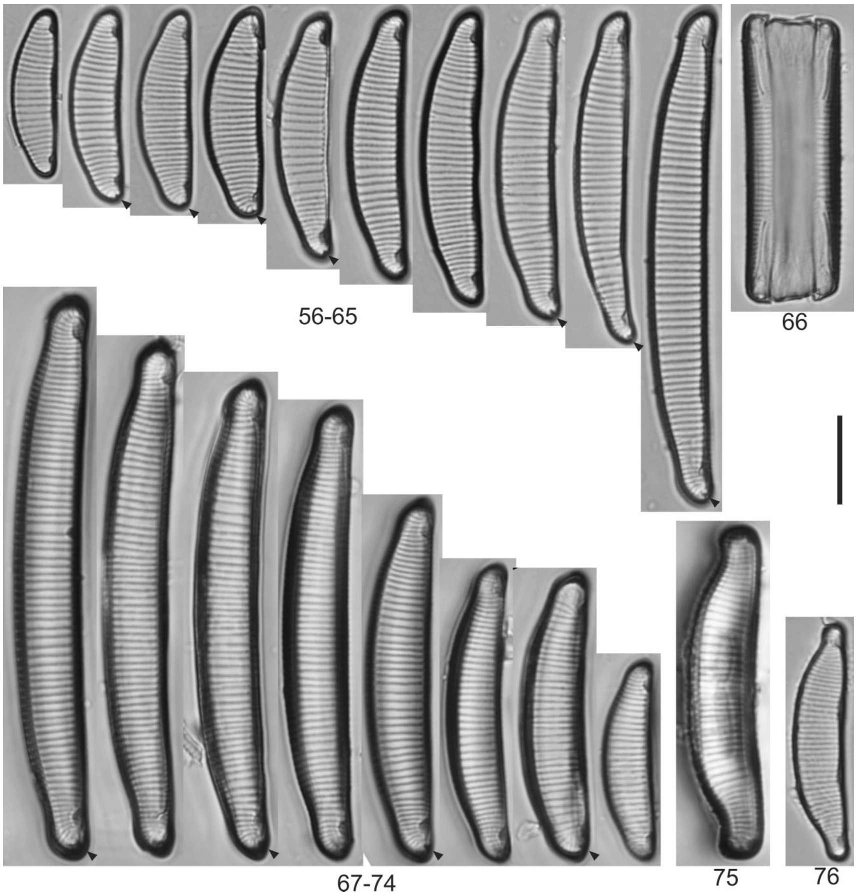
Fig. 46

Dorsiventral, isopolar valves; dorsal margin convex, ventral margin almost straight; ends rounded, slightly detached from the valve; parallel striae, equidistant; areolae, terminal nodules and rimoportula inconspicuous in LM. Length 11.2 µm, width 2.7 µm, 22 striae in 10 µm.

Occurrence in the samples: UPCB 76024

Comments: Krammer & Lange-Bertalot (1991) show a highly variable population for *E. paludosa*; but certainly, the specimens designated there should be reviewed. Our exemplar is especially similar to fig. 4 in plate 155 from those authors and is also in accordance to the description provided by Lange-Bertalot et al. (2011), based on lectotypified specimens of Grunow's protologue, and to the population illustrated in plate 147, figs. 24–29.

Eunotia parallela Ehrenberg, 1843, Abh. Kön. Akad. Wiss. Berlin, p. 414.



Figures. 56-76. *Eunotia* spp. in LM. **Figs 56-66.** *E. pseudosudetica* var. *pseudosudetica*. **Figs 56-65.** Valve view. Arrowheads indicate rimoportula position. **Fig. 66.** Ventral view. **Figs. 67-74.** *E. pseudosudetica* var. *rotundata* var. nov., valve view. Arrowheads indicate rimoportula position. **Fig. 75.** *E. tropico-arcus*. **Fig. 76.** *E. veneris*. Scale bar: 10 µm.

Figs. 47–48

Dorsiventral, isopolar valves; dorsal margin convex, ventral margin slightly concave, margins parallel in the most of valve length; ends broadly rounded, not detached from the valve; striae parallel in the middle, becoming radiate towards the ends, not equidistant; short striae in the ends accompanying the apex curvature; areolae inconspicuous in LM; thick terminal nodules; terminal raphe fissures simple, almost reaching the apex center (Fig. 33); rimoportula visible in LM, one per valve, located between pole and ventral margin. Length 68.9-75.3 µm, width 7.7-8.2 µm, 11-15 striae in 10 µm.

Occurrence in the samples: UPCB 76024

Comments: Lange-Bertalot et al. (2011) remarked the difficulty of leptotypifying *E. paralella* from Ehrenberg's (1843) syntypes. Mayama in Lange-Bertalot et al. (2011), proposed a lectotype, based on analysis of one only valve, notably observed by Ehrenberg, but without illustrations. Our specimens agrees with his morphological description (Lange-Bertalot et al., 2011), except by the lectotype is longer (ca. 130 µm), wider (ca. 16 µm) and has less striae density (7-8.5 in 10 µm). Nevertheless, the ''sausage-like'' shape, with rounded, not protracted ends, are typical features of the species. Moreover, our data agree with *E. paralella* populations described in Patrick & Reimer (1966) and Furey et al. (2011), based in many specimens. *E. faba* Ehrenberg is a similar taxon but differs by the typical bean-like outline and shorter length (16-65 µm) (Furey et al. 2011, Lange-Bertalot et al. 2011). *Eunotia paralelladubia* Lange-Bertalot et Mayama also differs by being wider (8-11 µm) and having terminal nodules closer to ends (Lange-Bertalot et al. 2011).

Eunotia pirla Carter et Flower, 1988, Diat. Res. 3, p. 2, figs 5–48, 51–56.

Figs. 49–53

Dorsiventral, isopolar valves; dorsal margin convex, ventral margin straight with irregular margin thickening (slightly triundulate); ends attenuate-rounded, detached from the valve; striae parallel in the middle, becoming radiate toward the ends, not equidistant; short striae near to dorsal margin; areolae punctuated, hardly observed in LM; terminal nodules distinct; one rimoportula per valve, located on valve extremity (Figs. 35–37). Length 20.8-32.1 µm, width 6.0-8.3 µm, 13-17striae in 10 µm.

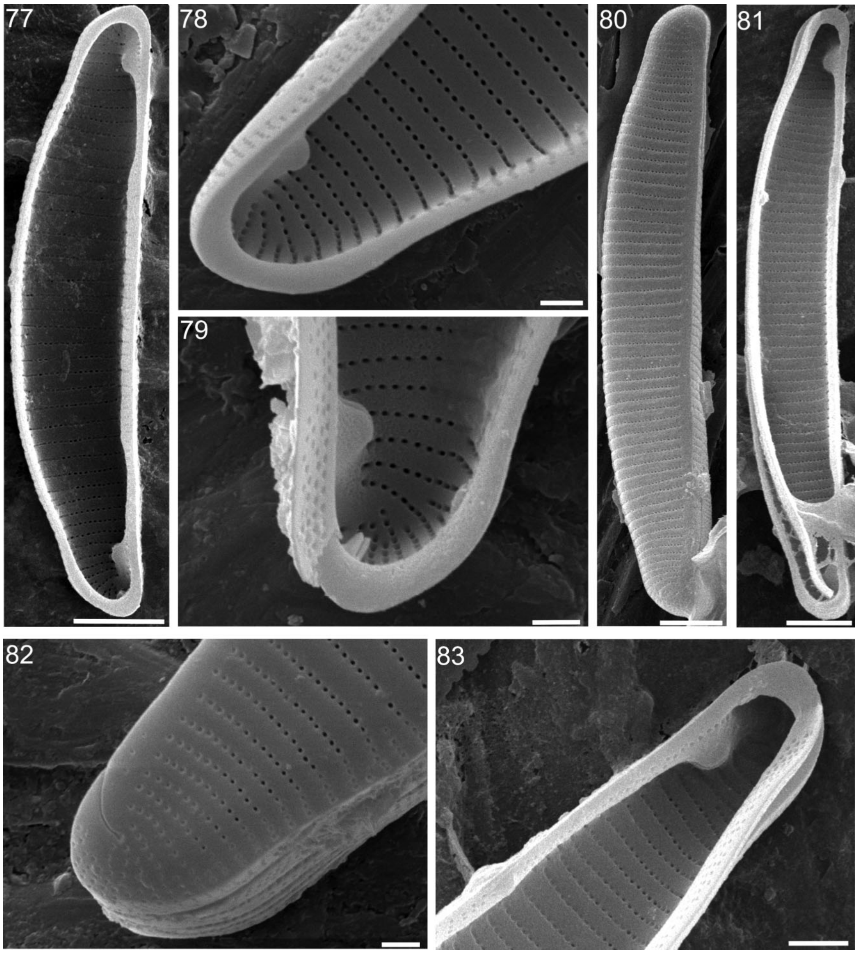
Occurrence in the samples: UPCB 76024

Comments: the type population showed by Carter & Flower (1988) and Lange-Bertalot et al. (2011) is similar to our material. Carter & Flower (1988) presented individuals with ventral margin sharply triundulate (central region of ventral margin is convex) and discussed that this feature is highly variable into this species.

Eunotia pseudosudetica Metzeltin, Lange-Bertalot et García-Rodríguez **var. *pseudosudetica***, 2005, Icon. Diat.15, p. 57, pl. 24, figs. 15-18.

Figs. 56-66, 77-79

Rectangular frustules in lateral view, valve surface straight; dorsiventral, isopolar valves; dorsal margin convex, ventral



Figures 77-79. *Eunotia pseudosudetica* var. *pseudosudetica* in SEM. **Fig. 77.** Internal valve overview. **Figs 78, 79.** Detail of valve ends in internal view, showing helictoglossa and rimoportula. **Figs 80-83.** *E. pseudosudetica* var. *rotundata* var. nov. in SEM. **Figs 80, 81.** External and internal valve overview, respectively. **Fig. 82.** Detail of valve end in external view. **Fig. 83.** Detail of valve end in internal view. Scale bars: 5 µm (77, 80, 81), 2 µm (83), 1 µm (78, 79, 82).

margin straight, thickened in the median region, in the proximal raphe endings located in the mantle; ends nose-like, ventrally flexed, detached from the valve; striae parallel in the middle, radiate in the ends, not equidistant; punctuate, delicate areolae, hardly resolved in LM; terminal nodules distinct; one rimoportula per valve, close to the valve extremity (Figs. 43-51). Length 18.7-55.8 µm, width 5.5-7.8 µm, 11-15 striae in 10 µm.

In SEM, areolae in round poroids, 36-38 in 10 µm; ventral margin thickened in the median portion (Fig. 77); helictoglossae are developed; rimoportula is small and sessile, located in the valve surface-mantle junction, and can be hidden by the thick valve wall (Fig. 77-79).

Occurrence in the samples: UPCB 76024

Eunotia pseudosudetica* var. *rotundata Cavalcante, Tremarin et T. Ludwig var. nov.

Figs. 67-74, 80-83

Diagnosis: It differs from typical variety by the more rounded ends, less marked dorsiventrality of the valve and ventral margin without thickening.

Description: dorsiventral, isopolar valves; dorsal margin convex to slightly convex, ventral margin straight to slightly concave; rounded ends, detached from the valve; striae parallel, radiate in the ends, not equidistant; areolae punctuate, discernible in LM in some individuals; terminal nodules distinct; one small rimoportula per valve, near to poles. Length 22.6-63.7 µm, width 5.2-6.8 µm, 11-14 striae in 10 µm, 34-41 areolae in 10 µm.

In SEM, areolae externally open in round poroids, 36-38 in 10 µm (Fig. 80); terminal raphe fissures are simple, almost in the center of valve end (Fig. 82); helictoglossae are developed, septum ornated by small poroids (Fig. 83); rimoportula not observed.

Holotype: BRAZIL, Santa Catarina: Herval D'oeste, Irani River. UPCB 76024. Periphyton attached to *Podostemum muelleri*. 09.III.2010. A.S. Melo & A. Nuernberg. Holotype represented by fig. 67.

Comments: this taxon is usually recorded as *Eunotia pectinalis* (Kützing) Rabenhorst in Brazilian researches (eg. Moro et al. 1994, Raupp et al. 2006, Talgatti et al. 2007) or *E. sudetica* O. Müller (eg. Ludwig & Flôres 1995). *E. pectinalis* has undulate dorsal margins and narrower ends (Tuji & Williams 2005). Regarding *E. sudetica*, this is a taxon often illustrated with high morphological variability (eg. Krammer & Lange-Bertalot 1991, Levkov et al. 2007, Lange-Bertalot et al. 2011). As the type material of this species is unknown, it is difficult to define the original forms of *E. sudetica stricto sensu*. In contrast, *E. pseudosudetica* is a well-defined taxon (Metzeltin et al. 2005), and the population analyzed here is more similar to this taxon than to the former. Valve contour and apices are sufficient to propose this taxon as a new variety.

Our population was very similar to those determined as *E. sudetica* by Frenguelli (1933, pl. 8, figs. 8, 9), from northern

Argentina, as "*Eunotia* (?nov.) spec." by Metzeltin & Lange Bertalot (1998, pl. 58, figs. 5, 8) from northern Brazil and as "*Eunotia* sp. 1" by Bertolli et al. (2010), from southern Brazil. All of them probably correspond to *Eunotia pseudosudetica* var. *rotundata*. *Eunotia* aff. *donatoi* recorded in Santos et al. (2011) is similar to this taxon, but differs by lower striae density (9-10 in 10 µm) and narrower ends.

Eunotia tropico-arcus Metzeltin et Lange-Bertalot, 2007, Icon. Diat. 18, p. 123, pl. 74, figs. 1–10.

Fig. 75

Dorsiventral, isopolar valve; dorsal margin convex with median region almost straight; ventral margin slightly concave, especially in central region; truncate ends, dorsally flexed, detached from the valve; parallel striae, becoming radiate towards the ends, equidistant; areolae inconspicuous in LM; terminal nodules delicate, near to ends; rimoportula not observed. Length 37.4 µm, width 8.1 µm, 15 striae in 10 µm.

Occurrence in the samples: UPCB 76020

Comments: the single specimen found conforms to the protologue's description (Metzeltin & Lange-Bertalot 2007), except for striae density (11-12 in 10 µm). However, a venezuelan taxon identified as *E. praerupta* Ehrenberg (Lange-Bertalot 1993, pl 33, fig. 5), but reinterpreted by Metzeltin & Lange-Bertalot (2007) as *E. tropico-arcus*, possesses very similar measurements to our exemplar (length 40 µm, width 9 µm, 15 striae in 10µm). Hence, we believe that the striae density may reach15 striae in 10 µm. It differs from *E. praerupta* by the latter is wider (10-18 µm, Lange-Bertalot et al. 2011) and shows strongly convex dorsal margin. Also differs from *E. bidens* Ehrenberg especially by dorsal margin strongly biundulate and more truncate ends of the latter. Finally, *Eunotia tropico-arcus* differs from *E. arcus* Ehrenberg because the latter shows less arcuate valves, ventral margins less concave and ends narrower than central region (Metzeltin & Lange Bertalot 2007).

Eunotia veneris (Kützing) De Toni, 1892, Syll. Alg. Omn. Hucusque Cogn. 2(2), p. 794.

Fig. 76

Dorsiventral, isopolar valve; dorsal margin convex, ventral margin almost straight, with slight central tumescence; subcapitate ends, detached from the valve; striae parallel in the middle, radiate towards the ends, equidistant; areolae inconspicuous; terminal nodules distinct; rimoportula inconspicuous. Length 26.5 µm, width 5.6 µm, 17 striae in 10 µm.

Occurrence in the samples: UPCB 76024

Comments: identification of this taxon was based on Kützing's type material of *Eunotia veneris* illustrated in Lange-Bertalot et al. (2011). *E. veneris* had ever been cited in state of Santa Catarina, in Conceição lagoon (Souza-Mosimann 1980) and Tubarão river (Rodrigues 1984), but without illustrations.

Acknowledgments

We thank MSc. Edimar F. Lopes for making macrophytes samples available, and MSc. Anderson S. de Mello for providing us the sampling data; the Electron Microscopy Center of UFPR provides the SEM analysis; the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) granted a scientific productivity scholarship to TAV. Ludwig, and REUNI, CAPES and CNPq granted master scholarship to CEJA.Tibiriçá, J. Wojciechowski and EC. Castro, respectively; we are also grateful to Dr. John Patrick Kociolek for valuable comments on the manuscript.

References

BARTOZEK, E.C.R, BUENO, N.C., LUDWIG, T.A.V., TREMARIN, P.I., NARDELLI, M.S. & ROCHA, A.C.R. 2013. Diatoms (Bacillariophyceae) of Iguaçu National Park, Foz do Iguaçu, Brazil. Acta Bot. Bras. 27: 108–123, http://dx.doi.org/10.1590/S0102-33062013000100012

BERTOLLI, L.M., TREMARIN, P.I. & LUDWIG, T.A.V. 2010. Diatomáceas perifíticas em *Polygonum hydropiperoides* Michaux, reservatório do Passaúna, Região Metropolitana de Curitiba, Paraná, Brasil. Acta Bot. Bras. 24:1065–1081, http://dx.doi.org/10.1590/S0102-33062010000400022

BICCA, A.B., TORGAN, L.C. & SANTOS, C.B. 2011. Eunotiaceae (Eunotiales, Bacillariophyta) em ambientes lacustres na Planície Costeira do Sul do Brasil. Rev. Bras. Bot. 34: 1–19, http://dx.doi.org/10.1590/S0100-84042011000100002

BICCA, A.B. & TORGAN, L.C. 2009. Novos registros de *Eunotia* Ehrenberg (Eunotiaceae-Bacillariophyta) para o Estado do Rio Grande do Sul e Brasil. Acta Bot. Bras. 23: 427–435, http://dx.doi.org/10.1590/S0102-33062009000200014

BURLIGA, A.L. & KOCIOLEK, J.P. 2012. Four new *Eunotia* Ehrenberg species (Bacillariophyceae) from pristine regions of Carajás National Forest, Amazonia, Brazil. Nova Hedwigia 141: 53–70.

BURLIGA, A.L., TORGAN, L.C. & BEAUMORD, A.C. 2007. *Eunotia ariengae* sp. nov., an epilithic diatom from Brazilian Amazon. Diatom Res. 22: 247–253.

CARTER, J.R. & FLOWER, R.J. 1988. A new species of *Eunotia*, *E. pirla* sp. nov., from Woolmer pond, an acid pool in the southeast of England. Diatom Res. 3: 1–8.

EHRENBERG, C.G. 1843. Verbreitung und Einfluß des mikroskopischen Lebens in Süd- und Nord-Amerika. Abhand. König. Akad. Wiss., Berlin.

FERNANDES, L.F., SOUZA-MOSIMANN, R.M. & FELÍCIO-FERNANDES, G. 1990. Diatoms (Bacillariophyceae) of Ratones river, Florianópolis, Santa Catarina, Brazil I – Low current and estuary. Insula 20: 11–112.

FERRARI, F., PROCOPIAK, L.K., ALENCAR, Y.B. & LUDWIG, T.A.V. 2007. Eunotiaceae (Bacillariophyceae) em igarapés da Amazônia Central, Manaus e Presidente Figueiredo, Brasil. Acta Amazonica 37: 1–16.

FERRARI, F., WETZEL, C.E., ECTOR, L., BLANCO, S., VIANA, J.C.C., SILVA, E.M. & BICUDO, D.C. 2009. *Perinotia diamantina* sp. nov., a new diatom species from the Chapada Diamantina, northeastern Brazil. Diatom Res. 24: 79–100.

FERREIRA, F.A, MORMUL, R.P., BIOLO, S. & RODRIGUES, L. 2011. *Podostemum rutifolium* subsp. *rutifolium* como estruturador da comunidade de algas perifíticas em um rio neotropical. Rodriguésia 62: 813–825.

FRENGUELLI, J. 1933 Diatomeas de la región de los esteros del Yberá (en la provincia de Corrientes). Anales Mus. Nac. Hist. Nat. 37: 365–476.

FUHRMANN, A, METZELTIND & TREMARIN, P.I. 2013. A triangular *Eunotia* (Bacillariophyceae) in southeastern Brazil: *Eunotia trigona* sp. nov. Acta Bot. Bras. 27: 694–697, http://dx.doi.org/10.1590/S0102-33062013000400007

FUREY, P.C., LOWE, R.L. & JOHANSEN, J.R. 2011. *Eunotia* Ehrenberg (Bacillariophyta) of the Great Smoky Mountains National Park, USA. Bibliot. Diatomol. 56: 1–133.

HUSTEDT, F. 1949. Süsswasser-diatomeen aus dem Albert-Nationalpark in Belgisch-Kongo. Exploration du Parc National Albert, Mission H. Damas (1935-1936). Vol. 8. Institut des Parcs Nationaux du Congo Belge, Brussels.

HUSTEDT, F. 1952. Neue und wenig bekannte Diatomeen. III. Phylogenetische Variationen bei den rhaphidioiden Diatomeen. Ber. Deutsch. Bot. Ges. 56: 133–145.

KRAMMER, K. & LANGE-BERTALOT, H. 1991. Bacillariophyceae, Teil. 3: Centrales, Fragilariaceae, Eunotiaceae. In Süßwasserflora von Mitteleuropa 2/3 (H. Ettl, I. Gerloff, H. Heynig & D. Mollenhauer, eds). Gustav Fischer Verlag, Stuttgart-Jena, p.1–576.

KOBAYASI, H., ANDO, K. & NAGUMO, T. 1981. On some endemic species of the genus *Eunotia* in Japan. Proc. 6th Diatom Symp. 1980: 93–114.

KOCIOLEK, J.P., LYON, D. & SPAULDING, S. 2001. Revision of the South American Species of *Actinella*. In Lange-Bertalot-Festschrift (R. Jahn, J.P. Kociolek, A. Witkowski & P. Compère, eds). Gantner, Ruggell, p.131–165.

LANGE-BERTALOT, H. 1993. 85 Neue taxa und über 100 weitere neu definierte taxa ergänzend zur süßwasserflora von Mittleleuropa Vol. 2/1–4. Bibliot. Diatomol. 27: 1–454, http://dx.doi.org/10.1515/bd.1993.27.1.1

LANGE-BERTALOT, H., BAK, M. & WITKOWSKI, A. 2011. *Eunotia* and some related genera. In Diatoms of Europe 6 (H. Lange-Bertalot, ed.). Gantner, Ruggell, p.1–747.

LANGE-BERTALOT, H., KÜLBS, K., LAUSER, T., NÖRPEL-SCHEMPP, M. & WILLMANN, M. 1996. Diatom Taxa introduced by Georg Krasske – Documentation and Revision. In Iconographia Diatomologica 3 (H. Lange-Bertalot, ed.). Koeltz Scientific Books, Königstein, p.1–358.

LEVKOV, Z., KRSTIC, S., METZELTIN, D. & NAKOV, T. 2007. Diatoms of Lakes Prespa and Ohrid – About 500 taxa from ancient lake system. – In Iconografia Diatomologica 16 (H. Lange-Bertalot, ed.). Gantner, Ruggell, p.1–613.

LOZOVEI, A.L. & SHIRATA, M.T. 1990. Diatomáceas (Chrysophyta, Bacillariophyceae) no rio Passaúna, Curitiba, Paraná, Brasil: levantamento qualitativo da diatomoflórula em um segmento do manancial. Estudos de Biologia 27: 5–56.

LUDWIG, T.A.V. & FLÔRES, T.L. 1995. Diatomoflórula dos rios da região a ser inundada para a construção da usina hidrelétrica de Segredo, PR. I. Coscinodiscophyceae, Bacillariophyceae (Achnanthales e Eunotiales) e Fragilariophyceae (*Meridion* e *Asterionella*). Arq. Biol. Tecnol. 38: 631–650.

METZELTIN, D. & LANGE-BERTALOT, H. 1998. Tropical Diatoms of South America I: About 700 predominantly rarely known or new taxa representative of the neotropical flora. In Iconographia Diatomologica 5 (H. Lange-Bertalot, ed.). Koeltz Scientific Books, Königstein, p.1–695.

METZELTIN, D. & LANGE-BERTALOT, H. 2007. Tropical Diatoms of South America II: Special Remarks on Biogeographic Disjunction. In Iconographia Diatomologica 18 (H. Lange-Bertalot, ed.). Gantner, Ruggell, p.1–877.

METZELTIN, D. & TREMARIN, P.I. 2011. Uma nova espécie de *Eunotia* para o sudeste do Brasil: *Eunotia fuhrmannii*. Iheringia Sér. Bot. 62: 201–208.

METZELTIN, D., LANGE-BERTALOT, H. & GARCÍA-RODRIGUEZ, F. 2005. Diatoms of Uruguay. In Iconographia Diatomologica 15 (H. Lange-Bertalot, ed.). Koeltz Scientific Books, Königstein, p.1–736.

MOREIRA FILHO H, & VALENTE-MOREIRA, I.M. 1981. Avaliação taxonômica e ecológica das diatomáceas (Bacillariophyceae) epífitas em algas pluricelulares obtidas nos litorais dos Estados do Paraná, Santa Catarina e São Paulo. Bol. Mus. Bot. Munic. 47: 1–17.

MORO, R.S., GARCIA, E. & OLIVEIRA-JR, HF. 1994. Diatomáceas (Bacillariophyceae) da represa de Alagados, Ponta Grossa, Paraná, Brasil (exclusive cêntricas). Iheringia Sér. Bot. 45: 5–9.

PATRICK, R. & REIMER, C.W. 1966. The Diatoms of United States: exclusive of Alaska and Hawaii. Academy of Natural Sciences, Philadelphia.

RAUPP, S.V., TORGAN, L.C. & BAPTISTA, L.R.M. 2006. Composição e variação temporal de diatomáceas (Bacillariophyta) no plâncton da represa de Canastra, sul do Brasil. Iheringia Sér. Bot. 61: 105–134.

REICHARDT, E. 2007. Neue und wenig bekannte *Gomphonema*-Arten (Bacillariophyceae) mit Areolen in Doppelreihen. Nova Hedwigia 85: 103–137.

REICHARDT, E. 2008. *Gomphonema intermedium* Hustedt sowie drei neue, ähnliche Arten. Diatom Res. 23: 105–115, http://dx.doi.org/10.1080/0269249X.2008.9705740

RODRIGUES, L. 1984. Contribuição ao conhecimento das diatomáceas do rio Tubarão – Santa Catarina – Brasil. Insula 14: 47–120.

ROUND, F.E., CRAWFORD, R.M. & MANN, D.G. 1990. The diatoms: biology and morphology of the genera. Cambridge University Press, New York.

SANTOS, E.M., TREMARIN, P.I. & LUDWIG, T.A.V. 2011. Diatomáceas perifíticas em *Potamogeton polygonus* Cham. & Schltdl.: citações pioneiras para o estado do Paraná. Biota Neotrop. 11: 303–315 http://biotaneotropica.org.br/v11n3/en/abstract?inventory+bn01611032011.

SAUPE, A.C. & SOUZA-MOSIMANN, R.M.S. 2003. Diatomáceas (Bacillariophyta) preservadas nos sedimentos holocênicos da Lagoa do Peri- Florianópolis-SC- Brasil. Insula 21: 33–61.

SCHMIDT, A. 1913. Atlas der Diatomaceen-Kunde. Tafel 287. O. R. Reisland, Leipzig.

SILVA, W.J., NOGUEIRA, I.S. & SOUZA, M.G.M. 2011. Catálogo de diatomáceas da região Centro-Oeste brasileira. Iheringia Sér. Bot. 66: 1–32.

SIMONSEN, R. 1974. The diatom plankton of the Indian Ocean expedition of the R/V Meteor 64-65. Meteor Forsch. D. 19: 1–107.

SIMONSEN R. 1987. Atlas and catalogue of the Diatom types of Friedrich Hustedt. Vol. 1–3. J Cramer, Berlin.

SOUZA-MOSIMANN, R.M. 1980. Diatomáceas da represa da Lagoa da Conceição. Insula 11: 1–36.

SOUZA-MOSIMANN, R.M. & LAUDARES-SILVA, R. 2005. Diatoms (Bacillariophyta) from the southern lagoon complex of Santa Catarina, Brazil. Insula 34: 39–82.

SOUZA-MOSIMANN, R.M., LAUDARES-SILVA, R., TALGATTI, D.M. & D'AQUINO-ROSA, V. 2011. The diatom flora in Conceição Lagoon, Florianópolis, SC, Brazil. Insula 40: 25–54.

TALGATTI, DM, GARCIA, M & SCHEFFER, L. 2007. O gênero *Eunotia* Ehrenberg (Bacillariophyta) do arroio do Ecocamping Municipal de Pelotas, RS, Brasil. Rev. Bras. Bioc. 5: 756–758.

TORGAN, L.C. & BECKER, V. 1997. *Eunotia densistriata* sp. nov.: a subaerial diatom from Southern Brazil. Diatom Res. 12: 115–124.

TORGAN, L.C., BECKER, V & PRATES, M. 1999. Checklist das diatomáceas (Bacillariophyta) de ambientes de águas continentais e costeiros o estado do Rio Grande do Sul, Brasil. Iheringia Sér. Bot. 52: 89–144.

TREMARIN, P.I., LUDWIG, T.A.V. & MOREIRA-FILHO, H. 2008. *Eunotia* Ehrenberg (Bacillariophyceae) do rio Guaraguaçu, litoral do Paraná, Brasil. Acta Bot. Bras. 22: 845–862, http://dx.doi.org/10.1590/S0102-33062008000300020

TREMARIN, P.I., FREIRE, E.G, BERTOLLI, L.M. & LUDWIG, T.A.V. 2009. Catálogo das diatomáceas (Ochrophyta-Diatomeae) continentais do estado do Paraná. Iheringia Sér. Bot. 64: 79–107.

TREMARIN, P.I., WETZEL, C.E., LUDWIG, T.A.V. & ECTOR, L. 2011. *Encyonema exuberans* sp. nov. (Bacillariophyceae) from southern Brazilian lotic systems. Nova Hedwigia 92: 107–120.

TUJI, A. & WILLIAMS, D.M. 2005. Observation of the type materials for *Eunotia pectinalis* (Kütz.) Rabenh. var. *pectinalis* and *Eunotia pectinalis* var. *undulata* (Ralfs) Rabenh. Diatom 21: 57–60.

WETZEL, C.E., ECTOR, L., HOFFMANN, L. & BICUDO, D.C. 2010. Colonial planktonic *Eunotia* (Bacillariophyceae) from Brazilian Amazon: Taxonomy and biogeographical considerations on the *E. asterionelloides* species complex. Nova Hedwigia 91: 49–86.

WETZEL, C.E., ECTOR, L., HOFFMANN, L., LANGE-BERTALOT, H. & BICUDO, D.C. 2011. Two new periphytic *Eunotia* species from the neotropical Amazonian 'black waters', with a type analysis of *E. braunii*. Diatom Res. 26: 135–146, http://dx.doi.org/10.1080/0269249X.2011.587644

Received 25/02/2014
Revised 09/06/2014
Accepted 01/09/2014

Biota Neotropica 14(3): e20140052, 2014
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
inventory

High termite richness in an urban fragment of Atlantic Forest in northeastern Brazil

Matilde Vasconcelos Ernesto[1,3], *Elaine Folly Ramos*[1], *Flávia Maria da Silva Moura*[2] &

Alexandre Vasconcellos[3,4]

[1]*Centro de Ciências Aplicadas e Educação, Universidade Federal da Paraíba, 58297–000, Rio Tinto, PB, Brazil. e-mail: matildeernesto@gmail.com e-mail: elafolly@yahoo.com.br*
[2]*Unidade Acadêmica de Ciências Biológicas, Centro de Saúde e Tecnologia Rural, Universidade Federal de Campina Grande, 58708–110, Patos, PB, Brazil. e-mail: fmsmoura@yahoo.com.br*
[3]*Laboratório de Termitologia, Departamento de Sistemática e Ecologia, Centro de Ciências Exatas e da Natureza, Universidade Federal da Paraíba, 58051-900, João Pessoa, PB, Brazil.*
e-mail: avasconcellos@dse.ufpb.br
[4]*Corresponding author: Alexandre Vasconcellos, e-mail: avasconcellos@dse.ufpb.br*

ERNESTO, M.V., RAMOS, E.F., MOURA, F.M.S., VASCONCELLOS, A. **High termite richness in an urban fragment of Atlantic Forest in northeastern Brazil.** Biota Neotropica. 14(3): e20140052. http://dx.doi.org/10.1590/1676-06032014005214

Abstract: Termites are very abundant in tropical ecosystems and have active roles in nutrient cycling and soil formation, but few studies of their assemblages have been undertaken in the Brazilian Atlantic Forest. The present study analyzed the composition of termite assemblages in a fragment of Atlantic Forest embedded within an urban matrix in northeastern Brazil. Five standardized sampling protocols of termites were applied, with a total sampling effort of 1500m^2. We encountered 45 species of termites belonging to 25 genera and three families. The soil-feeders group demonstrated the greatest species richness, while wood-feeders species were the most abundant. The species richness of termite in the study area was the greatest yet recorded for an Atlantic forest site in Brazil, indicating the importance of urban fragments for conserving tropical biodiversity.

Keywords: *Isoptera, Feeding Groups, Biodiversity, Neotropical Region.*

ERNESTO, M.V., RAMOS, E.F., MOURA, F.M.S., VASCONCELLOS, A. **Alta riqueza de térmitas em um fragmento urbano de Floresta Atlântica do Nordeste Brasileiro.** Biota Neotropica. 14(3): e20140052. dx.doi.org/10.1590/1676-06032014005214

Resumo: Térmitas são muito abundantes em ecossistemas tropicais e possuem papéis ativos na ciclagem de nutrientes e formação do solo, mas poucos estudos sobre suas taxocenoses têm sido realizados na Floresta Atlântica. O presente estudo analisou a composição das taxocenoses de térmitas em um fragmento de Floresta Atlântica inserida dentro de uma matriz urbana no Nordeste do Brasil. Cinco protocolos padronizados de amostragem de térmitas foram aplicados, o que totalizou um esforço amostral de 1500m^2. Foram encontradas 45 espécies de térmitas pertencentes a 25 gêneros e três famílias. O grupo alimentar dos humívoros demonstrou a maior riqueza de espécies, enquanto as espécies xilófagas foram as mais abundantes. A riqueza de espécies de térmitas na área de estudo foi a maior registrada até o momento para uma área de Floresta Atlântica no Brasil, indicando a importância dos fragmentos urbanos para a conservação da biodiversidade tropical.

Palavras-chave: *Isoptera, Grupo alimentar, Biodiversidade, Região Neotropical.*

Introduction

The Atlantic Forest is one of the principal humid forests in South America. It is isolated from the Amazonian and Andean forests by a corridor of open and semi-open vegetation formations including Caatinga (dryland), Cerrado (neotropical savanna), and Chaco (arid lowland savanna) (Costa 2003, Pennington et al. 2006). More than 500 years of anthropogenic disturbances have caused the Atlantic Forest to lose more than 88.3% of its original cover (Ribeiro et al. 2009). Currently, the Atlantic Forest is considered one of the priority areas for global biodiversity conservation (Myers et al. 2000).

Termites are eusocial insects that have important roles in ecosystem functioning by influencing the processes of nutrient cycling and altering the physical and chemical structures of the soil (Lee & Wood 1971, Wood & Sands 1978, Holt & Lepage 2000). More than 2,900 living species have been described globally (Krishna et al. 2013), and the greatest values of α-diversity and biomass have been recorded in humid tropical forests (Vasconcellos 2010, Jones & Eggleton 2011). Up to 50%

of the decomposition of organic detritus of plant origin has been attributed to termite action in these forests (Bignell & Eggleton 2000).

In spite of the importance of termites to tropical and subtropical ecosystems, our knowledge of their diversity and functional roles in their respective ecosystems are still relatively scarce. Very few studies have been published concerning the structure of termite assemblages in northeastern Brazil, especially in the Caatinga and Atlantic Forest ecosystems (Bandeira et al. 1998, Bandeira et al. 2003, Mélo & Bandeira 2004, Vasconcellos 2010, Vasconcellos et al. 2010). Furthermore, although some studies have been performed in fragments of Atlantic Forest in Brazil, including the study area of this work, the species richness of termites was clearly underestimated. Studies of termite assemblages are considered basic prerequisites for understanding the important functions of these insects in the ecosystem, and for identifying their patterns of diversity and endemism. The present study therefore sought to describe the composition of termite assemblages in a fragment of Atlantic Forest embedded within an urban matrix in northeastern Brazil with regard to their species richness, numbers of encounters, and feeding groups.

Materials and methods

Study sites

The present study was undertaken in the Mata do Buraquinho Permanent Protection Area (PPA) (07°08'42''S x 34°51'54''W) located within the urban perimeter of the municipality of João Pessoa, Paraíba State, Brazil. The PPA occupies an area of 515 ha of a slightly rolling landscape, with altitudes varying from 35 to 85 m. The topsoils sandy red-yellow podzol, and the climate there is hot and humid (classified as "As" in the Köppen [1936] system), with rainfall principally from March to August (Barbosa 2008). The average annual temperature and humidity are 27°C and 80%, respectively, and the mean annual rainfall is 1,482 mm (Governo do Estado da Paraíba 1985). The regional vegetation is characterized as Seasonal Semideciduous Forest (IBGE 1992), and this area is considered one of the largest and most important remnants of that domain in Paraíba State (Barbosa 2008; Oliveira & Melo 2009).

Sampling protocols

The termite assemblages were inventoried in December/2010 and from May through November/2012 using standard protocols for rapid evaluations of termite diversity. Each protocol was performed by establishing six 65 m x 2 m transects within forest areas without any apparent human impacts, at minimum distances of 50 m from the forest borders and from other transects. Five 5 m x 2 m plots were established 10 m one from the other in each transect, totaling 30 plots (300 m^2) per protocol. The sampling effort in each plot was 1person-hour. During this period, the termites were searched in active and abandoned nests (up to 2 m above ground level), in tunnels and live and dead tree trunks, in the leaf litter and the interiors of fallen branches, in the soil (at depths up to 15 cm), under rocks, and in dead roots. The specimens collected were stored in labeled glass vials containing 75% alcohol and were subsequently deposited in the Isopteran collection of the Federal University of Paraíba.

Feeding groups

Based on *in situ* observations of feeding and on information available in the literature concerning Neotropical termites (DeSouza & Brown 1994, Constantino 1999, Bandeira et al. 2003, Sena et al. 2003,Vasconcellos et al. 2005, Vasconcellos 2010), the species were classified as belonging to the following feeding groups: (i) wood-feeders, that consume the wood of live or dead trees in various stages of decomposition; (ii) soil-feeders, that feed on humus and generally live in the ground; (iii) wood/soil feeders, that consume humus as well as wood in different stages of decomposition, and usually carrying soil into the wood they are consuming; and (iv) wood/leaf feeders, that consume both wood in advanced stages of decomposition and leaf litter on the forest floor.

Analyses

Species accumulation curve was prepared using the Mao Tau method considering the 95%, confidence intervals for 1,000 randomizations without replacement the original data. These analyses were performed using Estimate S9.1.0 software (Colwell 2013).

Results

Forty-five species of termites (624 encounters), belonging to 25 genera and three families were recorded in the Mata do Buraquinho Permanent Preservation Area (Table 1). The family Termitidae was the best-represented in terms of the numbers of species (40; 88.9%), followed by Kalotermitidae (3; 6.7%) and Rhinotermitidae (2; 4.4%). The Termitidae were also dominant in terms of the frequencies of encounters (86.1%). In relation to subfamilies, Termitinae and Nasutitermitinae were the most representative in terms of the numbers of species (12; 30%, each) and Termitinae in terms of the frequencies of encounters (34.5%). The soil-feeders group was dominant in terms of the number of species (19; 42.2%) and the wood-feeders group in terms of the frequency of encounters (41.2%).

The species that constructed conspicuous nests in the area were: *Anoplotermes banksi* Emerson, 1925, *Embiratermes neotenicus* (Holmgren, 1906), *Labiotermes labralis* (Holmgren, 1906), *Microcerotermes indistinctus* Mathews, 1977, *M. strunckii* (Sörensen, 1884), *Nasutitermes corniger* (Motschulsky, 1855), *N. ephratae* (Holmgren, 1910), *N. macrocephalus* (Silvestri, 1903), *N. obscurus* Holmgren, 1906, and *Silvestritermes holmgreni* (Snyder, 1926).

The species accumulation curve in the Mata do Buraquinho PPA is almost stabilized (Figure 1). The upper confidence level was 46.84 species and the lower 43.16, very close to the actual number observed in the field (45 spp.).

Discussion

Forty-five species were encountered in the Mata do Buraquinho PPA, of which 31 could be identified to the species level. Including four new records of this study (*Ibitermes inflatus*, *Orthognathotermes longilamina*, *O. tubesauassu* e *Termes hispaniolae*), a total of 46 species are now known from the Atlantic Forest (Bandeira et al. 1998, Brandão 1998, Vasconcellos et al. 2005, Reis & Cancello 2007, Vasconcellos 2010, Souza et al. 2012). Considering only the region north of the São Francisco River, 37 species have been identified to the species level. Studies examining termite assemblages in the

Table 1. Termite species found in the Mata do Buraquinho Permanent Preservation Area in an Atlantic Forest fragment in northeastern Brazil. W, wood; S, subterranean; L, litter; A, arboreal; E, epigeal; I, inquiline.

Species	Number of encounters	Place of sampling	Feeding group
KALOTERMITIDAE			
Calcaritermes rioensis Krishna, 1962	8	W	Wood
Neotermes sp.	1	W	Wood
Rugitermes sp.	3	W	Wood
RHINOTERMITIDAE			
Coptotermes testaceus (Linnaeus, 1758)	19	S	Wood
Heterotermes longiceps (Snyder, 1924)	56	S	Wood
TERMITIDAE			
Apicotermitinae			
Apicotermitinae sp. 1	4	S	Soil
Apicotermitinae sp. 2	6	S	Soil
Apicotermitinae sp. 3	19	S	Soil
Apicotermitinae sp. 4	6	S	Soil
Apicotermitinae sp. 5	3	S	Soil
Anoplotermes banksi Emerson, 1925	19	A	Soil
Anoplotermes sp. 1	6	S	Soil
Anoplotermes sp. 2	25	W/S	Soil
Anoplotermes sp. 3	11	W/S	Soil
Anoplotermes sp. 4	18	S	Soil
Ruptitermes sp.	4	L	Wood/Leaf
Nasutitermitinae			
Atlantitermes sp.	2	W	Wood/Soil
Convexitermes sp.	1	W	Wood/ Soil
Diversitermes diversimiles (Silvestri, 1901)	4	L	Wood/Leaf
Nasutitermes callimorphus Mathews, 1977	24	W	Wood
Nasutitermes corniger (Motschulsky, 1855)	15	A	Wood
Nasutitermes ephratae (Holmgren, 1910)	16	A	Wood
Nasutitermes gaigei Emerson, 1925	25	W	Wood
Nasutitermes jaraguae (Holmgren, 1910)	11	W	Wood
Nasutitermes macrocephalus (Silvestri, 1903)	3	A	Wood
Nasutitermes obscurus Holmgren, 1906	3	A	Wood
Subulitermes microsoma (Silvestri, 1903)	3	S/I	Soil
Velocitermes velox (Holmgren, 1906)	2	L	Wood/Leaf
Syntermitinae			
Embiratermes neotenicus (Holmgren, 1906)	13	E	Wood/ Soil
Embiratermes parvirostris Constantino, 1992	55	S	Soil
Ibitermes inflatus Vasconcellos, 2002	2	S	Soil
Labiotermes labralis (Holmgren, 1906)	19	A	Soil
Silvestritermes holmgreni (Snyder, 1926)	33	A	Wood/Soil
Termitinae			
Amitermes amifer Silvestri, 1901	54	W	Wood/Soil
Cavitermes tuberosus (Emerson, 1925)	7	I	Soil
Cylindrotermes sapiranga Rocha & Cancello, 2007	27	W	Wood
Dentispicotermes conjunctus Araujo, 1969	1	S	Soil
Microcerotermes indistinctus Mathews, 1977	41	A	Wood
Microcerotermes strunckii (Sörensen, 1884)	5	A	Wood
Neocapritermes opacus Hagen, 1858	5	S	Wood/Soil
Neocapritermes talpoides Krishna & Araujo, 1968	14	S	Soil
Orthognathotermes longilamina Rocha & Cancello, 2009	2	S/I	Soil
Orthognathotermes tubesauassu Rocha & Cancello, 2009	7	S/I	Soil
Termes hispaniolae (Banks, 1918)	2	W/I	Wood/Soil
Termes medioculatus Emerson in Snyder, 1949	20	W/I	Wood/Soil
Total of species	**45**		
Total of encounters	**624**		

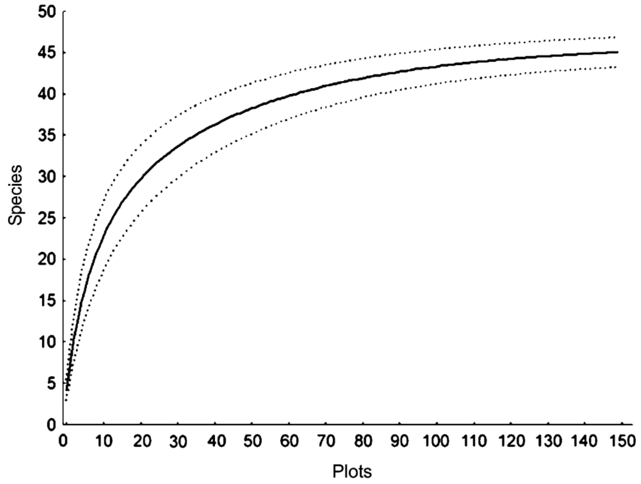


Figure 1. Termite species accumulation curves and 95% confidence intervals for an urban fragment of Atlantic Forest in northeastern Brazil.

Atlantic Forest have reported between 21% and 100% non-determined species (Bandeira et al.1998, Silva & Bandeira 1999, Brandão 1998, Vasconcellos et al. 2005, Reis & Cancello 2007, Vasconcellos 2010, Souza et al. 2012). The sampling effort used in this study was the highest ever made in a Brazilian Atlantic Forest fragment, which could partly explain the high species richness in relation to other studies in the same biome.

The termite fauna of the Mata do Buraquinho PPA has been previously investigated using various methodologies (Bandeira et al. 1998, Silva & Bandeira 1999, Vasconcellos 2010). The number of species collected in the present study (45 spp.) was greater than in any previous publication. In these earlier works, only 0% to 12% of the insects were identified to the species level (Bandeira et al. 1998, Silva & Bandeira 1999). More recently, Vasconcellos (2010) evaluated the biomass and abundance of termites and reported 29 species in the PPA.

The number of species of termites in the Mata do Buraquinho PPA was greater than reports from other Atlantic Forest fragments, where richness varied from 11 to 38 species (Bandeira et al. 1998, Silva & Bandeira 1999, Brandão 1998, Vasconcellos et al. 2005, Reis & Cancello 2007, Vasconcellos 2010, Souza et al. 2012). In terms of other Brazilian morphoclimatic domains, the number of species encountered here was within the amplitude reported for areas of Cerrado vegetation (30 to 70 species) (Constantino 2005) and for the Amazon region (11 to 88 species) (Bandeira & Macambira 1988, Bandeira 1989, Constantino 1992, De Souza & Brown 1994), but higher than the amplitude reported for Caatinga areas (10 to 26 species) (Mélo & Bandeira 2004, Vasconcellos et al. 2010, Alves et al. 2011).

Equal numbers of species were found in the present study for the subfamilies Nasutitermitinae and Termitinae, with the Termitinae being more abundant. Other studies examining the termite fauna in Atlantic Forest areas have reported the subfamily Nasutitermitinae as dominant in terms of the numbers of species and relative abundance (Bandeira et al. 1998, Brandão 1998, Vasconcellos et al. 2005, Reis & Cancello 2007, Vasconcellos 2010, Souza et al. 2012). The low abundance observed for the family Kalotermitidae probably underestimates their true numbers, as most of the colonies of this termite group are quite small and form inside pieces of dried wood or in the canopies of trees, making their collection much more difficult when using rapid survey collection techniques (Roisin et al. 2006, Reis & Cancello 2007).

The soil-feeders group demonstrated the largest number of species – a pattern different from that observed in other studies conducted in the Atlantic Forest, where the wood-feeders group was responsible for from 39% to 64% of the total species richness (Bandeira et al. 1998, Brandão 1998, Vasconcellos et al. 2005, Reis & Cancello 2007, Souza et al. 2012). This fact is certainly associated with the huge sampling effort, which increases the sampling of rare soil-feeders species. In terms of abundance, on the other hand, the wood-feeders group dominated in the Mata do Buraquinho PPA. High species richness is therefore not always directly linked to high abundance and high biomasses in this ecosystem (Souza et al. 2012).

Approximately 35% of all termite species in humid tropical forests in South America construct conspicuous nests (Constantino 1992, Martius 1994, Bandeira & Vasconcellos 2002). All of the 10 species of termites known to construct conspicuous nests in Atlantic Forest sites north of the São Francisco River were encountered in the Mata do Buraquinho PPA, including *N. obscurus,* which was identified in previous studies as *Nasutitermes* sp. (Vasconcellos et al. 2005, Vasconcellos et al. 2008, Vasconcellos 2010, Souza et al. 2012). Vasconcellos (2010) reported nine species that constructed arboricolous nests in a previous study undertaken in the Mata do Buraquinho PPA. Additionally, the present study encountered a single species (*E. neotenicus*) that constructed epigeous nests.

The Mata do Buraquinho PPA contained an expressive number of termite species for an Atlantic Forest fragment embedded within an urban matrix. The effective conservation of many species will depend on their capacity to persist in fragmented habitats in the midst of modified landscapes (Holland & Bennett 2011). Extreme urbanization almost always reduces the species richness of vertebrates, invertebrates, and plants, although in cases of moderate urbanization these effects vary among the different taxonomic groups (Mckinney 2008). Brown (1997) reported that under conditions of low environmental disturbance termite species richness may even increase. The high diversity of the termite fauna in the Mata do Buraquinho PPA reinforces the importance of small forest fragments in conserving the biodiversity of the Atlantic Forest – even when these remnants sites are embedded within an urban matrix.

Acknowledgments

The authors would like to thank the directors of the Benjamin Maranhão Botanical Garden (located in the Mata do Buraquinho PPA) for their permission to undertake the termite collections; Reginaldo Constantino for the identification of some species; A Adriano M. Souza, Aline O. Lopes, Ana C.F. Alves, Carolina N. Liberal, Daniel D. Bó, Dayse P. Nascimento, Everton P. Lorenzo, Giácomo R.P. Monteiro, Gindomar G. Santana, José R.C. Barbosa, Luiz P.A. Silva, Ricardo A. Nink, Rozzanna E.C.R. Figueirêdo, Virgínia F.P. Araújo, and Wellington E. Santos for their help with the fieldwork; and CAPES for the Masters grant and financial support awarded to Matilde Vasconcelos Ernesto.

References

Alves, W.F., Mota, A.S., Lima, R.A.A., Bellezoni, R. & Vasconcellos, A. 2011. Termites as Bioindicators of Habitat Quality in the Caatinga, Brazil: Is There Agreement Between Structural Habitat Variables and the Sampled Assemblages? Neotrop. Entomol. 40(1):39-46, http://dx.doi.org/10.1590/S1519-566X2011000100006

Bandeira, A.G. & Macambira, M.L.J. 1988. Térmitas de Carajás, Estado do Pará, Brasil: composição faunística, distribuição e hábito alimentar. Bol. Mus. Para. Emílio Goeldi, Zool. 4(2): 175-190.

Bandeira, A.G. & Vasconcellos, A. 2002. A quantitative survey of termites in a gradient of disturbed highland forest in Northeastern Brazil (Isoptera). Sociobiology 39:429-439.

Bandeira, A.G. 1989. Análise da termitofauna (Insecta:Isoptera) de uma floresta primária e de uma pastagem na Amazônia Oriental. Bol. Mus. Para. Emílio Goeldi, Zool. 5:225-241.

Bandeira, A.G., Pereira, J.C.D., Miranda, C.S. & Medeiros, L.G.S. 1998. Composição da fauna de cupins (Insecta, Isoptera) em área de Mata Atlântica em João Pessoa, Paraíba, Brasil. Rev. Nordestina Biol. 12(1/2):9-17.

Bandeira, A.G., Vasconcellos, A., Silva, M.P. & Constantino, R. 2003. Effects of habitat disturbance on the termite fauna in a highland humid forest in the Caatinga domain, Brazil. Sociobiology 42(1):117-127.

Barbosa, M.R.V. 2008.Floristic composition of a remnant of Atlantic Coastal Forest in João Pessoa, Paraíba, Bazil. In The Atlantic Coastal Forest of Northeastern Brazil (W.W. Thomas & E.G. Briton, eds). New York Botanical Garden Press, New York, p.439-457.

Bignell, D.E. & Eggleton, P. 2000. Termites in ecosystems. In Termites: Evolution, Sociality, Symbiosis, Ecology (T. Abe, D.E. Bignell & M. Higashi, eds.). Kluwer Academic Publications, Dordrecht, p.363-387.

Brandão, D. 1998. Patterns of termite (Isoptera) diversity in the Reserve Florestal de Linhares, state of Espírito Santo, Brazil. Rev. Bras. Entomol. 41:151-153.

Brown JR., K.S. 1997. Diversity, disturbance, and sustainable use of Neotropical forests: insects as indicators for conservation monitoring. J. Insect. Conserv. 1:25-42, http://dx.doi.org/10.1023/A:1018422807610

Colwell, R.K. 2013. EstimateS: Statistical estimation of species richness and shared species from samples, Version 9.1.0. http://viceroy.eeb.uconn.edu/estimates/ (ultimo acesso em 15/08/2013).

Constantino, R. 1992. Abundance and diversity of termites (Insecta: Isoptera) in two sites of primary rain forest in Brazilian Amazonia. Biotropica 24(3):420-430.

Constantino, R. 1999. Chave ilustrada para identificação dos gêneros de cupins (Insecta: Isoptera) que ocorrem no Brasil. Pap. Avulsos Zool. (São Paulo) 40, 387-448.

Constantino, R. 2005. Padrões de diversidade e endemismo de térmitas no bioma Cerrado. In Biodiversidade, Ecologia e Conservação do Cerrado (A.O. Scariot, J.C.S. Silva & J.M. Felfili, eds.). Ministério do MeioAmbiente, Brasília, p.319-333.

de Souza O.F.F, & Brown, V.K. 1994. Effects of habitat fragmentation on Amazonian termite communities.J. Trop. Ecol. 10:197-206.

Governo do Estado da Paraíba. 1985. Atlas Geográfico do Estado da Paraíba. Grafset, João Pessoa.

Holland, G.J. & Bennett, A.F. 2011.Recolonization of forest fragments by a native rodent following experimental "extinctions". Austral Ecol. 36:521-529, http://dx.doi.org/10.1111/j.1442-9993.2010.02182.x

Holt, J.A. & Lepage, M. 2000.Termites and soil properties. In Termites: Evolution, Sociality, Symbiosis, Ecology (T. Abe, D.E. Bignell & M. Higashi, eds.). Kluwer Academic Publications, Dordrecht, p.389-407.

IBGE. Anuário estatístico do Brasil. 1992. 52 ed. Fundação Instituto Brasileiro de Geografia e Estatística, Rio de Janeiro.

Jones, D.T. & Eggleton, P. 2011. Global Biogeography of Termites: A Compilation of Sources. In Biology of Termites: A Modern Synthesis (D.E. Bignell, Y. Roisin & N. Lo, eds.). Springer SBM, Dordrecht, p.477-498.

Köppen, W. 1936. Das geographische system der klimate. In Handbuch der Klimatologie, part C. (W. Köppen& R. Geiger, eds.). Verlag von Gebrüder Borntraeger, Berlin, p.1-44.

Krishna, K., Grimaldi, D.A., Krishna, V. & Engel, M.S. 2013. Treatise on the Isoptera of the world. B. Am. Mus. Nat. Hist. 377:1-2704, http://dx.doi.org/10.1206/377.1

Lee, K.E. & Wood, T.G. 1971. Termites and soils. Academic Press, London and New York.

Martius, C. 1994. Diversity and ecology of termites in Amazonian forest. Pedobiology 38: 407-428.

Mckinney, M.L. 2008. Effects of urbanization on species richness: A review of plants and animals. Urban Ecosyst 11: 161-176.

Mélo, A.C.S. & Bandeira, A.G. 2004. A qualitative and quantitative survey of termites (Isoptera) in an open shrubby Caatinga in Northeast Brazil. Sociobiology 44(3):707-716.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., Fonseca, G.A.B. & Kent, J. 2000.Biodiversity hotspots for conservation priorities. Nature 403:853-858.

Oliveira, S.C.C. & Melo, R.S. 2009. As trilhas do Jardim Botânico Benjamim Maranhão (João Pessoa - PB) como recurso para interpretação ambiental. Cad. Virtual Tur. 9(2):113-125.

Pennington, R.T., Lewis, G.P. & Ratter, J.A. 2006. An overview of the plant diversity, biogeography and conservation of neotropical savannas and seasonally dry forests. In Neotropical savannas and dry forests: Plant diversity, biogeography, and conservation. (R.T. Pennington, G.P. Lewis, & J.A. Ratter, eds.). Taylor & Francis CRC Press, Oxford, p.1-29.

Reis, Y.T. & Cancello, E.M. 2007. Riqueza e diversidade de cupins (Insecta, Isoptera) numa área de mata primária e outra secundária, na Mata Atlântica do sudeste da Bahia. Iheringia, Zool. 97:229-234.

Ribeiro, M.C., Metzger, j.p., Martensen, A.C., Ponzoni, F.J. & Hirota, M.M. 2009. The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. Biol. Conserv. 142:1141-1153, http://dx.doi.org/10.1016/j.biocon.2009.02.021

Roisin, Y., Dejean, A., Corbara, B., Orivel, J., Samaniego, M. & Leponce, M. 2006. Vertical stratification of the termite assemblage in a Neotropical rainforest. Oecologia 149:301-311.

Sena, J.M., Vasconcellos, A., Gusmão, M.A.B. & Bandeira, A.G. 2003. Assemblage of termites in a fragment of cerrado on the coast of Paraiba State, Northeast Brazil (Isoptera). Sociobiology 42(3):753-760.

Silva, E.G. & Bandeira, A.G. 1999. Abundância e distribuição de cupins (Insecta, Isoptera) em solo de Mata Atlântica, João Pessoa, Paraíba. Rev. Bras. Biol. 13(1/2):13-36.

Souza, H.B.A., Alves, W.F. & Vasconcellos, A. 2012. Termite assemblages in five semideciduous Atlantic Forest fragments in the northern coastland limit of the biome. Rev. Bras. Entomol. 56(1):67-72, http://dx.doi.org/10.1590/S0085-56262012005000013

Vasconcellos, A. 2010. Biomass and abundance of termites in three remnant areas of Atlantic Forest in northeastern Brazil. Rev. Bras. Entomol. 54(3):455-461, http://dx.doi.org/10.1590/S0085-56262010000300017

Vasconcellos, A., Bandeira, A.G., Almeida, W.O. & Moura, F.M.S. 2008. Térmitas construtores de ninhos conspícuos em duas áreas de Mata Atlântica com diferentes níveis de perturbação antrópica. Neotrop. Entomol. 37:15-19, http://dx.doi.org/10.1590/S1519-566X2008000100003

Vasconcellos, A., Bandeira, A.G., Moura, F.M.S., Araujo, V.F.P., Gusmão M.A.B. & Constantino, R. 2010. Termite assemblages in three habitats under different disturbance regimes in the semi-arid Caatinga of NE Brazil. J. Arid Environ. 74:298-302, http://dx.doi.org/10.1016/j.jaridenv.2009.07.007

Vasconcellos, A., Mélo, C.A.S., Segundo, E.M.V. & Bandeira, A.G. 2005. Cupins de duas florestas de restinga do nordeste brasileiro. Iheringia, Zool. 95(2):127-131, http://dx.doi.org/10.1590/S0073-47212005000200003

Wood, T.G. & Sands, W.A. 1978.The role of termites in ecosystems. In Production Ecology of Ants and Termites (M.V. Brian, ed.). Cambridge University Press, Cambridge, p.245-292.

Received 27/03/2014
Revised 14/05/2014
Accepted 04/09/2014

Biota Neotropica 14(3): e20130077, 2014
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
article

Ichthyofauna of Seridó/Borborema: a semi-arid region of Brazil

Márcio Joaquim da Silva[1,3], *Telton Pedro Anselmo Ramos*[2], *Virginia Dantas Diniz*[2], *Robson Tamar da Costa Ramos*[2] *& Elvio Sergio Figueredo Medeiros*[1]

[1] *Grupo de Ecologia de Rios do Semiárido, Universidade Estadual da Paraíba, Centro de Ciências Biológicas e Sociais Aplicadas, and Rua Horácio Trajano de Oliveira, Cristo Redentor, CEP: 58020-540, João Pessoa, PB, Brasil.* www.uepb.edu.br

[2] *Laboratório de Sistemática e Morfologia de Peixes, Universidade Federal da Paraíba, Centro de Ciências Exatas e da Natureza, Jardim Cidade Universitária, CEP: 58059-900, João Pessoa, PB, Brasil.* www.ufpb.br

[3] *Corresponding author: Márcio Joaquim da Silva, e-mail:* silva.marcio.j@gmail.com

SILVA, M.J., RAMOS, T.P.A., DINIZ, V.D., RAMOS, R.T. da C, MEDEIROS, E.S.F. **Ichthyofauna of Seridó/Borborema: a semi-arid region of Brazil.** Biota Neotropica. 14(3): e20130077. dx.doi.org/10.1590/1676-06032014007713

Abstract: The Seridó/Borborema region is located between the States Paraíba and Rio Grande do Norte and a priority area for conservation of the Caatinga. This region is under the hydrological influence of the middle portion of Piranhas-Açu River basin. Previous systematic inventories of the ichthyofauna of this Caatinga's area are not significant. The Caatinga fish fauna is threatened due the advancement of historic anthropogenic activities, mainly agricultural. Furthermore, another relevant aspect is the recent governmental decision of transferring water from São Francisco River to other Northern river basins, which includes the Piranhas-Açu basin. This study performed a systematic survey of the Seridó/Borborema's ichthyofauna. The sites were sampled during the years 2006 and 2007 (four annual, diurnal samples), using three different types of gear: beach seine nets, cast net and gillnets. We captured 13,009 individuals of 5 orders, 14 families, 28 genera and 35 species of fish. The predominant orders were Characiformes (21 species), Siluriformes (6) and Perciformes (5). The greatest number of species (11) was catch from the family Characidae, followed by Cichlidae (5) and Loricariidae (4). This inventory produced a comprehensive sampling of the middle portion of Piranhas-Açu River basin and its result overcomes the diversity reported in previous studies (22 species) on the same region.
Keywords: *Inventory, Fish, Piranhas-Açu River, Caatinga, Northeastern Brazil.*

SILVA, M.J., RAMOS, T.P.A., DINIZ, V.D., RAMOS, R.T. da C, MEDEIROS, E.S.F. **Ictiofauna do Seridó/Borborema: uma região semiárida do Brasil.** Biota Neotropica. 14(3): e20130077. dx.doi.org/10.1590/1676-06032014007713

Resumo: A região Seridó/Borborema, localizada entre os Estados da Paraíba e do Rio Grande do Norte, é uma área prioritária para conservação da Caatinga e encontra-se sob domínio hidrológico da porção média da bacia do rio Piranhas-Açu. Levantamentos sistemáticos sobre a ictiofauna desta região da Caatinga realizados até esse momento são pouco expressivos. A ictiofauna da Caatinga sofre ameaças devido ao avanço histórico de atividades antrópicas, principalmente agropastoris. Além disso, outro aspecto que merece destaque é a recente implantação da obra de integração do rio São Francisco com as bacias hidrográficas do Nordeste Setentrional. O presente estudo realizou um levantamento sistemático da ictiofauna da região Seridó/Borborema. As amostragens foram realizadas entre os anos de 2006 e 2007 (quatro amostragens anuais diurnas), utilizando redes de arrasto, tarrafa e redes de emalhar. Foram capturados 13.009 indivíduos pertencentes a 5 ordens, 14 famílias, 28 gêneros e 35 espécies de peixes. As ordens predominantes foram Characiformes (21 espécies), Siluriformes (6) e Perciformes (5). Characidae foi a família com o maior número de espécies (11), seguida por Cichlidae (5) e Loricariidae (4). Este inventário produziu uma abrangente amostragem da porção média do rio Piranhas-Açu e seu resultado supera a diversidade registrada em estudos prévios (22 espécies) da mesma região.
Palavras-chave: *Inventário, Peixe, Rio Piranhas-Açu, Caatinga, Nordeste Brasileiro.*

Introduction

The knowledge related to the freshwater fishes of Brazil has increased during the last decades (Buckup et al. 2007, Menezes et al. 2007), as to the Neotropical region (Vari 1983, Reis et al. 2003, Kullander & Ferreira 2006). However, studies are unevenly distributed in Brazil. While some areas of the country are relatively well documented, studies available from the ecoregion Midle Eastern Portion of Northeastern Brazil (Nordeste Médio-Oriental) are still not expressive, either due to the low extension of the inventory, or due to the lack of clarity about the taxonomic identity of its components (Santos & Zanata 2006, Buckup et al. 2007, Langeani et al. 2009, Nogueira et al. 2010, Ramos 2012). The more comprehensive and cited general work on the diversity of Northeastern freshwater fish was carried out by Rosa et al. (2003), a study limited to the Caatinga region, that recorded 240 species. The authors cited above argue that the current scenario of knowledge about the fish fauna of Northeastern Brazil may only be changed with conducting extensive sampling programs in the various river basins of the region, and their results being analyzed from new systematic reviews. Therefore, estimates of diversity of freshwater fish in the Northeastern Brazil may be premature, considering the existence of the few representative collections from this region (Menezes 1996, Rosa et al. 2003, Ramos et al. 2005).

Subprojects as part of large projects like PROBIO (Project of the conservation and sustainable use of Brazilian biological diversity) and PPBio (Program of biodiversity research) are among recent programs aiming to increase knowledge of fish diversity of Brazilian Northeastern Region. Recently, Ramos (2012) conducted an inventory in the Parnaíba River basin in the States of Ceará, Piauí and Maranhão, and recorded 143 species of freshwater fish. Among those species 23 were new records for the basin, 25 are new species to science, and 50 are endemic species - almost 40% of 136 native species diagnosed by author.

The Caatinga extends along most of Northeastern region and is one of the most altered regions of Brazil, as well as its associated aquatic systems, mainly by human activities such as farming (Coimbra-Filho & Câmara 1996, Leal et al. 2003). Another relevant aspect is the recent governmental decision of transferring water from São Francisco River to other Northern river basins, which includes the Piranhas-Açu. Certainly this action will bring major changes, thus, it is urgent to expand knowledge about the ichthyic diversity of the region. Tabarelli and Silva (2003) identified priority areas for conservation of the Caatinga, classified according to the degree of biological importance and urgency of conserving species as: Extreme, Very High, and High Priority. Among those areas, the Seridó/Borborema stands out as the only Extreme Priority area in the Piranhas-Açu River basin, and incorporates one of its main tributaries, the Seridó river. The Seridó/Borborema region is addressed in this study, which aimed to survey the fish species occurring there and is a byproduct of the PPBio, a program of taxonomic and ecological assessment of the Northeastern Brazilian region.

Material and methods

1. *Study area*

This study was performed in the Seridó/Borborema region which lies within the middle portion of Piranhas-Açu river basin (Figure 1). The relief of Seridó/Borborema region dates from Precambrian, formed by igneous and metamorphic rocks (Felipe & Carvalho 1999). The characteristic climate of Brazilian Semi-arid is BSh, according to Köppen-Geiger's classification (Rubel & Kottek 2010), with rainfall between 400-800 mm (historical average of years 1998-2007; CPTEC 2011), average annual temperature 30.7°C, with minimum of 29.3°C (February) and maximum of 31.7°C (October) (Amorim et al. 2005). Leal et al. (2003) affirm that the climate of studied region is a result of high solar radiation, low nebulosity, high average annual temperature, low rates of relative humidity, high potential evapotranspiration, as well as low and irregular rainfall.

The vegetation of Seridó/Borborema region is the typical Caatinga's trees and low shrubs (Maia 2004). Species richness is low, with few rare or endangered species, showing associations of genera *Mimosa, Caesalpinia* and *Aristida* (Giulietti et al. 2003).

This study was conducted on five sampling sites during two years, one of them repeated in both years. The sites Cipo (CIP) and Recanto (REC) were sampled during 2006; Riacho da Serra (RSE) and Poço dos Patos (PPT), in 2007; Caturerê (CAT) was assessed during the hydrological cycles of 2006 and 2007 (Figure 1). The 2006 samples were performed in March/April, June, September and December; samples of 2007 were conducted in April, July, October and December.

2. *Collection and identification of specimens*

Four samples were performed at each sampling site (authorization n° 032-DIFAP/IBAMA) using gillnets (10 m long, mesh sizes 25, 35 and 45 mm), manuals trawls (20 m long, 2.5 m height, mesh sizes 10 mm; 4 m long, 2 m high, mesh sizes 5 mm, and cast nets (2 m height, mesh sizes 12 mm) with standardized effort (Medeiros et al. 2010). The fish were fixed in field with formalin 10% neutralized with sodium tetraborate.

The specimens were treated according to rules of scientific curation (Malabarba & Reis 1987). Sorting and identification of specimens were carried out at Laboratory of Systematics and Morphology of Fishes of Universidade Federal da Paraíba, according to specific literature. Voucher specimens were deposited after identification in the Ichthyological Collection of the same institution (see appendix).

Results and discussion

Thirty-five species in 28 genera of 14 families distributed in five orders of teleostean fishes were recorded (Table 1). The order Characiformes was the most representative, with 21 species (Table 1). Among the families registered, Characidae (Characiformes) had the highest number of species (11), followed by Cichlidae (Perciformes), with five species, Loricariidae (Siluriformes), with four species, and Curimatidae (Characiformes), with three; from the remaining families were registered one or two species (Table 1). Other studies in the rivers, streams and artificial environments of Brazilian Semi-arid region revealed this same pattern of dominance (Medeiros & Maltchik 2001, Ramos et al. 2005, Medeiros et al. 2006, Medeiros et al. 2008).

Among the registered species, four are introduced: *Cichla ocellaris* Bloch & Schneider, 1801, *Colossoma macropomum* (Cuvier, 1818), *Oreochromis niloticus* (Linnaeus, 1758) and *Poecilia reticulata* Peters, 1860 (Table 1), corresponding to 11.4% of the recorded ichthyofauna. Except for *Colossoma*

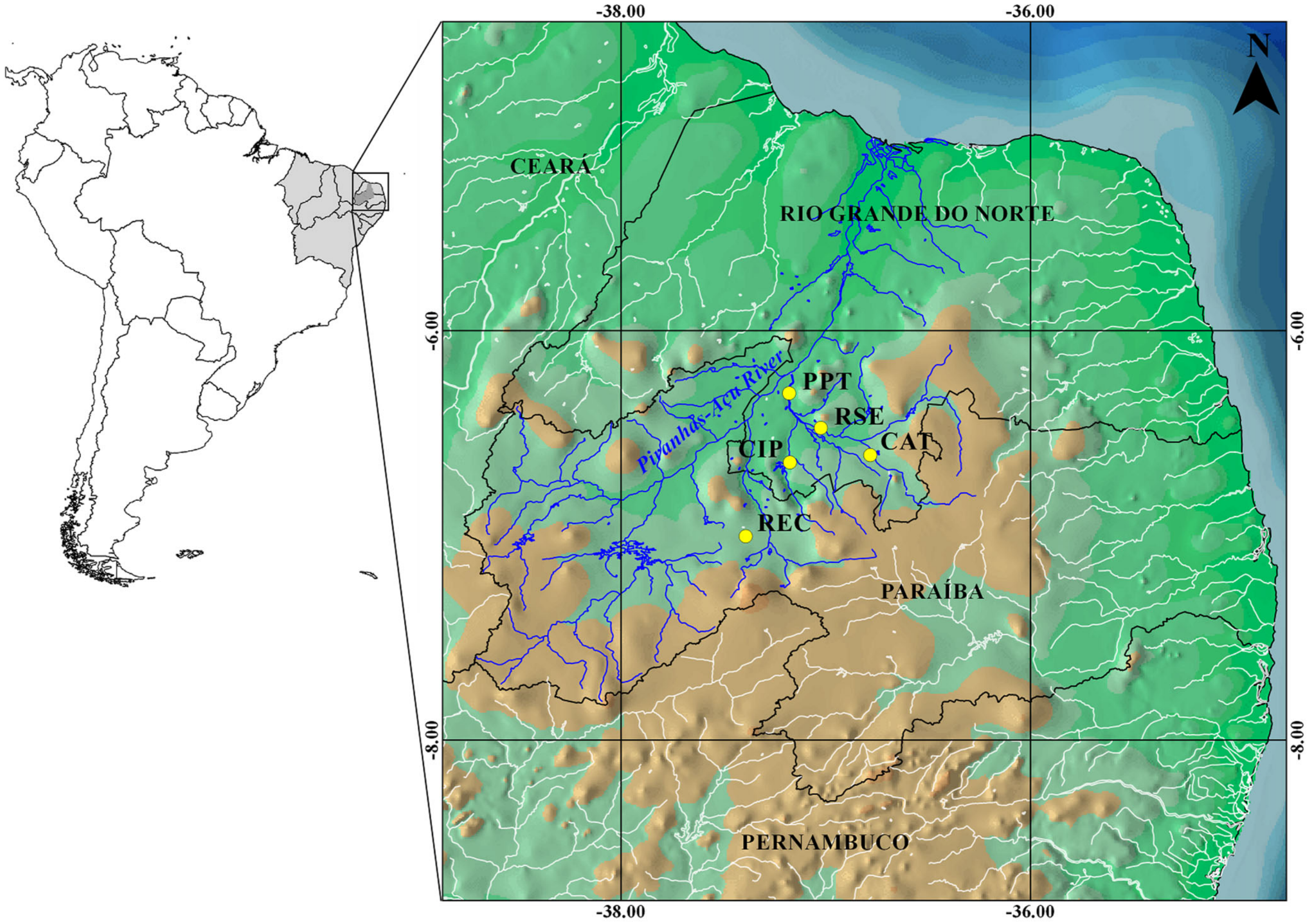


Figure 1. Study area showing major river systems and sampling sites in the semi-arid region of Brazil.

macropomum, which was registered only at the Recanto site, the three other aloctone species were sampled in three of the four study sites. There were no endangered species, when comparing the list of detected species with those contained in MMA (2008), IUCN (2012) and CITES (2013).

Astyanax aff. *bimaculatus* (Linnaeus, 1758), *Astyanax.* aff. *fasciatus* (Cuvier, 1819), *Hoplias malabaricus* (Bloch, 1794), *Prochilodus brevis* Steindachner, 1874 and *Serrapinnus heterodon* (Eigenmann, 1915) were the most widely distributed species in Seridó/Borborema region, being recorded in all sampling sites (Table 1). The four most abundant species in this inventory were *Oreochromis niloticus* (18.4% of specimens), *Astyanax* aff. *bimaculatus* (17.4%), *Poecilia vivipara* Schneider & Bloch, 1801 (12.7%), *Hemigrammus marginatus* Ellis, 1911 (12.3%) and *Serrapinnus heterodon* (12.1%) (Figure 2). *Oreochromis niloticus* (Tilapia) is an exotic species, as previously mentioned. The Tilapias are usually introduced from fish farming and have negatively impacted biodiversity at local level (GISP 2005, Attayde et al. 2007).

The collected specimens of *Astyanax* aff. *bimaculatus*, *Hemigrammus marginatus* and *Serrapinnus heterodon* accounted for 41.8% of all sampled individuals. These species belong to the group of "Piabas", of Characidae family. The reason for the high number of specimens of this group may be related to the habit of forming schools, which facilitates the capture of a larger number of individuals (Ramos 2012).

Other species with significant abundance was the "Guaru" *Poecilia vivipara*, a small fish able to survive in water with low oxygen content, and wide variation in salinity and temperature. This wide tolerance and its efficient reproductive strategy, allow the species to multiply rapidly and produce large numbers of individuals (Andreatta & Mendonça 2001); the species also forms schools.

Astyanax aff. *bimaculatus* was among the most abundant species and was collected in all sampling sites. This is a generalist species (Mazzoni 2010, Silva et al. 2010) that can reproduce during all year through partitioned or total spawning, depending on the characteristics of the environment (Ihering & Azevedo 1936, Geniari-Filho & Braga 1996).

Among the carnivorous species registered [*Serrasalmus rhombeus* (Linnaeus, 1766), *Hoplias malabaricus* and *Cichla ocellaris* Bloch & Schneider, 1801], *Hoplias malabaricus* was the most abundant and frequent (Figure 2). This result was expected and is in agreement with other studies in the region, which states *Hoplias malabaricus* as the top predator species commonly found in aquatic environments of Brazilian semi-arid (Paiva 1974, Chaves et al. 2009).

This inventory produced a comprehensive sampling of the ichthyofauna occurring at the Seridó/Borborema region, on the middle portion of Piranhas-Açu River basin. The number of 35 species registered in this study, overcomes in more than 50% that stated by previous work in the same basin (Nascimento et

Table 1. List of species collected in the Seridó/Borborema region. Taxonomic classification in accordance to Eschmeyer (2014); sampled sites: PPT = Poço dos Patos, RSE = Riacho da Serra, CAT = Caturerè, CIP = Cipó and REC = Recanto; * = Introduced species.

TAXON	PPT	RSE	CAT	CIP	REC	COMMON NAME
Characiformes						
Curimatidae						
Curimatella lepidura (Eigenmann & Eigenmann, 1889)			X			Branquinha
Psectrogaster rhomboides Eigenmann & Eigenmann, 1889	X		X			Branquinha
Steindachnerina notonota (Miranda-Ribeiro, 1937)			X			Branquinha
Prochilodontidae						
Prochilodus brevis Steindachner, 1874	X	X	X	X	X	Curimatã
Anostomidae						
Leporinus piau Fowler, 1941	X	X	X	X		Piau
Erythrinidae						
Hoplias malabaricus (Bloch, 1794)	X	X	X	X	X	Traíra
Serrasalmidae						
Colossoma macropomum (Cuvier 1816) *					X	Pacu
Serrasalmus brandtii Lütken, 1875	X	X	X			Pirambeba
Characidae						
Astyanax aff. *bimaculatus* (Linnaeus, 1758)	X	X	X	X	X	Piaba
Astyanax aff. *fasciatus* (Cuvier, 1819)	X	X	X	X	X	Piaba
Compsura heterura Eigenmann, 1915		X	X			Piaba
Hemigrammus brevis Ellis, 1911	X	X				Piaba
Hemigrammus marginatus Ellis, 1911	X	X	X			Piaba
Hemigrammus sp.		X				Piaba
Moenkhausia costae (Steindachner, 1907)		X				Piaba
Moenkhausia sp.	X	X	X			Piaba
Psellogrammus kennedyi (Eigenmann, 1903)		X				Maria-do-oião
Serrapinnus heterodon (Eigenmann, 1915)	X	X	X	X	X	Piaba
Serrapinnus piaba (Lütken, 1875)		X	X			Piaba
Triportheidae						
Triportheus signatus (Garman, 1890)	X	X	X			Sardinha
Crenuchidae						
Characidium bimaculatum Fowler, 1941	X	X	X		X	Canivete
Siluriformes						
Auchenipteridae						
Trachelyopterus galeatus (Linnaeus, 1766)	X	X				Cangati
Heptapteridae						
Pimelodella enochi Fowler, 1941			X			Mandi-mole
Loricariidae						
Hypostomus pusarum (Starks, 1913)	X	X	X	X		Cascudo
Pseudancistrus papariae Fowler 1941	X	X	X			Cascudo
Parotocinclus sp.	X	X				Cascudinho
Parotocinclus cf. *cearensis* Garavello, 1976	X					Cascudinho
Cyprinodontiformes						
Poecilidae						
Poecilia reticulata Peters, 1860 *	X	X	X			Guaru
Poecilia vivipara Bloch & Schneider, 1801	X	X	X	X		Guaru
Synbranchiformes						
Synbranchidae						
Synbranchus marmoratus Bloch,1785		X		X		Mussum
Perciformes						
Cichlidae						
Cichla ocellaris Bloch & Schneider, 1801 *	X	X		X		Tucunaré
Cichlasoma orientale Kullander, 1983	X	X	X	X		Corró-preto
Crenicichla menezesi Ploeg, 1991	X	X	X			Jacundá
Geophagus brasiliensis (Quoy & Gaimard, 1824)	X	X	X		X	Corró
Oreochromis niloticus (Linnaeus, 1758) *	X	X	X			Tilápia
Total of species	25	29	25	11	8	35

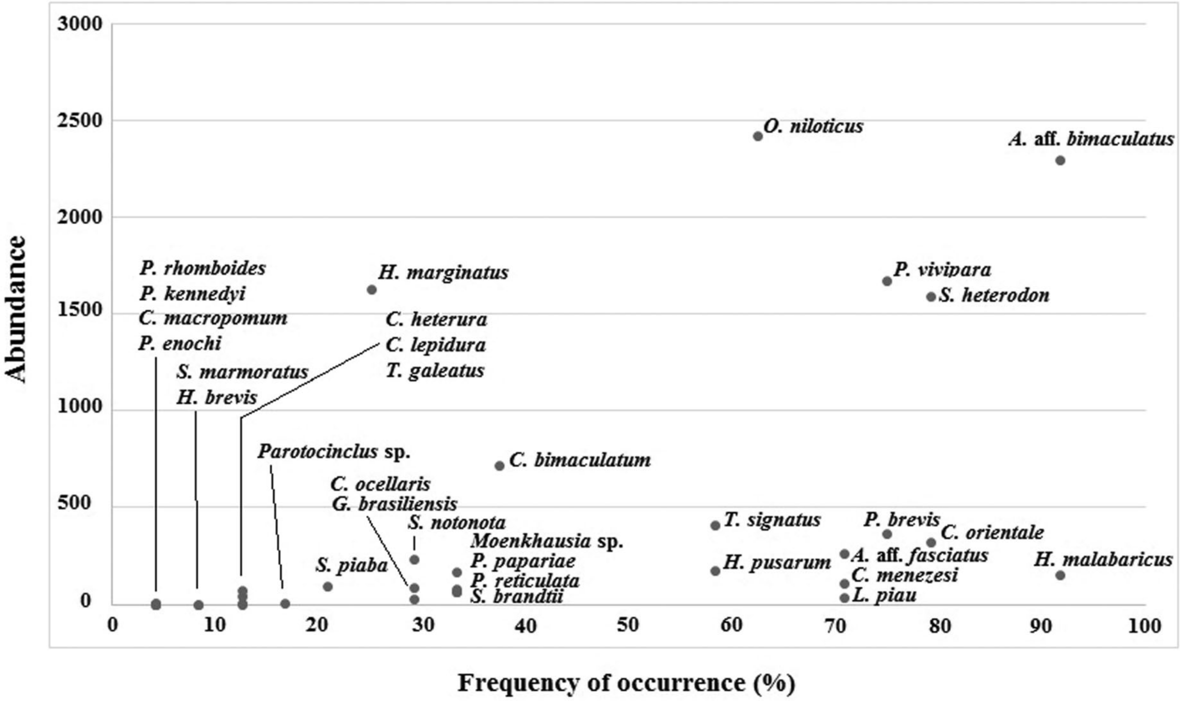


Figure 2. Most abundant fish species and their frequency of occurrence in the Seridó/Borborema region, Brazil.

al. 2011, 22 species) and others in the Curimataú River basin, adjacent to the study area (Ramos et al. 2005, Medeiros et al. 2006, 22 species). The results add data on the taxonomic composition and predominance of ichthyic elements in aquatic environments of Northeastern Brazil, in a priority area for the conservation of the Caatinga. Another relevant aspect is that this survey provides information on the ichthyofauna composition of Piranhas-Açu River basin before it receives water from the project of water transfer from São Francisco River to other Northern rivers basins.

Appendix

Listing of vouchers

UFPB6106, UFPB6107, UFPB6108, UFPB6109, UFPB6110, UFPB6111, UFPB6112, UFPB6113, UFPB6114, UFPB6121, UFPB6125, UFPB7878, UFPB7879, UFPB7880, UFPB7881, UFPB9684, UFPB9685, UFPB9686, UFPB9687, UFPB9688, UFPB9689, UFPB9690, UFPB9691, UFPB9692, UFPB9693, UFPB9694, UFPB9695, UFPB9696, UFPB9697, UFPB9698, UFPB9699, UFPB9700, UFPB9701, UFPB9702, UFPB9703, UFPB9704, UFPB9705, UFPB9706, UFPB9707, UFPB9708, UFPB9709, UFPB9710, UFPB9711, UFPB9712, UFPB9713.

Acknowledgements

The authors are grateful to Program for Research in Biodiversity (PPBio - Semi-Arid), to National Council of Technological and Scientific Development (MCT/CNPq 02/2006 - Universal 477545/2006-8), to Federal University of Paraíba (UFPB), to State University of Paraíba (UEPB) and to Foundation for Research Support of the State of Paraíba (Edital 02/2005 -FAPESQ/CNPq/UEPB - FAPESQ 68.0006/2006.0) by the logistical and financial support. MJS thanks the UEPB for scholarship granting for scientific initiation (2006/2007 and 2007/2008) and ESFM thanks CNPq for the scholarship of Regional Scientific and Technological Development (CNPq 350082/2006-5).

References

Amorim, I.L., Sampaio E.V.S.B., & Araújo, E.L. 2005. Flora e estrutura da vegetação arbustivo-arbórea de uma área de Caatinga do Seridó, RN, Brasil. Acta bot. bras. 19: 615-623, http://dx.doi.org/10.1590/S0102-33062005000300023

Attayde, J.L., Okun, N., Brasil, J., Menezes, R. & Mesquita, P. 2007. Impactos da introdução da tilápia do Nilo, *Oreochromis niloticus*, sobre a estrutura trófica dos ecossistemas aquáticos do Bioma Caatinga. Oecol. bras. 11 (3): 450-461, http://dx.doi.org/10.4257/oeco.2007.1103.13

Buckup, P.A., Menezes, N.A. & Ghazzi, M.S. 2007. Catálogo das Espécies de Peixes de Água Doce do Brasil. Rio de Janeiro: Museu Nacional.

Chaves, M.F., Torelli, J., Targino, C.H. & Crispim, M.C. 2009. Dinâmica reprodutiva e estrutura populacional de *Hoplias malabaricus* (Bloch, 1794) (Characiformes, Erythrinidae) em açude da bacia do rio Taperoá, Paraíba. Biotemas 22(2): 85-89.

CITES. 2013. http://www.cites.org/eng/app/appendices.php (last accessed 12/11/2013).

Coimbra-Filho, A.F. & Câmara, I.G. 1996. Os limites originais do bioma da Mata Atlântica na região Nordeste do Brasil. Rio de Janeiro: Fundação Brasileira para Conservação da Natureza.

CPTEC. 2011. www.cptec.inpe.br/proclima/ (last accessed 10/01/2012).

Eschmeyer, W.N. 2014. Catalog of fishes: genera, species, references. http://research.calacademy.org/research/ichthyology/catalog/fishcatmain.asp (last accessed 30/04/2014).

Felipe, J.L.A. & Carvalho, E.A. 1999. Atlas escolar do Rio Grande do Norte. João Pessoa: Grafset.

Geniari-Filho, O. & Braga, F.M.S. 1996. Fecundidade e desova de *Astyanax bimaculatus* e *A. schubarti* (Characidae, Tetragonopterinae) na represa de Barra Bonita, Rio Piracicaba (SP). Rev. UNIMAR 16(2): 241-254.

GISP. 2005. América do Sul invadida. A crescente ameaça das espécies exóticas invasoras. Secretaria do GISP. Giulietti, A.M., Du-Bocage-Neta, A.L., Paula, A.R.L., Barbosa, D.C., Nogueira, E., Sampaio, E.V.S.B., Silva, G.C., Machado, I.C., Vírginio, J.F., Maia, L.C., Griz, L.M.S., Queiroz, L.P., Lima, J.L.S., Silva, M.A., Figueiredo, M.A., Rodal, M.J.N., Barradas, M.M., Barbosa, M.R.V., Harley, R.M. & Chaves, S.M. 2003. Vegetação: áreas e ações prioritárias para a conservação da Caatinga In Ecologia e Conservação da Caatinga (I.R. Leal, J.M.C. Silva & M. Tabarelli, eds.). Recife: EDUFPE. p.112-131.

Ihering, R Von & Azevedo, P. 1936. As piabas dos açudes nordestinos (Characidae, Tetragonopterinae). Arquivos do Instituto Biológico. 7: 75-110.

IUCN. 2011. www.iucnredlist.org (last accessed 23/08/2011.

Kullander, S.O. & Ferreira, E.J.G. 2006. A review of the South American cichlid genus *Cichla*, with descriptions of nine new species (Teleostei: Cichlidae). Ichthyol. explor. freshw. 17: 289-398,

Langeani, F.L., Buckup, P.A., Malabarba, L.R., Py-Daniel, L.H.R., Lucena, C.A., Rosa, R.S., Zuanon, J.A.S., Lucena, Z.M.S., Britto, M.R., Oyakawa, O.T. & Gomes-Filho, G. 2009. Peixes de água doce In Estado da arte e perspectivas para a Zoologia no Brasil (R.M. Rocha & W.A.P. Boeger, eds.). Curitiba: p.211-230.

Leal, I.R., Silva, J.M.C. & Tabarelli, M. 2003. Ecologia e conservação da Caatinga: uma introdução ao desafio In Ecologia e Conservação da Caatinga (I.R. Leal, J.M.C. Silva & M. Tabarelli, eds.). Recife: EDUFPE. p.13-16.

Maia, G.N. 2004. Caatinga: árvores e arbustos e suas utilidades. São Paulo: D&Z.

Malabarba, L.R. & Reis, R.E. 1987. Manual de técnicas para a preparação de coleções zoológicas. Peixes. Campinas: Sociedade Brasileira de Zoologia.

Mazzoni, R. 2010. Alimentação e padrões ecomorfológicos das espécies de peixes de riacho do Alto Rio Tocantins, Goiás, Brasil. Iheringia 100(2): 162-168.

Medeiros, E.S.F. & Maltchik, L. 2001. Fish assemblage stability in an intermittently flowing stream from the Brazilian semiarid region. Austral Ecology 26: 156-164.

Medeiros, E.S.F., Ramos, R.T.C., Ramos, T.P.A. & Silva, M.J. 2006. Spatial variation in reservoir fish assemblages along semi-arid intermittent river, Curimataú River, northeastern Brazil. Campina Grande: Revista de biologia e ciências da terra. Suplemento Especial 1: 29-39.

Medeiros, E.S.F., Silva, M.J. & Ramos, R.T.C. 2008. Application of catchment- and local-scale variables of aquatic habitat characterization and assessment in the Brazilian Semi-arid Region. Neotrop. biol. conserv. 3(1): 13-20.

Medeiros, E.S.F., Silva, M.J., Figueiredo, B.R.S., Ramos, T.P.A. & Ramos, R.T.C. 2010. Effects of fishing technique on assessing species composition in aquatic systems in semi-arid Brazil. Braz. j. biol. 70: 255-262, http://dx.doi.org/10.1590/S1519-69842010000200004

Mendonça, J.P. & Andreata, J.V. 2001. Aspectos reprodutivos de *Poecilia vivipara* (Bloch & Schneider) (Poecilidae) da Lagoa Rodrigo de Freitas, Rio de Janeiro, Brasil. Rev. bras. zool. 18(4): 1041-1047, http://dx.doi.org/10.1590/S0101-81752001000400001

Menezes, N.A. 1996. Methods for assessing freshwater fish diversity. In: Biodiversity in Brazil: a first approach (C.E.M Bicudo & N.A Menezes, eds.). CNPq, São Paulo. p.289-295.

Menezes, N.A., Weitzman, S.H., Oyakawa, O.T., Lima, F.C.T., Castro, R.M.C. & Weitzman, M.J. 2007. Peixes de água doce da Mata Atlântica: lista preliminar das espécies e comentários sobre a conservação de peixes de água doce neotropicais. São Paulo, Museu de Zoologia, Universidade de São Paulo.

MMA. 2008. Livro vermelho da fauna brasileira ameaçada de extinção. 1.ed. - Brasília: MMA; Fundação Biodiversitas.

Nacimento, W.S., Araújo, A.S., Gurgel, L.L., Yamamoto, M.E., Chellappa, N.T., Rosa, R.S. & Chellappa, S. 2011. Endemic fish communities and environmental variables of the Piranhas-Assu Hydrografic Basin in the Brazilian Caatinga Ecoregion. Animal Biology Journal 2(3): 97-112.

Nogueira, C., Buckup, P.A., Menezes, N.A., Oyakawa, O.T., Kasecker, T.P., Ramos-Neto, M.B. & Silva, J.M.C. 2010. Restricted-range fishes and the conservation of Brazilian freshwaters. PLoS ONE, 5(6).

Paiva, M.P. 1974. Crescimento, alimentação e reprodução da traíra, Hoplias malabaricus (Bloch) no Nordeste brasileiro. Fortaleza: Imprensa universitária.

Ramos, T.P.A. 2012. Ictiofauna de Água Doce da Bacia do Rio Parnaíba. PhD Thesis, Federal University of Paraíba, João Pessoa.

Ramos, T.P.A., Ramos, R.T.C., Rosa, R.S., Groth, F. & Beltrão, G.B.M. 2005. Diversidade de peixes (Ictiofauna) da bacia do rio Curimataú, Paraíba In Análise das variações da biodiversidade do bioma Caatinga: suporte a estratégias regionais de conservação. (F.S. Araújo, M.J.N. Rodal & M.R.V. Barbosa, eds.). Brasília: Ministério do Meio Ambiente. p.291-317.

Reis, R.E., Kullander, S.O., Ferraris, J. & Carl, J. 2003. Check List of the freshwater fishes of South and Central America. Porto Alegre: EDIPUCRS.

Rosa, R.S., Menezes, N.A., Britski, H.A., Costa, W.J.E.M. & Groth, F. 2003. Diversidade, padrões de distribuição e conservação dos peixes da Caatinga In Ecologia e Conservação da Caatinga (I.R. Leal, J.M.C. Silva & M. Tabarelli, eds.). Recife: EDUFPE. p.135-181.

Rubel, F. & Kottek, M. 2010, Observed and projected climate shifts 1901-2100 depicted by world maps of the Köppen-Geiger climate classification. Meteorology. 19: 135-141, http://dx.doi.org/10.1127/0941-2948/2010/0430

Santos, A.C.A. & Zanata, A.M. 2006. Fishes in the Brazilian Semi-arid In Towards Greater Knowledge of the Brazilian Semi-arid Biodiversity. (L.P. Queiroz, A. Rapini & A.M. Giulietti, eds.). Brasília. p.97-102.

Silva, M.J., Figueiredo, B.R.S., Ramos, R.T.C. & Medeiros, E.S.F. 2010. Food resources used by three species of fish in the semi-arid region of Brazil. Neotrop. ichthyol. 4: 825-833.

Vari, R.P. 1983. Phylogenetic relationships of the families Curimatidae, Prochilodontidae, Anostomidae and Chilodontidae (Pisces: Characiformes). Smithsonian Contributions to Zoology, Smithsonian Institution Press. n. 378.

Tabarelli, M. & Silva, J.M.C. 2003. Áreas e ações prioritárias para a conservação da biodiversidade da Caatinga In Ecologia e Conservação da Caatinga. (I.R. Leal, J.M.C. Silva & M. Tabarelli, eds.). Recife: EDUFPE. p.777-796.

Received 29/11/2013
Revised 08/05/2014
Accepted 10/07/2014

Biota Neotropica 14(3): e20130078, 2014
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)

inventory

Herpetofauna of the Reserva Ecológica de Guapiaçu (REGUA) and its surrounding areas, in the state of Rio de Janeiro, Brazil

Mauricio Almeida-Gomes[1,5], Carla Costa Siqueira[2], Vitor Nelson Teixeira Borges-Júnior[2],
Davor Vrcibradic[3], Luciana Ardenghi Fusinatto[4] & Carlos Frederico Duarte Rocha[2]

[1] Departamento de Ecologia, Universidade Federal do Rio de Janeiro, Avenida Carlos Chagas Filho, 373, Cidade Universitária, CEP 21941-902, Rio de Janeiro, RJ, Brazil.
[2] Departamento de Ecologia, Universidade do Estado do Rio de Janeiro, R. São Francisco Xavier 524, CEP 20550-013, Rio de Janeiro, RJ, Brazil.
[3] Departamento de Zoologia, Universidade Federal do Estado do Rio de Janeiro, Av. Pasteur 458, Urca, CEP 22240-290, Rio de Janeiro, RJ, Brazil.
[4] Departamento de Ciências Biológicas, Universidade Federal de São Paulo, Campus Diadema, Rua Prof. Artur Ridel, 275, Eldorado, CEP 09972-270, Diadema, SP, Brazil.
[5] Corresponding author: Mauricio Almeida-Gomes, e-mail: almeida.gomes@yahoo.com.br

ALMEIDA-GOMES, M., SIQUEIRA, C.C., BORGES-JÚNIOR, V.N.T., VRCIBRADIC, D., FUSINATTO, L.A., ROCHA, C.F.D. **Herpetofauna of the Reserva Ecológica de Guapiaçu (REGUA) and its surrounding areas, in the state of Rio de Janeiro, Brazil.** Biota Neotropica. 14(3): e20130078. http://dx.doi.org/10.1590/1676-0603007813

Abstract: Species inventories are useful tools to improve conservation strategies, especially in highly threatened biomes such as the Brazilian Atlantic Forest. Here we present a species list of amphibians and reptiles for the Reserva Ecológica de Guapiaçu (REGUA), a forest reserve located in the central portion of Rio de Janeiro state, Brazil. The list results from an extensive sampling effort that lasted ten years. A total of 73 amphibian (of which ten are endemic to the state) and 37 reptile species was recorded from the area. Five amphibian species are presently categorized by the IUCN as "data-deficient", two as "near threatened" and one as "endangered", whereas one reptile species is categorized as "vulnerable". Our results showed that REGUA harbors about one-third of the herpetofauna species presently known to occur in state of Rio de Janeiro, adding more information to previously published lists of amphibians and reptiles from localities within the Serra dos Órgãos region, and highlighting the importance of this area for conservation of amphibians and reptiles of the Atlantic Forest.
Keywords: *amphibians, Atlantic Forest, conservation, hotspot, reptiles.*

ALMEIDA-GOMES, M., SIQUEIRA, C.C., BORGES-JÚNIOR, V.N.T., VRCIBRADIC, D., FUSINATTO, L.A., ROCHA, C.F.D. **Herpetofauna da Reserva Ecológica de Guapiaçu (REGUA) e das áreas do entorno, no estado do Rio de Janeiro, Brasil.** Biota Neotropica. 14(3): e20130078. http://dx.doi.org/10.1590/1676-0603007813

Resumo: Inventários de espécies constituem importantes subsídios para melhorar as estratégias de conservação, especialmente em biomas altamente ameaçados como a Mata Atlântica brasileira. Aqui, nós apresentamos uma lista de anfíbios e répteis para a Reserva Ecológica de Guapiaçu (REGUA), uma reserva florestal localizada na porção central do estado do Rio de Janeiro, Brasil. A lista é baseada em um esforço de amostragem extensivo de dez anos de duração. Um total de 73 espécies de anfíbios (das quais dez são endêmicas ao estado) e 37 espécies de répteis foi registrado na área. Cinco das espécies de anfíbios estão atualmente categorizadas pela IUCN como "deficiente em dados", duas como "quase ameaçadas" e uma como "em perigo", enquanto uma espécie de réptil está categorizada como "vulnerável". Nossos resultados mostram que a região da REGUA abriga cerca de um terço da herpetofauna atualmente conhecida para o estado do Rio de Janeiro, adicionando mais espécies em relação a listas de espécies anfíbios e répteis previamente publicadas para localidades na região da Serra dos Órgãos e enfatizando a importância desta área para a conservação dos anfíbios e répteis da Mata Atlântica.
Palavras-chave: *anfíbios, Mata Atlântica, conservação, hotspot, répteis.*

Introduction

The Atlantic Forest is one of the world's most threatened biodiversity "hotspots" (Mittermeier et al. 2011) and despite being currently reduced to only about 12% of its original area, it still harbors great diversity and high rates of endemism for several animal groups (Ribeiro et al. 2009). More than 500 amphibian and about 200 reptile species occur in this biome (Ribeiro et al. 2009, Haddad et al. 2013). For the state of Rio de Janeiro there are nearly 200 amphibian and over 130 reptile species currently reported (Vrcibradic et al. 2011b), with both new geographic distribution records (e.g. Vrcibradic et al. 2006a, b, Goyannes-Araújo et al. 2009, Silveira et al. 2009, Caram et al. 2011) and descriptions/revalidations of taxa (particularly for amphibians; e.g. Canedo & Pombal 2007, Pombal et al. 2008, Pombal 2010, Weber et al. 2011, Dias et al. 2013, Caramaschi & Cruz 2013, Mângia et al. 2014) having recently added more species to the amphibian and reptile lists previously compiled by Rocha et al. (2004).

Collections of field data and species inventories are urgent to guide species conservation in Brazil (Verdade et al. 2012). Despite the increasing number of amphibian and/or reptile species lists for various localities in the state of Rio de Janeiro in the last ten years (e.g. Rocha & Van Sluys 2006, Almeida-Gomes et al. 2008, 2010, Carvalho-e-Silva et al. 2008, Salles et al. 2009, 2010, Salles & Silva-Soares 2010, Siqueira et al. 2011a, b, Vrcibradic et al. 2011b, Telles et al. 2012, Bittencourt-Silva & Silva 2013, Rocha et al. 2013), there are some areas that still need more studies in order to provide a comprehensive characterization of their herpetofaunas.

The Reserva Ecológica de Guapiaçu (REGUA) is a private reserve, which has an area of nearly 7,600 ha. REGUA together with other conservation units such as Parque Estadual dos Três Picos (*ca.* 46,000 ha), Parque Nacional da Serra dos Órgãos (*ca.* 11,800 ha), and Estação Ecológica Estadual do Paraíso (*ca.* 5,000 ha) protects a large continuous area of wet Atlantic Forest (mostly represented by montane and low montane rainforest) covering much of the region of Serra dos Órgãos, which comprises one of the areas of highest biodiversity in the state (Rocha et al. 2003).

Information on the herpetofauna of REGUA currently consists of a first approach on the local forest floor leaf-litter amphibian community, derived from data obtained in a short-term inventory conducted in 2004 (Rocha et al. 2007), and a first approach on the local lizard fauna based on a study carried out between 2007 and 2010 (Almeida-Gomes & Rocha in press). Besides, there are isolated occurrence records and ecological observations on some species of frogs (Weber et al. 2007, 2009, Silva-Soares et al. 2008, Klaion et al. 2011, Maia-Carneiro et al. 2012a, b, 2013, Salles et al. 2012, Siqueira et al. 2013), lizards (Goyannes-Araújo et al. 2009, Maia et al. 2011, Almeida-Gomes et al. 2012), and snakes (Alves et al. 2005, Silveira et al. 2010, Vrcibradic et al. 2011a). Here, we present the list of amphibian and reptile species based on records obtained during nearly ten years of surveys at REGUA and its surroundings.

Material and Methods

1. *Study area*

The Reserva Ecológica de Guapiaçu (REGUA) (22° 24'S, 42° 44'W) is located at the slopes of the Serra dos Órgãos mountain range, in the municipality of Cachoeiras de Macacu, state of Rio de Janeiro, Brazil (Figure 1). In the continuous forest of REGUA, habitats range from secondary forests in early stages of ecological succession to areas of relatively little disturbed forest, in the higher and more inaccessible portions of the reserve (Rocha et al. 2007). In the surroundings of REGUA there are forest fragments of different sizes and degrees of regeneration and isolation, which are immersed in different types of matrix (mainly pastures) (Almeida-Gomes & Rocha 2014). The region has a mean annual precipitation of 2600 mm, with daily temperatures ranging from 14 to 37 °C (Bernardo et al. 2011).

2. *Data collection*

The amphibians and reptiles were sampled mainly within the continuous forest of REGUA, but also in 21 forest fragments (totaling nearly 1,300 ha of forested area), and in

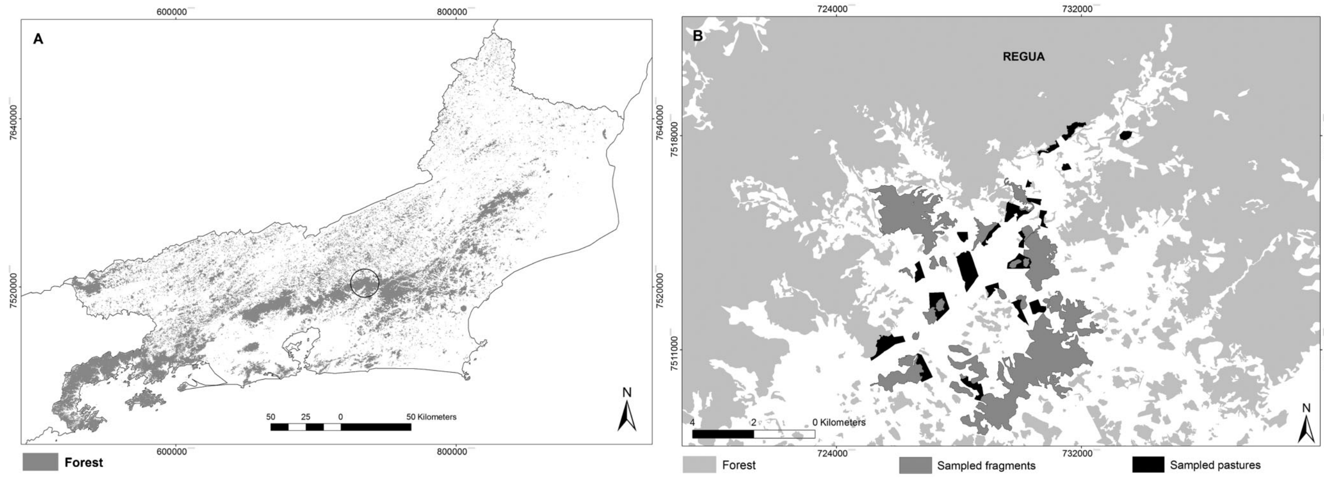


Figure 1. (A) Location of Reserva Ecológica de Guapiaçu (REGUA) in the state of Rio de Janeiro, Brazil (open circle) and (B) detail of the study area showing the continuous forest of REGUA, the sampled forest fragments and the sampled pasture areas (matrix).

the surrounding pasture matrix (besides some anthropogenic habitats such as roads). Samplings were done during September 2004 and from July 2007 to February 2014 at altitudes ranging from ca. 30 m to 700 m. We used three different methods for herpetofaunal sampling: pitfall traps with drift fences (Corn 1994), time-constrained visual encounter surveys (VES; Crump & Scott 1994) and quadrats (16 m^2 and 25 m^2 plots; Jaeger & Inger 1994). The total sampling effort for each method was 6600 bucket-days (for pitfalls), 2631 hours (for VES) and 4750 m^2 of forest floor surveyed (for quadrat method). Except for pasture areas (sampled only by VES), we used the three sampling methods in the continuous forest of REGUA and in forest fragments. Besides, we also recorded all specimens of amphibians and reptiles found during casual encounters (i.e. not using the above methodologies). Voucher specimens of all sampled species of the local herpetofauna were deposited at the Museu Nacional do Rio de Janeiro - MNRJ (Appendix 1), except for a few species of reptiles (the lizards *Ameiva ameiva* and *Salvator merianae*, the snakes *Boa constrictor*, *Chironius laevicollis*, *Pseustes sulphureus* and *Spilotes pullatus*, and the crocodylian *Caiman latirostris*); in all those cases the specimens found were too large for preservation and storage (considering the material we had available) or could not be collected (in the case of *Ameiva ameiva*). Also, one amphibian species (*Vitreorana* sp.) was recorded based on an observation of an individual that evaded capture. Identification of all specimens of amphibians and reptiles collected were confirmed by taxonomists from the Museu Nacional, Rio de Janeiro; the specimens that were not collected were identified in the field by the authors. For amphibian nomenclature used throughout the text we follow Frost (2013), and regarding reptile nomenclature we follow Carrasco et al. (2012) for viperid snakes, Harvey et al. (2012) for teiid lizards, and Pyron et al. (2013) for the remaining groups.

Results and Discussion

We recorded 73 species of amphibians (71 anurans and two caecilians) (Table 1; Figures 2-5) and 37 species of reptiles (10 lizards, 24 snakes, one amphisbaenian, one chelonian and one crocodylian) (Table 2; Figures 6-8). The species richness we recorded in the area corresponds to *ca.* 40% and *ca.* 30% of the total species richness of, respectively, amphibians and reptiles known to occur in the state of Rio de Janeiro (see Vrcibradic et al. 2011b). Regarding the anurofauna in particular, the species richness reported here for REGUA is the highest yet reported for Atlantic Forest areas in which anuran surveys have been conducted, with only the Estação

Table 1. Amphibian species of the Reserva Ecológica de Guapiaçu (REGUA) and its surroundings, municipality of Cachoeiras de Macacu, state of Rio de Janeiro, Brazil. The habitats where they have been recorded are represented by: CF – continuous forest of REGUA; FF – forest fragments; and M – matrix habitats (composed of pastures, wetlands and anthropogenic areas). * Species endemic to state of Rio de Janeiro.

TAXA	HABITAT
ANURA	
Brachycephalidae	
Brachycephalus didactylus (Izecksohn, 1971)	CF, FF
Brachycephalus ephippium (Spix, 1824)	CF
Ischnocnema guentheri (Steindachner, 1864)	CF, FF
Ischnocnema octavioi (Bokermann, 1965)	CF, FF
Ischnocnema parva (Girard, 1853)	CF
Ischnocnema cf. *venancioi* (Lutz, 1958)	CF
Bufonidae	
Dendrophryniscus brevipollicatus Jiménez de la Espada, 1870	CF
Rhinella hoogmoedi Caramaschi and Pombal, 2006	CF
Rhinella icterica (Spix, 1824)	CF, FF, M
Rhinella ornata (Spix, 1824)	CF, FF, M
Centrolenidae	
Vitreorana sp.	CF
Craugastoridae	
Euparkerella brasiliensis (Parker, 1926) *	CF, FF
Haddadus binotatus (Spix, 1824)	CF, FF
Cycloramphidae	
Cycloramphus brasiliensis (Steindachner, 1864) *	CF
Thoropa miliaris (Spix, 1824)	CF, FF
Zachaenus parvulus (Girard, 1853)	CF
Hemiphractidae	
Fritziana goeldii (Boulenger, 1895)	CF
Gastrotheca albolineata (Lutz and Lutz, 1939)	CF
Hylidae	
Aplastodiscus eugenioi (Carvalho-e-Silva and Carvalho-e-Silva, 2005)	CF, FF
Bokermannohyla circumdata (Cope, 1871)	CF
Dendropsophus anceps (Lutz, 1929)	FF, M
Dendropsophus berthalutzae (Bokermann, 1962)	CF, FF, M

Continued on next page

Table 1. Continued.

TAXA	HABITAT
Dendropsophus bipunctatus (Spix, 1824)	FF, M
Dendropsophus decipiens (Lutz, 1925)	FF, M
Dendropsophus elegans (Wied-Neuwied, 1824)	CF, FF, M
Dendropsophus giesleri (Mertens, 1950)	FF
Dendropsophus meridianus (Lutz, 1954)	FF, M
Dendropsophus microps (Peters, 1872)	M
Dendropsophus minutus (Peters, 1872)	FF, M
Dendropsophus pseudomeridianus (Cruz, Caramaschi and Dias, 2000)	FF, M
Dendropsophus seniculus (Cope, 1868)	CF, FF, M
Hypsiboas albomarginatus (Spix, 1824)	CF, FF, M
Hypsiboas albopunctatus (Spix, 1824)	M
Hypsiboas faber (Wied-Neuwied, 1821)	CF, FF, M
Hypsiboas pardalis (Spix, 1824)	CF, M
Hypsiboas secedens (Lutz, 1963) *	CF, FF
Hypsiboas semilineatus (Spix, 1824)	CF, FF, M
Itapotihyla langsdorffii (Duméril and Bibron, 1841)	FF
Phyllomedusa burmeisteri (Boulenger, 1882)	CF, FF, M
Phyllomedusa rohdei Mertens, 1926	CF, FF, M
Scinax albicans (Bokermann, 1967) *	CF
Scinax alter (Lutz, 1973)	M
Scinax argyreornatus (Miranda-Ribeiro, 1926)	CF, FF, M
Scinax cuspidatus (Lutz, 1925)	FF, M
Scinax flavoguttatus (Lutz and Lutz, 1939)	CF
Scinax hayii (Barbour, 1909)	CF
Scinax humilis (Lutz and Lutz, 1954) *	CF, FF, M
Scinax v-signatus (Lutz, 1968)*	CF
Scinax aff. *x-signatus*	CF, FF, M
Sphaenorhynchus planicola (Lutz and Lutz, 1938)	M
Trachycephalus mesophaeus (Hensel, 1867)	CF, FF, M
Trachycephalus nigromaculatus Tschudi, 1838	FF
Hylodidae	
Crossodactylus aeneus Müller, 1924	CF
Hylodes asper (Müller, 1924)	CF
Hylodes charadranaetes Heyer and Cocroft, 1986 *	CF
Hylodes lateristrigatus (Baumann, 1912)	CF
Hylodes pipilans Canedo and Pombal 2007 *	CF
Megaelosia goeldii (Baumann, 1912)	CF
Leptodactylidae	
Adenomera cf. *bokermanni* (Heyer 1973)	M
Adenomera marmorata Steindachner, 1867	CF, FF, M
Leptodactylus fuscus (Schneider, 1799)	M
Leptodactylus latrans (Steffen, 1815)	CF, FF, M
Leptodactylus mystacinus (Burmeister, 1861)	FF
Leptodactylus spixi Heyer, 1983	FF
Physalaemus signifer (Girard, 1853)	CF, FF, M
Microhylidae	
Chiasmocleis carvalhoi Cruz, Caramaschi and Izecksohn, 1997	CF, FF
Myersiella microps (Duméril and Bibron, 1841)	FF
Stereocyclops parkeri (Wettstein, 1934)	FF
Odontophrynidae	
Proceratophrys appendiculata (Günther, 1873) *	CF
Proceratophrys boiei (Wied-Neuwied, 1824)	CF
Ranidae	
Lithobates catesbeianus (Shaw, 1802)	M
GYMNOPHIONA	
Siphonopidae	
Siphonops hardyi Boulenger, 1888	CF
Typhlonectidae	
Chthonerpeton braestrupi Taylor, 1968	FF

http://dx.doi.org/10.1590/1676-0603007813

Figure 2. Some anurans recorded in the Reserva Ecológica de Guapiaçu, southeastern Brazil: a) *Brachycephalus ephippium*; b) *Ischnocnema guentheri; c) Rhinella hoogmoedi*; d) *Rhinella ornata*; e) *Euparkerella brasiliensis*; f) *Haddadus binotatus*; g) *Cycloramphus brasiliensis*; h) *Zachaenus parvulus*. Photos by M. Almeida-Gomes (a, b, c, h) and D. Vrcibradic (d, e, f, g).

Biológica de Boracéia (in São Paulo state) presenting a comparable richness (see review in Salles et al. 2009). This reflects the intensive and extensive sampling effort employed in our study and the diversity of habitats (including both forested and open areas) and altitudinal gradient (30 - 700m) surveyed, all of which likely maximized our potential to sample a representative portion of the region's anuran diversity. The reptile richness reported here for REGUA is likely underestimated, but is still among the highest recorded for inventoried areas of Atlantic Forest (see review in Salles et al. 2010). Our study represents a further contribution to the knowledge of the herpetofauna of the Serra dos Órgãos region, adding more information to previously published lists of amphibians and reptiles from other localities within that region (Siqueira et al. 2009, 2011b, Silva-Soares et al. 2010, Vrcibradic et al. 2011b).

Figure 3. Some anurans recorded in the Reserva Ecológica de Guapiaçu, southeastern Brazil: a) *Gastrotheca albolineata*; b) *Bokermannohyla circumdata*; c) *Dendropsophus anceps*; d) *Dendropsophus seniculus*; e) *Hypsiboas faber*; f) *Phyllomedusa burmeisteri*; g) *Scinax v-signatus*; h) *Trachycephalus nigromaculatus*. Photos by L. Fusinatto (a, f), M. Almeida-Gomes (b, c, d, g, h) and V. Borges-Júnior (e).

Of the 73 amphibian species recorded, 25 (34%) were found only in continuous forest, whereas 17 (23%) were found in all three types of habitats (continuous forest, forest fragments and matrix) (Table 1). Of the 37 reptile species recorded, 13 (35%) were found only in continuous forest, whereas four (11%) were found only in matrix habitat (Table 2). Landscape heterogeneity (continuous forest, forest fragments and matrix habitats) allows for different forms of occupation by species of the herpetofauna (Almeida-Gomes et al. 2010, Dixo & Metzger 2010; Almeida-Gomes & Rocha 2014, Almeida-Gomes & Rocha in press), as observed in the present study at REGUA. The amphibian community was dominated by species of the family Hylidae (33 spp.), many of them found in disturbed landscapes, where they commonly use permanent or temporary ponds for reproduction (e.g. Almeida-Gomes et al. 2008, 2010). Among reptiles, some species such as the crocodylian *Caiman*

Figure 4. Some anurans recorded in the Reserva Ecológica de Guapiaçu, southeastern Brazil: a) *Hylodes charadranaetes*; b) *Megaelosia goeldii*; c) *Adenomera marmorata*; d) *Leptodactylus mystacinus*; e) *Physalaemus signifer*; f) *Chiasmocleis carvalhoi*; g) *Myersiella microps*; h) *Proceratophrys boiei*. Photos by D. Vrcibradic (a, b, e), L. Fusinatto (c), M. Almeida-Gomes (d, f, h) and V. Borges-Júnior (g).

latirostris (broad-snouted caiman) were found only in the matrix habitat (specifically in wetlands and ponds). Thus, not only the forested habitats but also the matrix habitats are responsible for maintaining the great local diversity of amphibians and reptiles observed at REGUA.

We recorded two exotic species among the herpetofauna of REGUA: the African gekkonid lizard *Hemidactylus mabouia* (tropical house gecko) and the North American ranid frog *Lithobates catesbeianus* (American bullfrog). It is noteworthy that we recorded the presence of *H. mabouia* in natural areas inside some forest fragments sharing the habitat with native lizards. This indicates that *H. mabouia* has become an invasive species in remnants of Atlantic Forest in the area, as reported for other localities in Brazil (Anjos & Rocha 2008, Rocha et al. 2011). However, this species has not been recorded so far within the continuous forest of REGUA, only in fragments and in the matrix habitat. This suggests that *H. mabouia* may have a limited ability to colonize the more preserved forest habitats in

Figure 5. Gymnophiona recorded in the Reserva Ecológica de Guapiaçu, southeastern Brazil: a) *Siphonops hardyi*; b) *Chthonerpeton braestrupi*. Photos by D. Vrcibradic.

the area, especially far from forest edges. On the other hand, we found the bullfrog *L. catesbeianus* only in matrix habitats, with no records of the species occurring in natural habitats within the reserve. This suggests that *L. catesbeianus* may not have become an invasive species in the area and is possibly confined to perianthropic environments. The status of introduced American bullfrog populations in Brazil and their actual impact on natural ecosystems are currently not well known, despite this species having been reported in the wild from numerous localities in Brazil (Giovanelli et al. 2008, Both et al. 2011).

Regarding amphibians, nine species (*Euparkerella brasiliensis, Cycloramphus brasiliensis, Proceratophrys appendiculata, Hypsiboas secedens, Scinax albicans, Scinax humilis, Scinax v-signatus, Hylodes charadranaetes,* and *Hylodes pipilans*) are currently considered as endemic to state of Rio de Janeiro (Weber et al. 2009, Dias et al. 2013, Haddad et al. 2013, Silva & Alves-Silva 2013), representing 12% of the species herein recorded. Five amphibian species (*H. secedens, Crossodactylus aeneus, H. charadranaetes, H. pipilans* and *Chthonerpeton braestrupi*) are presently categorized by the IUCN (2013) as ''data-deficient'', two (*C. brasiliensis* and *Aplastodiscus eugenioi*) as ''near threatened'', and one (*Chiasmocleis carvalhoi*) as ''endangered''. Among the reptile species recorded, one (the turtle *Hydromedusa maximiliani*) is presently categorized by the IUCN (2013) as ''vulnerable''. The presence of endemic species and species listed in threatened categories points to the potential of REGUA for species conservation. Moreover, three of the ''data-deficient'' species (*Crossodactylus aeneus, H. charadranaetes,* and *H. pipilans*) and the two near-threatened ones (*C. brasiliensis* and *A. eugenioi*) have their life cycle restricted to streams inside forests, a life history characteristic that has been linked to amphibian declines in pristine areas (Stuart et al. 2004, Whiles et al. 2006), which points to the urgency of monitoring studies of these populations. A further interesting fact is that sites with ''data-deficient'' species may indicate high potential for discovery of new species (Trindade-Filho et al. 2012) suggesting that, besides the species recently described from the general area (e.g. *Hylodes pipilans*; Canedo & Pombal 2007), there may be more unknown species to be found or described at REGUA and its surroundings (e.g. Gehara et al. 2013). Furthermore, some species currently recognized as data deficient might have their extinction threat underestimated (Almeida-Gomes et al. 2014), which makes it urgent to conduct further studies to assess their real population status.

Some of the amphibians recorded at REGUA present taxonomic problems. One caecilian collected in a large forest fragment while moving on the ground during a rainy night was identified as *Chthonerpeton braestrupi* by Dr Mark Wilkinson (Natural History Museum, London, UK). This species is currently known only from the original description by Taylor (1968), who gives an imprecise type locality (''Brazil''). Also, the sole type specimen is apparently lost (see Frost 2013). More sampling effort and study of *Chthonerpeton* specimens in zoological collections are needed to determine to which population(s) the name *Chthonerpeton braestrupi* can be applied. Among the frogs, *Scinax* aff. *x-signatus* apparently represents an undescribed species (M.C.S. Cardoso, pers. comm.). One glass frog (*Vitreorana* sp.) was seen on a bromeliad at night, but evaded capture and therefore could not be identified to species (no other glass frogs were seen during the study). The taxonomic status of populations currently attributed to *Adenomera bokermanni* is problematic, as the type series of that taxon is composed of more than one species (J.P. Pombal Jr, pers. comm.). Also, what we are treating here under the name *Scinax v-signatus* may represent more than one taxon. Finally, taxa such as *Ischnocnema guentheri* (Gehara et al. 2013), *Euparkerella brasiliensis* (Fusinatto et al. 2013), *Adenomera marmorata* (C.S. Cassini, pers. comm.), and *Dendrophryniscus brevipollicatus* (C.A.G. Cruz, pers. comm.) represent species complexes, and the taxonomic status and/or the number of taxa recognized under those names that occur at REGUA are not known at present.

Based on our extensive sampling effort, using different collecting methodologies during a long-term study, we believe that REGUA currently constitutes one of the most well studied areas in the state of Rio de Janeiro, regarding its herpetofauna. We believe that our species list is quite comprehensive (especially for amphibians) and represents a good approximation of the composition of the local herpetofauna, and that REGUA can be considered as a reservoir of a considerable portion of the state's amphibian and reptile biodiversity.

Acknowledgments

This study was supported by research grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (processes 304791/2010-5 and 472287/2012-5) and from Fundação de Amparo à Pesquisa do Estado

Table 2. Reptile species of the Reserva Ecológica de Guapiaçu (REGUA) and its surroundings, municipality of Cachoeiras de Macacu, state of Rio de Janeiro. The habitats where they have been recorded are represented by: CF – continuous forest of REGUA; FF – forest fragments; and M – matrix habitats (composed of pastures, wetlands and anthropogenic areas).

TAXA	Habitat
AMPHISBAENIA	
Amphisbaenidae	
Amphisbaena microcephala (Wagler, 1824)	CF
LACERTILIA	
Anguidae	
Ophiodes striatus (Spix, 1824)	FF, M
Dactyloidae	
Anolis fuscoauratus D'Orbigny, 1837	FF
Anolis punctatus Daudin, 1802	CF, FF
Gekkonidae	
Hemidactylus mabouia (Moreau de Jonnès, 1818)	FF, M
Gymnophtalmidae	
Ecpleopus gaudichaudii Duméril and Bibron, 1839	CF, FF
Leiosauridae	
Enyalius brasiliensis (Lesson, 1830)	CF, FF
Phyllodactylidae	
Gymnodactylus darwinii (Gray, 1845)	FF
Scincidae	
Mabuya macrorhyncha Hoge, 1946	FF, M
Teiidae	
Ameiva ameiva (Linnaeus, 1758)	M
Salvator merianae Duméril and Bibron, 1839	CF, FF
SERPENTES	
Boidae	
Boa constrictor Linnaeus, 1758	M
Corallus hortulanus (Linnaeus, 1758)	CF, FF
Colubridae	
Chironius bicarinatus (Wied, 1820)	FF
Chironius foveatus Bailey, 1955	CF
Chironius fuscus (Linnaeus, 1758)	CF
Chironius laevicollis (Wied, 1824)	M
Echinanthera amoena (Jan, 1863)	CF
Echinanthera cephalostriata Di Bernardo, 1996	CF
Helicops carinicaudus (Wied-Neuwied, 1825)	CF
Imantodes cenchoa (Linnaeus, 1758)	CF
Liophis miliaris (Linnaeus, 1758)	FF, M
Liophis reginae (Linnaeus, 1758)	CF, FF
Oxyrhopus clathratus Duméril, Bibron & Duméril, 1854	CF
Oxyrhopus petolarius (Linnaeus, 1758)	CF, FF
Philodryas patagoniensis (Girard, 1858)	M
Pseustes sulphureus (Wagler, 1824)	CF
Sibynomorphus neuwiedi (Ihering, 1911)	CF,FF
Siphlophis compressus (Daudin, 1803)	CF
Spilotes pullatus (Linnaeus, 1758)	CF, M
Taeniophallus affinis (Günther, 1858)	CF, FF
Xenodon neuwiedii Günther, 1863	CF, FF
Elapidae	
Micrurus corallinus (Merrem, 1820)	CF
Viperidae	
Bothrops jararaca (Wied, 1824)	CF, FF
Bothrops jararacussu Lacerda, 1884	CF
CROCODYLIA	
Alligatoridae	
Caiman latirostris (Daudin, 1802)	M
TESTUDINES	
Chelidae	
Hydromedusa maximiliani (Mikan, 1820)	CF

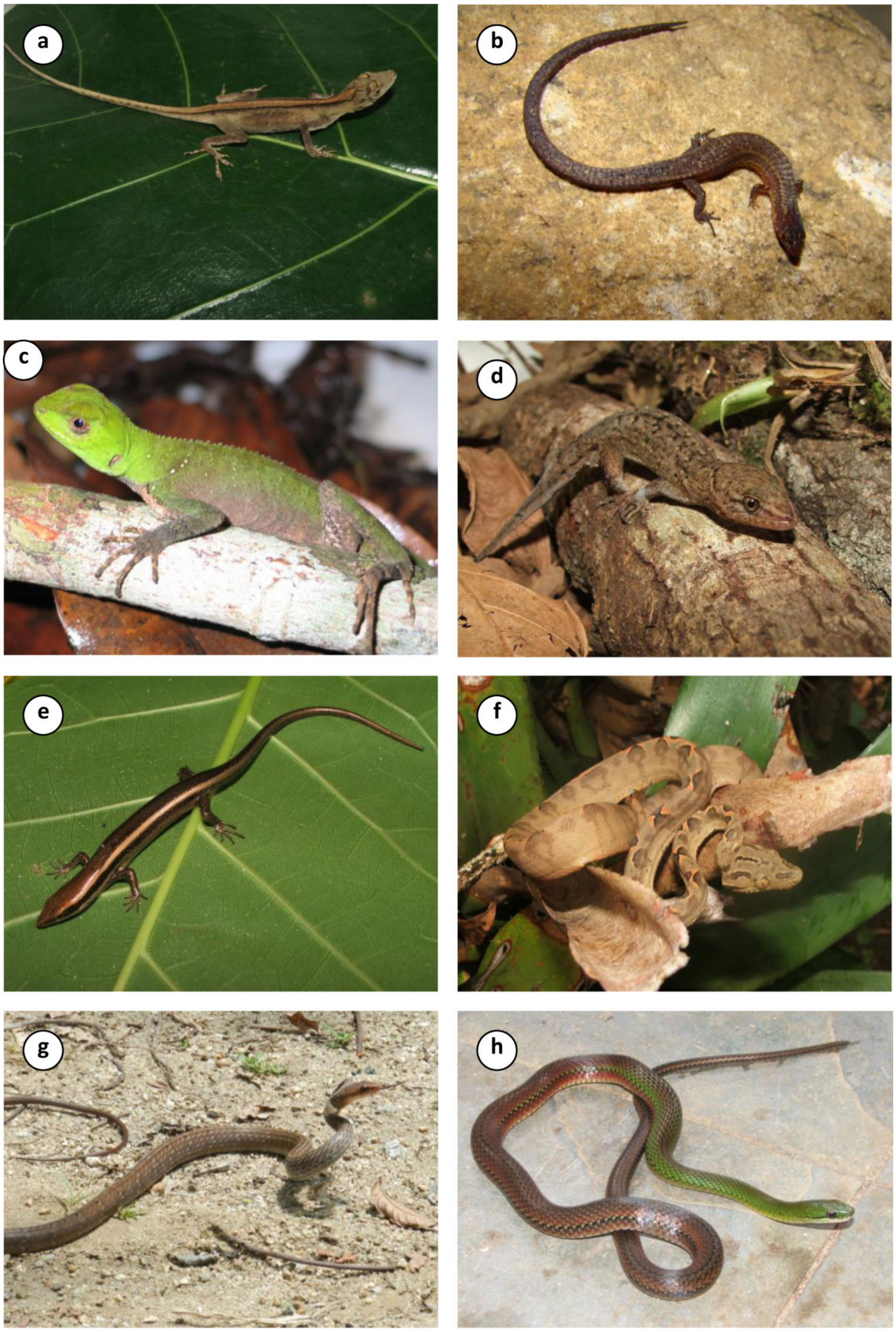


Figure 6. Some reptiles recorded in the Reserva Ecológica de Guapiaçu, southeastern Brazil: a) *Anolis fuscoauratus*; b) *Ecpleopus gaudichaudii*; c) *Enyalius brasiliensis*; d) *Gymnodactylus darwinii*; e) *Mabuya macrorhyncha*; f) *Corallus hortulanus*; g) *Chironius fuscus*; h) *Echinanthera amoena*. Photos by D. Vrcibradic (a, d, e, f, h), M. Almeida-Gomes (b), C.F. Rocha (c) and L. Fusinatto (g).

do Rio de Janeiro (FAPERJ) through ''Cientistas do Nosso Estado'' Program (process E-26/102.765/2012) to C. F. D. Rocha. This project also benefited from funding from the ''Edital Espécies Ameaçadas'' of Fundação Biodiversitas/CEPAN and RAN/ICMBio (Project No. 0158A/012006). M. Almeida-Gomes received PhD scholarship from Conservation International - Brazil and FAPERJ, and currently receives a Post-Doctoral scholarship from Programa Nacional de Pós Doutorado/Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – PNPD/CAPES. C.C. Siqueira received a PhD scholarship from CNPq, Post-Doctoral grants from CNPq, and currently receives a Post-Doctoral fellowship from PNPD-CAPES. V.N.T. Borges-Júnior received a PhD scholarship from CNPq, a ''sandwich'' PhD grant from Programa de Doutorado Sanduíche no Exterior – PDSE/CAPES, and currently receives a Post-Doctoral fellowship from Programa

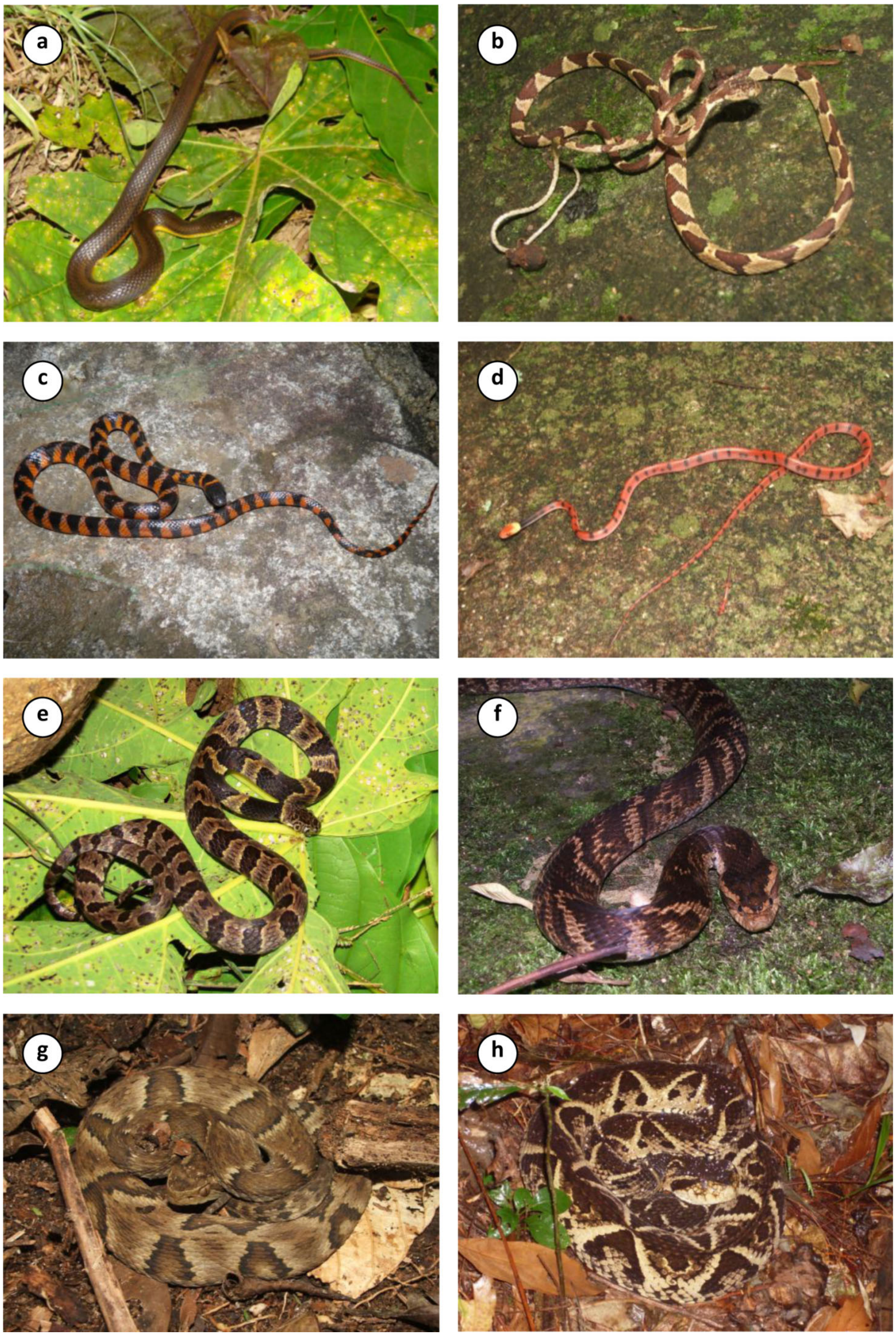


Figure 7. Some reptiles recorded in the Reserva Ecológica de Guapiaçu, southeastern Brazil: a) *Helicops carinicaudus*; b) *Imantodes cenchoa*; c) *Oxyrhopus clathratus*; d) *Siphlophis compressus*; e) *Sibynomorphus neuwiedi*; f) *Xenodon neuwiedii*; g) *Bothrops jararaca*; h) *Bothrops jararacussu*. Photos by M. Almeida-Gomes (a, e) and D. Vrcibradic (b, c, d, f, g, h).

de Apoio ao Pós-Doutorado – PAPD/FAPERJ. L. A. Fusinatto received a PhD scholarship from CNPq and a "sandwich" PhD grant from CAPES, and currently receives a Post-Doctoral fellowship from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). We are grateful to Nicholas J. Locke of the *Reserva Ecológica de Guapiaçu* (REGUA) for making many facilities available during our fieldwork in that area, and to all the various colleagues who helped us with data collection. We thank the Instituto Estadual do Ambiente (INEA) for permission to work in the area (licenses # 005/2008 and # 010/2008) and the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) for collection permits 11701-2, 13088-1, and 18684-2. We also thank the researchers of the Museu Nacional, Rio de Janeiro (MNRJ) for helping us with the identification of the amphibian and reptile species.

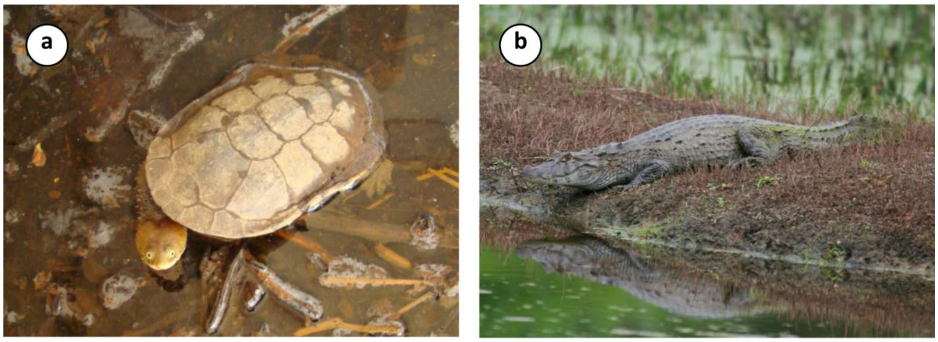


Figure 8. (a) The chelonian *Hydromedusa maximiliani* and (b) the crocodylian *Caiman latirostris* recorded in the Reserva Ecológica de Guapiaçu, southeastern Brazil. Photos by M. Almeida-Gomes (a) and Adilei da Cunha (b).

References

ALMEIDA-GOMES, M., ALMEIDA-SANTOS, M., GOYANNES-ARAÚJO, P., BORGES-JÚNIOR, V.N.T., VRCIBRADIC, D., SIQUEIRA, C.C., ARIANI, C.V., DIAS, A.S., SOUZA, V.V., PINTO, R.R., VANSLUYS, M & ROCHA, C.F.D. 2010. Anurofauna of an Atlantic Rainforest fragment and its surroundings in northern Rio de Janeiro State, Brazil. Braz. J. Biol. 70(3):871-877. http://dx.doi.org/10.1590/S1519-69842010000400018

ALMEIDA-GOMES, M., LORINI, M.L., ROCHA C.F.D. & VIEIRA, M.V. 2014. Underestimation of extinction threat to stream-dwelling amphibians due to lack of consideration of narrow area of occupancy. Conserv. Biol. 28(2):616-619. http://dx.doi.org/10.1111/cobi.12196

ALMEIDA-GOMES M. & ROCHA, C.F.D. 2014. Landscape connectivity may explain anuran species distribution in an Atlantic forest fragmented area. Landscape Ecol. 29(1):29-40. http://dx.doi.org/10.1007/s10980-013-9898-5

ALMEIDA-GOMES, M. & ROCHA, C.F.D. In press. Diversity and distribution of lizards in fragmented Atlantic Forest landscape in southeastern Brazil. J. Herpetol.

ALMEIDA-GOMES, M., VRCIBRADIC, D., MAIA-CARNEIRO, T. & ROCHA, C.F.D. 2012. Diet and endoparasites of the lizard *Gymnodactylus darwinii* (Gekkota, Phyllodactylidae) from an Atlantic Rainforest area in southeastern Brazil. Biotemas 25(1):203-206. http://dx.doi.org/10.5007/2175-7925.2012v25n1p203

ALMEIDA-GOMES, M., VRCIBRADIC, D., SIQUEIRA, C.C., KIEFER, M.C., KLAION, T., ALMEIDA-SANTOS, P., NASCIMENTO, D., ARIANI, C.V., BORGES-JÚNIOR, V.N.T., FREITAS-FILHO, R.F., VAN SLUYS, M & ROCHA, C.F.D. 2008. Herpetofauna of an Atlantic Rainforest area (Morro São João) in Rio de Janeiro State, Brazil. An. Acad. Bras. Ciênc. 80(2):291-300. http://dx.doi.org/10.1590/S0001-37652008000200007

ALVES, M.A.S., ESBERARD, C.E.L., VECCHI, M.B., LAURINDO, T.F.S.& TOMAZ, V.C. 2005. *Pseutes sulphureus* (South American Puffin Snake). Herpetol. Rev. 36(4):459.

ANJOS, L.A. & ROCHA, C.F.D. 2008. The *Hemidactylus mabouia* Moreau de Jonnes, 1818 (Gekkonidae) lizard: an invasive alien species broadly distributed in Brazil. Nat. Conservação 6(1):196-207.

BERNARDO, C.S.S., LLOYD, H., BAYLY, N. & GALETTI, M. 2011. Modelling post-release survival of reintroduced Red-billed Curassows *Crax blumenbachii*. Ibis 153(3):562-572. http://dx.doi.org/10.1111/j.1474-919X.2011.01142.x

BITTENCOURT-SILVA, G.B. & SILVA, H.R. 2013. Insular Anurans (Amphibia: Anura) of the coast of Rio de Janeiro, Southeast, Brazil. Check List 9(2):225-234.

BOTH, C., LINGNAU, R., SANTOS-JR, A.P., LIMA, L.P., MADALOZZO, B. & GRANT, T. 2011. Widespread occurrence of the American bullfrog, *Lithobates catesbeianus* (Shaw, 1802) (Anura: Ranidae), in Brazil. S. Am. J. Herpetol. 6(2):127-134. http://dx.doi.org/10.2994/057.006.0203

CANEDO, C. & POMBAL JR., J.P. 2007. Two new species of torrent frog of the genus *Hylodes* (Anura, Hylodidae) with nuptial thumb tubercles. Herpetologica 63(2):224-235. http://dx.doi.org/10.1655/0018-0831(2007)63[224:TNSOTF]2.0.CO;2

CARAM, J., LUNA-DIAS, C., GOMES, M.R. & CARVALHO-E-SILVA, S.P. 2011. Distribution extension of *Scinax hiemalis* (Haddad and Pombal, 1987) and new state record from Rio de Janeiro, southeastern Brazil (Amphibia: Anura: Hylidae). Herpetol. Notes 4:153-154.

CARAMASCHI, U. & CRUZ, C.A.G. 2013. A new species of the *Hypsiboas polytaenius* clade from southeastern Brazil (Anura: Hylidae). S. Am. J. Herpetol. 8(2):121-126. http://dx.doi.org/10.2994/SAJH-D-13-00009.1

CARRASCO, P.A., MATTONI, C.I., LEYNAUD, G.C. & SCROCCHI, G.J. 2012. Morphology, phylogeny and taxonomy of South American bothropoid pitvipers (Serpentes, Viperidae). Zool. Scr. 41(2):109-124. http://dx.doi.org/10.1111/j.1463-6409.2011.00511.x

CARVALHO-E-SILVA, A.M.T., SILVA, G.R. & CARVALHO-E-SILVA, S.P. 2008. Anuros da Reserva Rio das Pedras, Mangaratiba, RJ, Brasil. Biota Neotrop. 8(1):199-209. http://dx.doi.org/10.1590/S1676-06032008000100021

CORN, P.S. 1994. Straight-line drift fences and pitfall. In Measuring and monitoring biological diversity: standard methods for amphibians (R.W. Heyer, M.A. Donelly, R.A. McDiarmind, L.C. Heyek & M.S. Foster, eds.). Smithsonian Institution Press, Washington DC, 109-117.

CRUMP, M.L. & SCOTT, N.J. 1994. Visual encounter surveys. In Measuring and monitoring biological diversity: standard methods for amphibians (R.W. Heyer, M.A. Donelly, R.A. McDiarmind, L.C. Heyek & M.S. Foster, eds.). Smithsonian Institution Press, Washington DC, 84-92.

DIAS, P.H.S., AMARO, R.C., CARVALHO-E-SILVA, A.M.P.T. & RODRIGUES, M.T. 2013. Two new species of *Proceratophrys* Miranda-Ribeiro, 1920 (Anura; Odontophrynidae) from the Atlantic Forest, with taxonomic remarks on the genus. Zootaxa 3682(2):277-304. http://dx.doi.org/10.11646/zootaxa.3682.2.5

DIXO, M. & METZGER, J.P. 2010. The matrix-tolerance hypothesis: an empirical test with frogs in the Atlantic Forest. Biodivers. Conserv. 19:3059-3071. http://dx.doi.org/10.1007/s10531-010-9878-x

FROST, D.R. 2013. Amphibian Species of the World: an Online Reference. Version 5.6 (9 January 2013). Electronic Database accessible at http://research.amnh.org/herpetology/amphibia/index.html. American Museum of Natural History, New York, USA. (last accessed on 20 August 2013)

FUSINATTO, L.A., ALEXANDRINO, J., HADDAD, C.F.B., BRUNES, T.O., ROCHA, C.F.D., SEQUEIRA, F. 2013. Cryptic genetic diversity is paramount in small-bodied amphibians of the genus *Euparkerella* (Anura: Craugastoridae) endemic to the Brazilian Atlantic Forest. PLoS One 8(11): e79504. http://dx.doi.org/10.1371/journal.pone.0079504

GEHARA, M., CANEDO, C., HADDAD, C.F.B. & VENCES M. 2013. From widespread to microendemic: molecular and acoustic analyses show that *Ischnocnema guentheri* (Amphibia: Brachycephalidae) is endemic to Rio de Janeiro, Brazil. Conserv. Genet. 14. doi: 10.1007/s10592-013-0488-5, http://dx.doi.org/10.1007/s10592-013-0488-5

GIOVANELLI, J.G.R., HADDAD, C.F.B & ALEXANDRINO, J. 2008. Predicting the potential distribution of the alien invasive American bullfrog (*Lithobates catesbeianus*) in Brazil. Biol. Invasions 10(5):585-590. http://dx.doi.org/10.1007/s10530-007-9154-5

GOYANNES-ARAÚJO, P., ALMEIDA-GOMES, M., BORGES-JÚNIOR, V.N.T., ALBUQUERQUE, H.G., VRCIBRADIC, D. & ROCHA, C.F.D. 2009. Reptilia, Polychrotidae, *Anolis fuscoauratus*: Distribution extension. Check List 5(3):746-748.

HADDAD, C.F.B., TOLEDO, L.F., PRADO, C.P.A., LOEBMANN, D., GASPARINI, J.L. & SAZIMA, I. 2013. Guia dos Anfíbios da Mata Atlântica: Diversidade e Biologia. Anolis Books, São Paulo, 543p.

HARVEY, M.B., UGUETO, G.N. & GUTBERLET, R.L. 2012. Review of teiid morphology with a revised taxonomy and phylogeny of the Teiidae (Lepidosauria: Squamata). Zootaxa, 3459:1-156.

IUCN, 2013.IUCN Red List of Threatened Species. Version 2013.1. <www.iucnredlist.org>. (last accessed on 12 August 2013).

JAEGER, R. & INGER, R.F. 1994. Standard techniques for inventory and monitoring: Quadrat Sampling. In Measuring and monitoring biological diversity: standard methods for amphibians (R.W. Heyer, M.A. Donelly, R.A. McDiarmind, L.C. Heyek & M.S. Foster, eds.). Smithsonian Institution Press, Washington DC, 97-102.

KLAION, T., ALMEIDA-GOMES, M., TAVARES, L.E.R., ROCHA, C.F.D. & VAN SLUYS, M. 2011. Diet and nematode infection in *Proceratoprhys boiei* (Anura: Cycloramphidae) from two Atlantic rainforest remnants in southeastern Brazil. An. Acad. Bras. Cienc. 83(4):1303-1312. http://dx.doi.org/10.1590/S0001-37652011000400017

MAIA, T., ALMEIDA-GOMES, M., SIQUEIRA, C.C., VRCIBRADIC, D., KIEFER, M.C. & ROCHA, C.F.D. 2011. Diet of the leaf litter lizard *Ecpleopus gaudichaudii* (Gymnophtalmidae) in an Atlantic Rainforest area in Rio de Janeiro state, Brazil. Zoologia 28(5):587-592. http://dx.doi.org/10.1590/S1984-46702011000500006

MAIA-CARNEIRO, T., DORIGO, T.A., ALMEIDA-GOMES, M., VAN SLUYS, M & ROCHA, C.F.D. 2012a. New altitudinal records of the endemic species *Cycloramphus brasiliensis* (Anura, Cycloramphidae) from the Atlantic Rainforest in the state of Rio de Janeiro, southeastern Brazil. Braz. J. Biol. 72(1):225-226. http://dx.doi.org/10.1590/S1519-69842012000100029

MAIA-CARNEIRO, T., DORIGO, T.A., ALMEIDA-GOMES, M., VAN SLUYS, M & ROCHA, C.F.D. 2012b. Feeding habits, microhabitat use, and daily activity of *Cycloramphus brasiliensis* (Anura: Cycloramphidae) from the Atlantic Rainforest, Brazil. Zoologia 29(3):277-279. http://dx.doi.org/10.1590/S1984-46702012000300007

MAIA-CARNEIRO, T., KIEFER, M.C., VAN SLUYS, M & ROCHA, C.F.D. 2013. Feeding habits, microhabitat use, and daily activity period of *Rhinella ornata* (Anura, Bufonidae) from three Atlantic rainforest remnants in southeastern Brazil. North-West. J. Zool. 9(1):157-165.

MÂNGIA, S., SANTANA, D.J., CRUZ, C.A.G. & FEIO, R.N. 2014. Taxonomic review of *Proceratophrys melanopogon* (Miranda-Ribeiro, 1926) with description of four new species (Amphibia, Anura, Odontophrynidae). Bol. Mus. Nac., N.S., Zool. 531:1-33.

MITTERMEIER, R.A., TURNER, W.R., LARSEN, F.W., BROOKS, T.M. & GASCON, C. 2011. Global biodiversity conservation: the critical role of hotspots. In Biodiversity Hotspots (F.E. Zachos & J.C. Habel, eds.). Springer-Verlag, Berlin, 3-22.

POMBAL JR., J.P. 2010. A posição taxonômica das "variedades" de *Brachycephalus ephippium* (Spix, 1824) descritas por Miranda-Ribeiro, 1920 (Amphibia: Anura: Brachycephalidae). Bol. Mus. Nac., N.S., Zool. 526:1-12.

POMBAL JR., J.P., SIQUEIRA, C.C., DORIGO, T.A., VRCIBRADIC, D., & ROCHA, C.F.D. 2008. A third species of the rare frog genus *Holoaden* (Terrarana, Strabomantidae) from a montane rainforest area of southeastern Brazil. Zootaxa 1938:61-68.

PYRON, R.A., BURBRINK, F.T. & J.J WIENS. 2013. A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. BMC Evol. Biol. 13(93):1-53. http://dx.doi.org/10.1186/1471-2148-13-93

RIBEIRO, M.C., METZGER, J.P., MARTENSEN, A.C., PONZONI, F.J. & HIROTA, M.M. 2009. The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. Biol. Conserv. 142(6):1141-1153. http://dx.doi.org/10.1016/j.biocon.2009.02.021

ROCHA, C.F.D. & VAN SLUYS, M. 2006. New records of reptiles from Ilha Grande Island in Rio de Janeiro State, Brazil. Herpetol. Rev. 37(1):112-114.

ROCHA, C.F.D., BERGALLO, H.G., ALVES, M.A.S. & VAN SLUYS S,. 2003. A Biodiversidade nos Grandes Remanescentes Florestais do Estado do Rio de Janeiro e nas Restingas da Mata Atlântica. RiMa, São Carlos, 160p.

ROCHA, C.F.D., BERGALLO, H.G., POMBAL JR., J.P., GEISE, L., VAN SLUYS, M., FERNANDES, R., & CARAMASCHI, U. 2004. Fauna de anfíbios, répteis e mamíferos do Estado do Rio de Janeiro, sudeste do Brasil. Publ. Avul. Mus. Nac. 104:3-23.

ROCHA, C.F.D., ANJOS, L.A. & BERGALLO, H.G. 2011. Conquering Brazil: the invasion by the exotic gekkonid lizard *Hemidactylus mabouia* (Squamata) in Brazilian natural environments. Zoologia 28(6):747-754. http://dx.doi.org/10.1590/S1984-46702011000600007

ROCHA, C.F.D., VRCIBRADIC, D., KIEFER, M.C., ALMEIDA-GOMES, M., BORGES-JÚNIOR, V.N.T., CARNEIRO, P.C.F., MARRA, R.V., ALMEIDA-SANTOS, P., SIQUEIRA, C.C., GOYANNES-ARAÚJO, P., FERNANDES, C.G.A., RUBIÃO, E.C.N. & VAN SLUYS, M. 2007. A survey of the leaf-litter frog assembly from an Atlantic forest area (Reserva Ecológica de Guapiaçu) in Rio de Janeiro State, Brazil, with an estimate of frog densities. Trop. Zool. 20(1):99-108.

ROCHA, C.F.D., VRCIBRADIC, D., KIEFER, M.C., ALMEIDA-GOMES, M., BORGES-JÚNIOR, V.N.T., MENEZES, V.A., ARIANI, C.V., PONTES, J.A.L., GOYANNES-ARAÚJO, P., MARRA, R.V., GUEDES, D.M., SIQUEIRA, C.C. & VAN SLUYS, M. 2013. The leaf-litter frog community from Reserva Rio das Pedras, Mangaratiba, Rio de Janeiro State, Southeastern Brazil: species richness, composition and densities. North-West. J. Zool. 9(1):151-156.

SALLES, R.O.L. & SILVA-SOARES, T. 2010. Répteis do Município de Duque de Caxias, Baixada Fluminense, RJ, Brasil. Biotemas 23(2):135-144. http://dx.doi.org/10.5007/2175-7925.2010v23n2p135

SALLES, R.O.L., PONTES, R.C. & SILVA-SOARES, T. 2012. New records and geographic distribution of *Aplastodiscus eugenioi* (Anura: Hylidae) in southeastern Brazil. Herpetol. Notes 5:431-433.

SALLES, R.O.L., WEBER, L.N. & SILVA-SOARES, T. 2009. Amphibia, Anura, Parque Natural Municipal da Taquara, Municipality of Duque de Caxias, Rio de Janeiro State, Southeastern Brazil. Check List 5(4):840-854.

SALLES, R.O.L., WEBER, L.N. & SILVA-SOARES, T. 2010. Reptiles, Squamata, Parque Natural Municipal da Taquara, Municipality of Duque de Caxias, State of Rio de Janeiro, Southeastern Brazil. Check List 6(2):280-286.

SILVA, H.R. & ALVES-SILVA, R. 2013. Predictive-like distribution mapping using Google Earth: Reassessment of the distribution of the bromeligenous frog, *Scinax v-signatus* (Anura: Hylidae). Zootaxa 3609:213-222. http://dx.doi.org/10.11646/zootaxa.3609.2.7

SILVA-SOARES, T., HEPP, F., COSTA, P.N., LUNA-DIAS, C., GOMES, M.R., CARVALHO-E-SILVA, A.M.P.T. & CARVALHO-E-SILVA, S.P. 2010. Anfíbios Anuros da RPPN Campo Escoteiro Geraldo Hugo Nunes, Município de Guapimirim, Rio de Janeiro, sudeste do Brasil. Biota Neotrop. 10(2):225-233. http://dx.doi.org/10.1590/S1676-06032010000200025

SILVA-SOARES, T., WEBER, L.N. & SALLES, R.O.L. 2008. Amphibia, Anura, Hylidae, *Hylodes pipilans*: Distribution extension. Check List 4(3):295-296.

SILVEIRA, A.L., SALLES, R.O.L. & PONTES, R.C. 2009. Primeiro registro de *Rhinella pombali* e novos registros de *R. crucifer* e *R. ornata* no Estado do Rio de Janeiro, Brasil (Amphibia, Anura, Bufonidae). Biotemas 22(4):231-235. http://dx.doi.org/10.5007/2175-7925.2009v22n4p231

SILVEIRA, A.L., VRCIBRADIC, D., ROCHA, C.F.D. & SIQUEIRA, C.C. 2010. Geographic Distribution. *Echinanthera amoena*. Herpetol. Rev. 41(1):109.

SIQUEIRA, C.C., VRCIBRADIC, D., ALMEIDA-GOMES, M., BORGES-JÚNIOR, V.N.T., ALMEIDA-SANTOS, P., ALMEIDA-SANTOS, M., ARIANI, C.V., GUEDES, D.M., GOYANNES-ARAÚJO, P., DORIGO, T.A., VAN SLUYS, M & ROCHA, C.F.D. 2009. Density and richness of leaf litter frogs (Amphibia: Anura) of an Atlantic Rainforest area in the Serra dos Órgãos, Rio de Janeiro State, Brazil. Zoologia 26(1):97-102. http://dx.doi.org/10.1590/S1984-46702009000100015

SIQUEIRA, C.C., VRCIBRADIC, D., ALMEIDA-GOMES, M., MENEZES, V.A., BORGES-JÚNIOR, V.N.T., HATANO, F.H., PONTES, J.A.L., GOYANNES-ARAÚJO, P., GUEDES, D.M., VAN SLUYS M, & ROCHA, C.F.D. 2011a. Species composition and density estimates of the anurofauna of a site within the northernmost large Atlantic Forest remnant (Parque Estadual do Desengano) in the state of Rio de Janeiro, Brazil. Biota Neotrop. 11(4):131-137. http://dx.doi.org/10.1590/S1676-06032011000400014

SIQUEIRA, C.C., VRCIBRADIC D., DORIGO, T.A. & ROCHA, C.F.D. 2011b. Anurans from two high-elevation areas of Atlantic Forest in the state of Rio de Janeiro, Brazil. Zoologia 28(4):457-464. http://dx.doi.org/10.1590/S1984-46702011000400007

SIQUEIRA, C.C., VRCIBRADIC D. & ROCHA, C.F.D. 2013. Altitudinal records of data-deficient and threatened frog species from the Atlantic Rainforest of the Serra dos Órgãos mountains, in southeastern Brazil. Braz. J. Biol. 73(1):229-230. http://dx.doi.org/10.1590/S1519-69842013000100027

STUART, S.N., CHANSON, J.S., COX, N.A., YOUNG, B.E., RODRIGUES, A.S.L., FISCHMAN, D.L. & WALLER, R.W. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1783-1785. http://dx.doi.org/10.1126/science.1103538

TAYLOR, E.H. 1968. The Caecilians of the World. A Taxonomic Review. University of Kansas Press, Lawrence. 848p.

TELLES, F.B.S., MENEZES, V.A., MAIA-CARNEIRO, T., DORIGO, T.A., WINCK, G.R. & ROCHA, C.F.D. 2012. Anurans from the 'Restinga' of Parque Natural Municipal de Grumari. Check List 8(6):1267-1273.

TRINDADE-FILHO, J., CARVALHO, R.A., BRITO, D. & LOYOLA, R.D. 2012. How does the inclusion of Data Deficient species change conservation priorities for amphibians in the Atlantic Forest? Biodivers. Conserv. 21(10):2709-2718. http://dx.doi.org/10.1007/s10531-012-0326-y

VERDADE, V.K., VALDUJO, P.H., CARNAVAL, A.C., SCHIESARI, L., TOLEDO, L.F., MOTT, T., ANDRADE, G.V., ETEROVICK, P.C., MENIN, M., PIMENTA, B.V.S., NOGUEIRA, C., LISBOA, C.S., DE PAULA C.D. & SILVANO, D.L. 2012. A leap further: the Brazilian amphibian conservation action plan. Alytes 29(1-4):28-43.

VRCIBRADIC, D., ALMEIDA-GOMES, M., BORGES-JÚNIOR, V.N.T., KIEFER, M.C., VAN SLUYS, M & ROCHA, C.F.D. 2006a. Reptilia, Scincidae, *Mabuya frenata*: Distribution Extension. Check List 2(2):57-58.

VRCIBRADIC, D., HATANO, F.H., ROCHA, C.F.D. & VAN SLUYS, M. 2006b. Geographic Distribution. *Phyllodytes luteolus*. Herpetol. Rev. 37(4):489.

VRCIBRADIC, D., ALMEIDA-GOMES, M., SIQUEIRA, C.C., BORGES-JÚNIOR, V.N.T. & ROCHA, C.F.D. 2011a. *Oxyrhopus petola digitalis* (False Coral Snake). Prey. Herpetol. Rev. 42(2):299-300.

VRCIBRADIC, D., ROCHA, C.F.D., KIEFER, M.C., HATANO, F.H., FONTES, A.F., ALMEIDA-GOMES, M., SIQUEIRA, C.C., PONTES, J.A.L., BORGES-JÚNIOR, V.N.T., GIL, L.O., KLAION, T., RUBIÃO, E.C.N. & VAN SLUYS M.,. 2011b. Herpetofauna, Estação Ecológica Estadual do Paraíso, state of Rio de Janeiro, southeastern Brazil. Check List 7(6):745-749.

WEBER, L.N., BILATE, M., PROCACI, L.S. & SILVA, S.P. 2007. Amphibia, Anura, Hylodidae, *Hylodes charadranaetes*: Distribution extension and notes on advertisement calls. Check List 3(4):336-337.

WEBER, L.N., SILVA-SOARES, T. & SALLES, R.O.L. 2009. Amphibia, Anura, Hylidae, *Hypsiboas secedens*: reassessment of type locality coordinates and distribution extension. Check List 5(2):218-221.

WEBER, L.N.,VERDADE, V.K., SALLES, R.O.L., FOUQUET, A. & CARVALHO-E-SILVA, S.P. 2011. A new species of *Cycloramphus* Tschudi (Anura: Cycloramphidae) from the Parque Nacional da Serra dos Órgãos, Southeastern Brazil. Zootaxa 2737:19-33.

WHILES, M.R., LIPS, K.R., PRINGLE, C.M., KILHAM S.S., BIXBY, R.J., BRENES, R., CONNELLY, S., COLON-GAUD, J.C., HUNT-BROWN, M., HURYN, A.D., MONTGOMERY, C. & PETERSON, S. 2006. The effects of amphibian population declines on the structure and function of Neotropical stream ecosystems. Front. Ecol. Environ. 4(1):27-37. http://dx.doi.org/10.1890/1540-9295(2006)004[0027:TEOAPD]2.0.CO;2

Received 12/01/2014
Revised 21/01/2014
Accepted 01/08/2014

Appendix 1

Voucher specimens of amphibians and reptiles from the Reserva Ecológica de Guapiaçu deposited at the Museu Nacional, Rio de Janeiro (MNRJ).

AMPHIBIANS: GYMNOPHIONA: Siphonopidae: *Siphonops hardyi* (MNRJ 65728); Typhlonectidae: *Chthonerpeton braestrupi* (MNRJ 70119); ANURA: Brachycephalidae: *Brachycephalus didactylus* (MNRJ 55473-74, 57736, 68818-21, 86330); *Brachycephalus ephippium* (MNRJ 38104, 56517-18); *Ischnocnema guentheri* (MNRJ 53764, 55471-72, 59158, 66774-87, 68704, 86746-49); *Ischnocnema octavioi* (MNRJ 38129-32, 56613, 62328, 86767-68); *Ischnocnema parva* (MNRJ 56603, 66769-73, 73201-02); *Ischnocnema* cf. *venancioi* (MNRJ 60163); Bufonidae: *Dendrophryniscus brevipollicatus* (MNRJ 58294-95, 60701-02); *Rhinella hoogmoedi* (MNRJ 79893); *Rhinella icterica* (MNRJ 54372-74, 86314-16); *Rhinella ornata* (MNRJ 61075-91, 86737-43); Craugastoridae: *Euparkerella brasiliensis* (MNRJ 37317-18, 38212-21, 49300, 56932-35, 56943-51, 57561-63, 57735, 57737-41, 57787-90, 68702-3, 86845-47); *Haddadus binotatus* (MNRJ 53620, 55476-78, 66160, 66767-68, 86729-36); Cycloramphidae: *Cycloramphus brasiliensis* (MNRJ 55468, 76433-38); *Thoropa miliaris* (MNRJ 55447, 86710); *Zachaenus parvulus* (MNRJ 55448, 56938-39); Hemiphractidae: *Fritziana goeldii* (MNRJ 51516, 53758, 67315); *Gastrotheca albolineata* (MNRJ 59537, 65495, 86336); Hylidae: *Aplastodiscus eugenioi* (MNRJ 45783, 58950, 60197-98); *Bokermannohyla circumdata* (MNRJ 57018-22, 57292, 59536); *Dendropsophus anceps* (MNRJ 86317, 86722-25); *Dendropsophus berthalutzae* (MNRJ 79895, 86812-16); *Dendropsophus bipunctatus* (MNRJ 56936-37, 86804-07); *Dendropsophus decipiens* (MNRJ 67307); *Dendropsophus elegans* (MNRJ 49307, 86744-45); *Dendropsophus giesleri* (MNRJ 64156); *Dendropsophus meridianus* (MNRJ 63949, 86809-11); *Dendropsophus microps* (MNRJ 61050); *Dendropsophus minutus* (MNRJ 56940-41, 86801-03); *Dendropsophus pseudomeridianus* (MNRJ 86817-23); *Dendropsophus seniculus* (MNRJ 63947, 86726-28); *Hypsiboas albomarginatus* (MNRJ 86808); *Hypsiboas albopunctatus* (MNRJ 86896); *Hypsiboas faber* (MNRJ 86685-92); *Hypsiboas pardalis* (MNRJ 40610-11); *Hypsiboas secedens* (MNRJ 40609, 61475-77, 86331-34, 86337); *Hypsiboas semilineatus* (MNRJ 54013, 86339, 86750); *Itapotihyla langsdorffii* (MNRJ 86770-71); *Phyllomedusa burmeisteri* (MNRJ 47954, 68866, 86711-13); *Phyllomedusa rohdei* (MNRJ 64632; 86714-17); *Scinax albicans* (MNRJ 40080-82, 57243-44, 86751-66); *Scinax alter* (MNRJ 57084, 86782); *Scinax argyreornatus* (MNRJ 49514-25, 64634, 68692, 86783-90); *Scinax cuspidatus* (MNRJ 67397-98); *Scinax flavoguttatus* (MNRJ 53311); *Scinax hayii* (MNRJ 57600-02); *Scinax humilis* (MNRJ 40083-95, 49278-79, 58951, 61213, 63948); *Scinax v-signatus* (MNRJ 51800, 53902-03, 68691); *Scinax* aff. *x-signatus* (MNRJ 40809-11, 57783-86, 63950, 86318);*Sphaenorhynchus planicola* (MNRJ 87098-101); *Trachycephalus mesophaeus* (MNRJ 49301, 79894); *Trachycephalus nigromaculatus* (MNRJ 86709); Hylodidae: *Crossodactylus aeneus* (MNRJ 75172, 82563, 86838-41); *Hylodes asper* (MNRJ 60169-70, 60181, 86892); *Hylodes charadranaetes* (MNRJ 59064-65, 60167-68, 60174-77, 60180); *Hylodes lateristrigatus* (MNRJ 68986); *Hylodes pipilans* (MNRJ 59038-46, 59066, 60173, 68735); *Megaelosia goeldii* (MNRJ 86296); Leptodactylidae: *Adenomera* cf. *bokermanni* (MNRJ 86893); *Adenomera marmorata* (MNRJ 66561, 86327-29, 86772-81); *Leptodactylus fuscus* (MNRJ 86718); *Leptodactylus latrans* (MNRJ 51742-46, 61217); *Leptodactylus mystacinus* (MNRJ 86720-21); *Leptodactylus spixi* (MNRJ 57294, 86719); *Physalaemus signifer* (MNRJ 59897, 66441, 86791-800); Microhylidae: *Chiasmocleis carvalhoi* (MNRJ 38105-07, 49302, 53464, 68736, 68739-40, 74592, 86335); *Myersiella microps* (MNRJ 49311, 86837); *Stereocyclops parkeri* (MNRJ 54768, 57295, 60553, 86338); Odontophrynidae: *Proceratophrys appendiculata* (MNRJ 54012, 55470); *Proceratophrys boiei* (MNRJ 55469, 68764-83, 68810-11, 86769); Ranidae: *Lithobates catesbeianus* (MNRJ 86693-94). REPTILES: SQUAMATA: Amphisbaenidae: *Amphisbaena microcephala* (MNRJ 16474); Anguidae: *Ophiodes striatus* (MNRJ 19410); Boidae: *Corallus hortulanus* (MNRJ 18550, 19231); Colubridae: *Chironius bicarinatus* (MNRJ 18066); *Chironius foveatus* (MNRJ 15375, 18479); *Chironius fuscus* (MNRJ 16909, 19743, 24134); *Echinanthera amoena* (MNRJ 18068); *Echinanthera cephalostriata* (MNRJ 12347, 19288); *Helicops carinicaudus* (MNRJ 18085); *Imantodes cenchoa* (MNRJ 19289, 23207); *Liophis miliaris* (MNRJ 18528, 24389); *Liophis reginae* (MNRJ 16364, 18462); *Oxyrhopus clathratus* (MNRJ 23509); *Oxyrhopus petolarius* (MNRJ 12349, 15370, 18211); *Philodryas patagoniensis* (MNRJ 12350-51, 16365); *Sibynomorphus neuwiedi* (MNRJ 12345-46, 18086); *Siphlophis compressus* (MNRJ 19389); *Taeniophallus affinis* (MNRJ 16899, 19165-66); *Xenodon neuwiedii* (MNRJ 16367, 18097, 19388); Dactyloidae: *Anolis fuscoauratus* (MNRJ 16548); *Anolis punctatus* (MNRJ 15373, 19133, 19245-46); Elapidae: *Micrurus corallinus* (MNRJ 24391); Gekkonidae: *Hemidactylus mabouia* (MNRJ 12353, 19243-44); Gymnophtalmidae: *Ecpleopus gaudichaudi* (MNRJ 12352, 24276-300); Leiosauridae: *Enyalius brasiliensis* (MNRJ 12354-58, 21569-82); Phyllodactylidae: *Gymnodactylus darwini* (MNRJ 20558-72); Scincidae: *Mabuya macrorhyncha* (MNRJ 19241-42); Viperidae: *Bothrops jararaca* (MNRJ 16366); *Bothrops jararacussu* (MNRJ 12348); TESTUDINES: Chelidae: *Hydromedusa maximiliani* (MNRJ 21127).

Biota Neotropica 14(3): e20130016, 2014
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)

article

Importance of allochthonous resources in the diet of *Astyanax* aff. *fasciatus* (Osteichthyes: Characidae) in streams: a longitudinal approach

Marlene R. Silva[1], *Rosemara Fugi*[1,2], *Natália Carniatto*[1,3] & *Maria Júlia M. Ganassin*[1]

[1]*Programa de Pós-Graduação em Ecologia de Ambientes Aquáticos Continentais (PEA) - Universidade Estadual de Maringá, Av. Colombo 5790, Jardim Universitário, Bloco G-90, CEP 87020-900, Maringá, Paraná, Brazil. e-mail: marleners@ibest.com.br (MRS); rosemarafugi@gmail.com (RF); nati_carniatto@hotmail.com (NC)*

[2]*Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (Nupélia) - Universidade Estadual de Maringá, Av. Colombo 5790, Jardim Universitário, Bloco G-90, CEP 87020-900, Maringá, Paraná, Brazil. e-mail: mjganassin@hotmail.com (MJMG)*

[3]*Corresponding author: Natália Carniatto, e-mail: nati_carniatto@hotmail.com*

SILVA, M.R., FUGI, R., CARNIATTO, N., GANASSIN, M.J.M. **Importance of allochthonous resources in the diet of *Astyanax* aff. *fasciatus* (Osteichthyes: Characidae) in streams: a longitudinal approach.** Biota Neotropica. 14(3): e20130016. http://dx.doi.org/10.1590/1676-06032014001613

Abstract: The aim of this investigation was to respond the following question: is the consumption of allochthonous resources by *Astyanax* aff. *fasciatus* influenced by the longitudinal gradient in streams? To respond this question we sampled fish in the headwater, middle and mouth stretches of four streams (Upper Paraná River basin). Samplings were carried out from July 2007 to June 2008 using electrical fishing. Fish were identified, measured and their stomach contents identified and quantified. Spatial variations (among longitudinal stretches) in the diet were summarized using a principal coordinate analysis (PCoA). Spatial differences in the diet were tested with a multiresponse permutation procedure (MRPP). Allochthonous resources had the highest percentage of the *A.* aff. *fasciatus* diet in all stretches; however, the increased importance of autochthonous resources in the fish diet in the stream mouths made these stretches to differ significantly from the middle and headwater ones. The ordination analysis separated the fish diet sampled in the stream mouth from the diet of most fish samples in the headwater and middle stretches. The results highlight the importance of allochthonous resources, mainly insects, in the diet of *A.* aff. *fasciatus*. However, the diet was affected by longitudinal gradient and followed the pattern described for these ecosystems, where fish depend basically of allochthonous material in the headwaters and the importance of these resources decrease along the longitudinal gradient.

Keywords: *Fish, feeding resources, terrestrial subsidies, Pirapó River, Paraná River.*

SILVA, M.R., FUGI, R., CARNIATTO, N., GANASSIN, M.J.M. **Importância dos recursos alóctones na dieta de *Astyanax* aff. *fasciatus* (Osteichthyes: Characidae) em riachos: uma abordagem longitudinal.** Biota Neotropica. 14(3): e20130016. http://dx.doi.org/10.1590/1676-06032014001613

Resumo: O objetivo deste estudo foi responder a seguinte pergunta: o consumo de recursos alóctones por *Astyanax* aff. *fasciatus* é influenciado pelo gradiente longitudinal dos riachos? Para responder esta questão, foram amostrados peixes na cabeceira, no meio e na foz de quatro riachos (bacia do alto Rio Paraná). As amostragens foram realizadas entre julho/2007 e junho/2008, utilizando-se pesca elétrica. Os peixes foram identificados, medidos e seus conteúdos estomacais identificados e quantificados. Variações espaciais (entre os trechos) na dieta foram sumarizadas através da análise de ordenação de coordenadas principais (PCoA). Diferenças espaciais na dieta foram testadas através do procedimento de permutação de multiresposta (MRPP). Recursos alimentares alóctones compuseram a maior parcela da dieta de *A.* aff. *fasciatus* em todos os trechos, porém, na foz dos riachos a dieta diferiu significativamente do meio e da cabeceira, sendo esta diferença causada pelo incremento de recursos autóctones na dieta na foz. A análise de ordenação mostrou a separação da dieta dos peixes amostrados na foz da dieta da maioria dos peixes amostrados na cabeceira e no meio. Estes resultados evidenciam a importância de recursos alóctones, principalmente insetos, na dieta de *A.* aff. *fasciatus*. No entanto, a dieta foi influenciada pelo gradiente longitudinal, seguindo o padrão descrito para estes ambientes, onde na cabeceira os peixes dependem primariamente de material alóctone, e ao longo do gradiente ocorre um decréscimo na importância destes recursos.

Palavras-chave: *Peixes, recursos alimentares, subsídios terrestres, rio Pirapó, Rio Paraná.*

Introduction

Fish inhabiting streams depend on some environmental factors for the success of their ecological functions, such as the size of the stream and drainage basin (Oliveira & Bennemann 2005), morphological characteristics of the environment, flow velocity, cover of riparian vegetation (Vannote et al. 1980, Ferreira & Casatti 2006, Lorion & Kennedy 2009), availability of autochthonous (*in situ*) and allochthonous food resources (*ex situ*) (Vannote et al. 1980, Polis et al. 1997, Lancaster et al. 2008, Lorion & Kennedy 2009).

Streams and adjacent areas are ecosystems closely connected by the flow of matter and by the movement of organisms (Baxter et al. 2005), and this exchange allows the input of resources to aquatic ecosystem (Power et al. 2004, Lorion & Kennedy 2009). Subsidies comprise the flow of energy biologically fixed and nutrients from an ecosystem to another, that is, allochthonous resources (Polis et al. 1997, Richardson et al. 2009), and the vegetation adjacent to rivers and streams potentially has a large impact on aquatic communities (Erös et al. 2012). Thus, as important as the high internal productivity of the aquatic ecosystem, which sustains consumer populations, are the subsidies coming from the interface of terrestrial habitats, which ensures the supply of food resources to local populations even in environments where primary productivity is low (Rose & Polis 1998, Pace et al. 2004, Richardson et al. 2009). Subsidies as invertebrates can represent up to half of the energy in the diet of stream fish (Masson & Macdonald 1982), indicating that in these environments the availability of prey induce higher consumption (Rezende & Mazzoni, 2006), or the fishes have a preference for these organisms (Main & Lyon 1988, Garman 1991). The importance of subsidies in the diet of stream fish has been frequently reported in tropical regions (Henry et al. 1994, Vitule et al. 2008, Wolff et al. 2009, Tófoli et al. 2010, Manna et al. 2012, Small et al. 2013).

The input of subsidies to lotic ecosystems is associated with a gradient of physical conditions and biotic adjustments, from the headwaters to the mouth (River Continuum Concept, *sensu* Vannote et al. 1980). The headwaters suffers greater influence from the riparian vegetation than the mouth, decreasing the autotrophic production due to shading of the water, and at the same time contributing to the input of allochthonous material (mainly plants and insects) (Vannote et al. 1980). In rivers and streams there is a remarkable longitudinal gradient of food resources dominant in the fish diet, and in headwaters regions fish depend primarily on allochthonous material, made up of terrestrial insects and plant debris (Lowe-McConnell 1975). Manna et al. (2012) observed that the diet of *Astyanax taeniatus* varied according to physical characteristics of the environment, showing a reduced amount of allochthonous resources (mostly plant debris) along the longitudinal gradient of the stream.

Species of the genus *Astyanax* are considered flexible in the use of food resources in streams, and have been described as opportunistic with high feeding plasticity (Wolff et al. 2009, Manna et al. 2012). However several studies have shown the predominance of allochthonous resources, especially insects and higher plants (leaves, fruits and seeds) in the diet of these fish in streams (Bennemann et al. 2005, Borba et al. 2008, Ferreira et al. 2011), even those with little vegetation cover (Borba et al. 2008). On the other hand, some species of this genus have also consumed predominantly autochthonous resources (Mazzoni & Costa 2007, Mazzoni et al. 2010). According to Mazzoni et al. (2010) the almost absolute predominance of autochthonous items in the diet of *Astyanax janeiroensis* in closed sites of a coastal stream highlights the importance of internal processes in the maintenance of low order stream systems.

In this context, this study aimed to respond the following question: is the consumption of allochthonous resources by *Astyanax* aff. *fasciatus* (Cuvier, 1829) influenced by the longitudinal gradient in streams? To answer this question, fishes were sampled in rural streams belonging to the sub-basin of the Pirapó River, Upper Paraná River basin.

Material and Methods

Study area

This study was conducted in four low order rural streams (Água da Roseira, Remo, Romeira and Zaúna - Figure 1), belonging to the Pirapó River sub-basin, Upper Paraná River basin. This sub-basin is bounded by latitude 22°30 'and 23°30' South and longitude 51°15' and 52°15' West, with a drainage area of approximately 5,076 km^2 (Peruço 2004). Sampling was performed in three stretches of each stream: headwaters (He), middle (Mi) and mouth (Mo) (Figure 1).

The Água da Roseira stream has steep banks, narrow bands of arboreal riparian vegetation (He = 14.3 km; Mi = 16.0 km; Mo = 11.0 km), drainage area of 867 km^2 and is located at 23°20'56,30''S - 51°54'52,31''W. The Remo stream has heterogeneous arboreal vegetation, alternating native and exotic species. The width of the vegetation along its banks is variable (He = 54.0 km; Mi = 19.0 km ; Mo = 15.0 km), its drainage area is of 792 km^2 and is located between coordinates 23°21'39,26''S - 52°01'02,48''W. The Romeira stream has riparian vegetation with variable width along its steep banks (He = 24.5 km; Mi = 20.0 km; Mo = 9.5 km); its drainage area has 895 km^2 and is located at 23°22'04,05''S - 51°58'43,50''W. In the Zaúna stream the riparian vegetation is extensive along almost its entire course (He = 27.0 km; Mi = 30.3 km; Mo = 34.5 km); it is the smallest stream among the studied streams, with drainage area of 297 km^2, and is located at 23°23'47,36''S - 51°51'02,09''W. The headwaters and middle stretches were more structured considering the presence of trunks, branches and leaves in the bed, when compared to the mouth region. The width of the riparian vegetation was larger in the headwaters stretches, compared with the mouth, except for the Zaúna stream, where the vegetation width varied little between stretches.

Sampling and data analysis

Bimonthly samples were taken from July 2007 to June 2008, in three stretches of the streams, headwaters, middle and mouth, totaling 12 sampling sites. Samples were collected using electrofishing equipment (AC portable generator, 2.5 KW, 400 V, 2A), through three successive catches with constant unit effort over a segment of approximately 20-fold the average width of the stream bed (Lyons 1992), and the stretches were delimited by blocking nets with 2 mm between knots.

Fish caught were anesthetized with eugenol, preserved in 10% formalin and later identified, counted, measured (standard length), weighed (total weight) and eviscerated. *Astyanax* aff. *fasciatus* was not caught in enough number for analyses in the mouth of the Romeira stream. Voucher specimens were

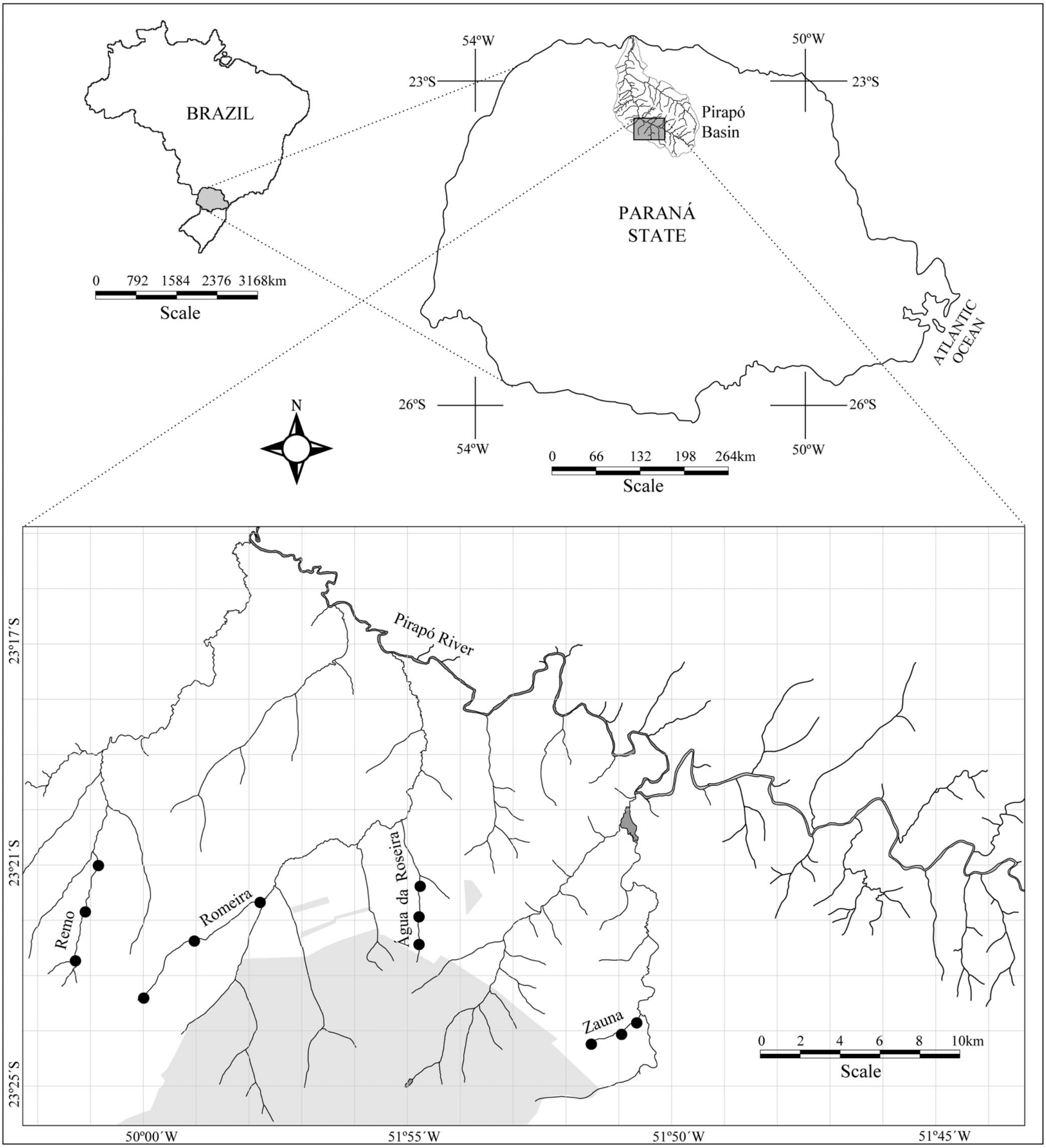


Figure 1. Location of streams in the sub-basin of the Pirapó River, Upper Paraná River basin, and sampling sites (•).

deposited in the Fish Collection of the Center for Research in Limnology, Ichthyology and Aquaculture – State University of Maringá (NUP 5580; NUP 6370). Stomachs with food were preserved in 70% alcohol and their contents were examined under stereoscopic and optical microscopes. Food items were identified and quantified by the volumetric method (Hyslop 1980), and the volume of each item was obtained using a graduated plate in which the volume is obtained in mm^3 and thereafter converted into ml (Hellawel & Abel 1971).

In order to verify the importance of allochthonous resources in the diet of *A.* aff. *fasciatus*, food items were grouped into autochthonous and allochthonous. To check the influence of the longitudinal gradient in the consumption of autochthonous and allochthonous resources, differences in diet composition between the headwaters, middle and the mouth stretches of each streams were tested using the nonparametric MRPP (Multi-Response Permutation Procedure – significance $p < 0.05$), which tests multivariate differences between

Table 1. Diet composition (% volume) of *Astyanax* aff. *fasciatus* in four streams of the sub-basin of the Pirapó River, Upper Paraná River basin (He= headwater; Mi= middle; Mo= mouth; value in parentheses represents the number of analyzed stomachs). Aquatic invert.= other aquatic invertebrates; Terrestrial invert.= other terrestrial invertebrates.

	Água Roseira			**Remo**			**Romeira**		**Zaúna**		
Resources/items	**He (13)**	**Mi (28)**	**Mo (16)**	**He (25)**	**Mi (21)**	**Mo (48)**	**He (5)**	**Mi (40)**	**He (3)**	**Mi (32)**	**Mo (14)**
Aquatic insects	**13.79**	**2.60**	**23.90**	**7.40**	**0.89**	**26.34**	**14.61**	**4.88**	**7.03**	**6.28**	**20.56**
Diptera	0.87	1.87	9.36	0.25	0.25	8.64	1.69	0.68	3.36	2.16	2.62
Trichoptera	1.59	0.50	11.07	0.07	0.07	7.57	1.13	2.33	0.61	2.66	12.71
Coleoptera				0.08	0.06	2.04	0.28	0.36		0.19	2.78
Ephemeroptera		0.13	2.60	1.68	0.42	3.77	0.56		3.05	0.92	1.64
Hemiptera				0.00			10.93	0.96		0.01	0.79
Odonata	11.32	0.09	0.23	2.46	0.07	1.77		0.52		0.19	
Plecoptera				0.07							
Neuroptera				2.64		2.53					
Aquatic invert.	**0.44**	**0.19**	**9.04**	**5.04**	**3.66**	**1.23**	**1.13**	**0.81**	**3.36**	**0.98**	**0.66**
Amphipoda				3.93		0.24	1.13				0.31
Microcrustáceo			0.63	0.03		0.45			3.05	0.54	
Oligochaeta	0.42	0.18	8.38	1.08	3.65	0.03		0.81		0.15	0.31
Tecameba	0.01	0.01	0.02			0.50				0.28	0.03
Bryozoa									0.30		
Detritus/sediment	**0.18**	**0.20**	**6.15**	**6.31**		**6.22**	**0.28**	**0.35**			**0.03**
Terrestrial insects	**54.01**	**83.11**	**32.97**	**29.99**	**75.41**	**42.52**	**76.88**	**51.93**	**86.54**	**69.41**	**40.89**
Hymenoptera	27.87	52.92	31.30	17.69	15.71	14.67	36.82	9.45	47.40	28.55	10.97
Coleoptera	2.05	1.36	0.34	3.48	1.21	5.04	23.79	22.32		11.94	15.45
Homoptera	2.40	0.13		1.35	1.08	3.73		0.05		1.93	
Lepidoptera	5.90	28.01		5.03	45.24	11.53	1.59	15.44	36.69	22.52	10.91
Diptera			0.17	1.09	0.04	0.11	11.33	0.21		0.59	
Hemiptera	0.08	0.36		0.04	0.72	0.02		1.60		1.15	2.54
Orthoptera	14.83				10.10	2.28	1.13	1.72		1.93	
Trichoptera					0.57			0.29		0.19	
Thysanoptera		0.02				0.10					
Isoptera						1.50		0.14			
Dermaptera		0.01									
Remains	0.85	0.27	1.15	1.29	0.69	3.51	2.26	0.67	2.44	0.57	1.00
Terrestrial invert.	**11.32**	**0.13**		**0.36**	**0.46**	**1.66**	**1.13**	**0.81**		**5.60**	**0.07**
Aranea	11.32	0.13		0.36	0.46	0.64	1.13	0.08		1.54	0.07
Isopoda						1.01		0.73		4.05	
Terrestrial plants	**20.24**	**13.74**	**27.91**	**50.87**	**19.56**	**22.01**	**5.94**	**41.19**	**3.05**	**17.70**	**37.75**

predefined groups (McCune & Grace 2002). The null hypothesis tested is that there is no difference in diet composition between stretches of streams.

To summarized the patterns of longitudinal variation (headwaters, middle, and mouth) in the diet composition of *A.* aff. *fasciatus* we used a Principal Coordinates Analysis (PCoA) (Legendre & Legendre 1998), applied to the diet data matrix, for each stream, controlling the longitudinal gradient (headwaters, middle and mouth). For this analysis, food items were grouped into: aquatic insects, other aquatic invertebrates, detritus/sediment, terrestrial insects, other terrestrial invertebrates and terrestrial plants (see Table 1 of *Results*), and these groups were referred to as food resources. This analysis was run in R Programming Environment using the Vegan package (*The R Project for Statistical Computing*, http://www.r-project.org/).

Differences in diet composition, considering different food resources (Table 1), between the headwaters, middle and mouth of each stream, were tested by a MRPP (Multi-Response Permutation Procedure) as previously described. Analyses were run using the software PC-Ord 4.0 (McCune & Mefford 2006).

Results

To describe the diet were analyzed the stomach content of 245 individuals of *A.* aff. *fasciatus*, whose standard length ranged between 2.0 and 10.0 cm. The species showed varied diet, totaling 28 food items, grouped into six types of food resources (Table 1). Considering the origin of food resources, in general, allochthonous resources comprised the largest portion of the diet in all stretches of the four streams (Figure 2), but, significant differences were detected in relation to the longitudinal gradient (Table 2).

At the headwaters and middle stretches of the four streams sampled, allochthonous resources were dominant in the diet of *A.* aff. *fasciatus*, whereas in the mouth, although allochthonous resources also have been used most frequently, autochthonous resources represented a significant portion of the diet (Figure 2). Significant differences were registered in the composition of allochthonous and autochthonous resources in the diet of *A.* aff. *fasciatus* along the longitudinal gradient, with differences detected between the headwaters and mouth for two, of the

http://dx.doi.org/10.1590/1676-06032014001613

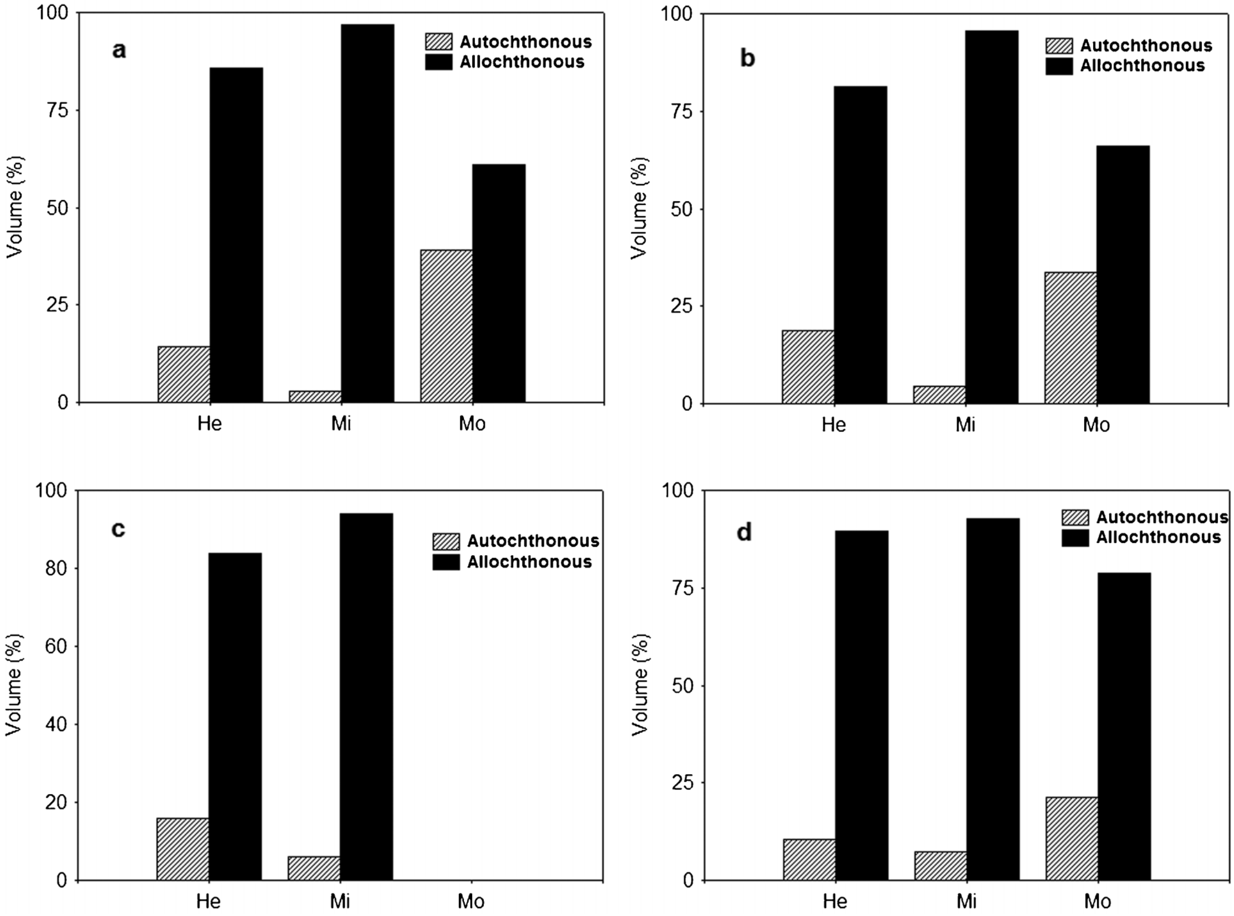


Figure 2. Percentage composition (volume) of autochthonous and allochthonous resources in the diet of *Astyanax* aff. *fasciatus* in four streams (a= Água da Roseira; b= Remo; c= Romeira; d= Zaúna) of the sub-basin of the Pirapó River, Upper Paraná River basin (He= headwaters; Mi= middle; Mo= mouth).

three streams sampled in mouth (Table 2). In only one stream, the diet was similar. There were significant differences between the middle and mouth for all streams sampled in mouth, showing that the diet can vary a lot between these two stretches (Table 2). There were no significant differences in the consumption of autochthonous and allochthonous resources between the headwaters and middle at the four streams sampled (Figure 2; Table 2).

Table 2. Multi Response Permutation Procedure (MRPP) used to test differences in the composition of autochthonous and allochthonous items in the diet of *Astyanax* aff. *fasciatus*, along the longitudinal gradient (He= headwater; Mi= middle; Mo= mouth) of four streams of the sub-basin of the Pirapó River, Upper Paraná River basin.

Stream		A	*p*
Água da Roseira	He × Mi	0.022	0.082
	He × Mo	0.044	0.037
	Mi × Mo	0.169	<0.001
Remo	He × Mi	0.008	0.192
	He × Mo	0.141	<0.001
	Mi × Mo	0.172	<0.001
Romeira	He × Mi	0.004	0.301
Zaúna	He × Mi	0.009	0.246
	He × Mo	0.02	0.243
	Mi × Mo	0.159	<0.001

The results of the ordination analysis indicated an important spatial separation in the diet of *A.* aff. *fasciatus* when considering the items grouped into food resources, and indicated that the longitudinal gradient was important in grouping the individuals analyzed (Figure 3). The first axis of the PCoA explained 82.2% of data variability, and so it was the only one analyzed. The variation revealed by this ordination showed the separation of the diet of fish sampled in the mouth stretches (positive scores) from the diet of most fish sampled at the headwaters and in middle stretches (negative scores). The food resource most positively correlated were aquatic insects, responsible for the grouping of individuals sampled in the mouth, and negatively, terrestrial insects, which grouped the majority of individuals caught in the headwaters and middle stretches of the streams (Figure 3).

Considering the food items grouped into food resources (Table 1) the diet composition of *A.* aff. *fasciatus* in the headwaters varied significantly from middle in two of the four streams sampled (Table 3). Between the headwaters and mouth differences were registered in only one stream. Significant differences were registered between the middle and mouth for all streams sampled in mouth. In general, in the headwaters and middle, terrestrial insects, was the most consumed resource

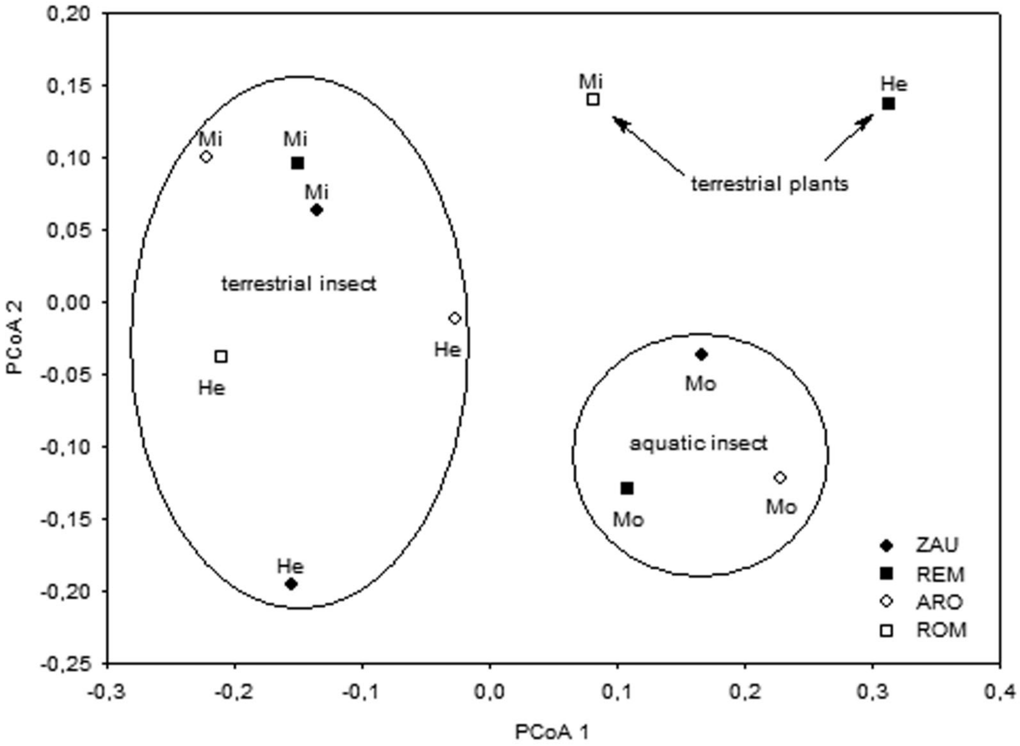


Figure 3. PCoA analysis of volumetric data of food resources in the diet of *Astyanax* aff. *fasciatus* in four streams (ARO= Água da Roseira; REM= Remo; ROM= Romeira; ZAU= Zaúna) of the sub-basin of the Pirapó River, Upper Paraná River basin (He= headwaters; Mi= middle; Mo= mouth).

(Table 1). Hymenoptera, Coleoptera and Diptera were important in the headwaters, and Coleoptera, Lepidoptera and Hymenoptera in the middle, in order of importance. Besides, in the headwaters, terrestrial plants and aquatic insects were also important in the diet (Table 3). In the mouth the diet of *A.* aff. *fasciatus* was composed of similar percentages of terrestrial insects, aquatic insects and terrestrial plants (Table 1).

Considering only main insect groups consumed by *A.* aff. *fasciatus*, it was observed an increasing trend of aquatic insects (Diptera and Trichoptera) in the diet in the mouth stretch (Figure 4). Hymenoptera was the most consumed terrestrial insect in the headwaters of all streams, followed by Lepidoptera in the Zaúna stream, and Coleoptera in the Romeira stream, while aquatic insects were insignificant in this stretches (Figure 4a). In the middle stretch, terrestrial insects also predominated in all streams, with Hymenoptera being more consumed in Água da Roseira and Zaúna streams, Coleoptera in the Romeira, and Lepidoptera in the Remo stream, whereas aquatic insects were little consumed (Figure 4b). In the mouth stretch, aquatic Diptera and Trichoptera showed a relevant increment in the diet of *A.* aff. *fasciatus*, reaching percentages similar to those of terrestrial insects, except for Hymenoptera in the Água da Roseira stream (Figure 4c).

Table 3. Multi Response Permutation Procedure (MRPP) used to test differences in the composition of items in the diet of *Astyanax* aff. *fasciatus*, along the longitudinal gradient (He= headwater; Mi= middle; Mo= mouth) of four streams of the sub-basin of the Pirapó River, Upper Paraná River basin.

Stream		A	*P*
Água da Roseira	He × Mi	0.03	0.032
	He × Mo	0.032	0.065
	Mi × Mo	0.051	<0.001
Remo	He × Mi	0.047	0.006
	He × Mo	0.147	<0.001
	Mi × Mo	0.148	<0.001
Romeira	He × Mi	0.008	0.23
Zaúna	He × Mi	0.011	0.068
	He × Mo	0.025	0.22
	Mi × Mo	0.087	<0.001

Discussion

Our results showed that *A.* aff. *fasciatus* consumed a wide range of food items and there was a predominance of allochthonous resources in the diet, particularly insects and plants, along the longitudinal gradient. Several studies in streams, involving the diet of fish fauna, reveal the importance of allochthonous subsidies to many species (Esteves & Aranha 1999, Lowe-McConnell 1999, Casatti 2002, Silva et al. 2012), especially for the genus *Astyanax* (Bennemann et al. 2005, Borba et al. 2008, Wolff et al. 2009, Ferreira et al. 2011, Ferreira et al. 2012, Manna et al. 2012). Specifically for *A.* aff. *fasciatus* it was reported a high consumption of terrestrial plants and insect remains in streams of the Atlantic forest (Villela et al. 2002, Wolff et al. 2009).

Nevertheless, the substantial consumption of allochthonous resources in the headwaters and middle stretches and the expressive consumption of aquatic insects in the mouth evidenced the influence of the longitudinal gradient in the diet of *A.* aff. *fasciatus*. These differences were caused by the

http://dx.doi.org/10.1590/1676-06032014001613

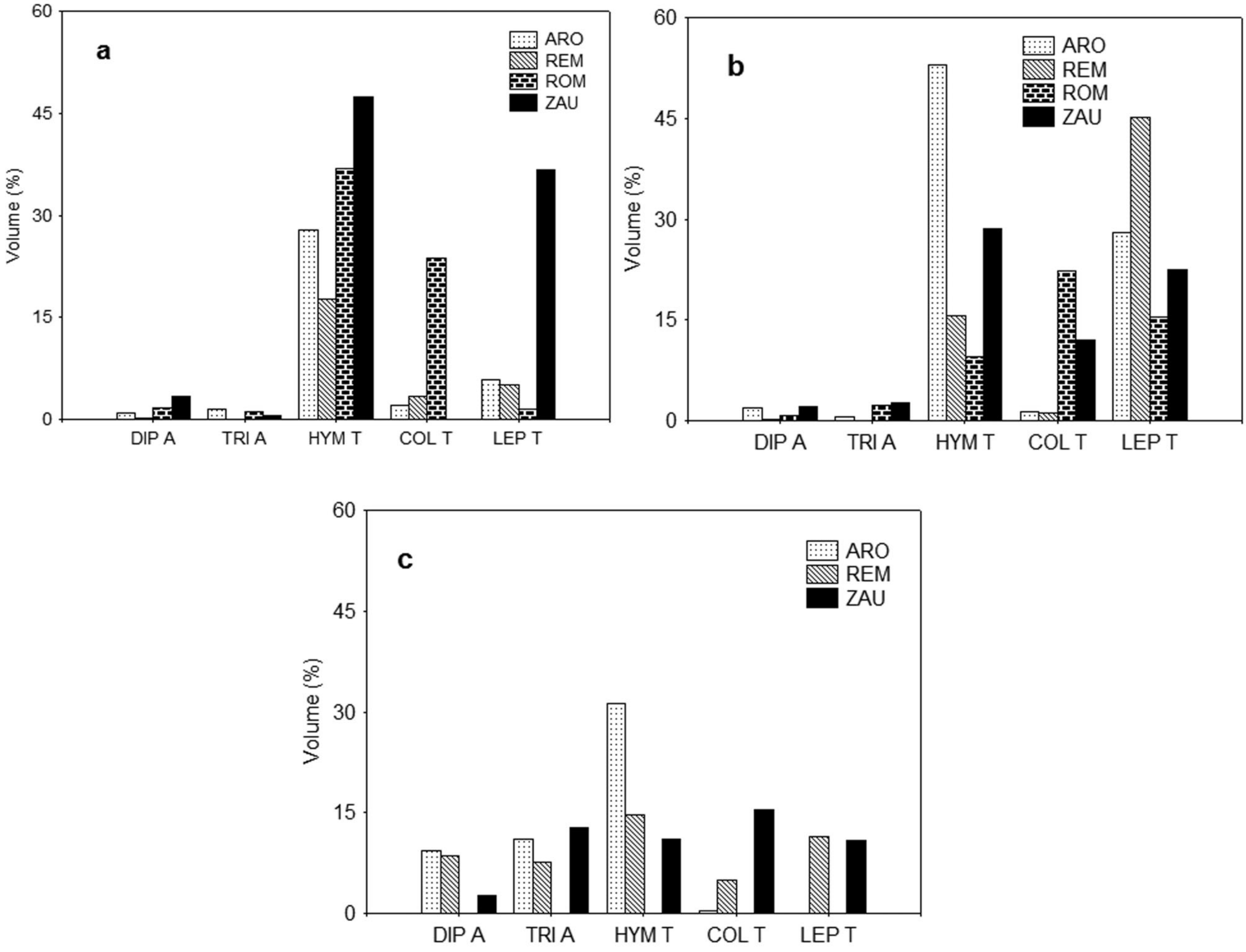


Figure 4. Participation (% volume) of main insect groups in the diet of *Astyanax* aff. *fasciatus* in four streams (ARO= Água da Roseira; REM= Remo; ROM= Romeira; ZAU= Zaúna) of the sub-basin of the Pirapó River, Upper Paraná River basin (a= headwaters; b= middle, c= mouth; DIP A= aquatic Diptera; TRI A= aquatic Trichoptera; HYM T= terrestrial Hymenoptera; COL T= terrestrial Coleoptera; LEP T= terrestrial Lepidoptera).

increase of autochthonous resources, mainly aquatic insects represented by immature Trichoptera and Diptera, and may be related to factors such as the presence of riparian vegetation along the gradient, greater internal structure of the bed at the headwaters and middle, and larger width of the channel in the mouth stretch. Vannote et al. (1980) stated that in lotic ecosystems, from the headwaters to the mouth, one can find a continuous gradient of physical and biological conditions. The headwaters region is influenced by the surrounding vegetation, which contributes with allochthonous material (plant material and terrestrial insects), as the stream enlarges, decreases the importance of importing terrestrial organic matter. The riparian vegetation is well documented in the literature as a major supplier of food for aquatic organisms (Vannote et al. 1980, Polis et al. 1997, Baxter et al. 2005, Manna et al. 2012, Silva et al. 2012), in turn controlling the flow of nutrients. The shading produced by riparian vegetation limits the autotrophic production, and fish depend on resources from the slopes for feeding (Lowe-McConnell 1999). Moreover, Polis et al. (1997) considered the spatial scale, emphasizing the ration of edge to the water body (i.e. perimeter-to-area - P/A), which declines from the headwaters towards the mouth, with a corresponding decline in the relative importance of local allochthonous inputs.

In this context, the internal structure (trunk, branches and leaves) of the headwaters and middle stretches increases the environmental heterogeneity, increases the substrate area for aquatic invertebrates, creating shelters (Wohl et al. 1995), which probably makes it difficult the capture of these organisms by fish. For *A. taeniatus*, Manna et al. (2012) demonstrated a reduction in the consumption of allochthonous resources, especially plant debris, and an increase for an aquatic Curculionidae larvae (Coleoptera) along the gradient of the stream.

Therefore in streams examined, the separation of the diet of fish sampled at the mouth section from the diet of most fish sampled at the headwaters and middle stretches is mainly the consequence of the consumption of aquatic insects, responsible for the grouping of individuals sampled at the mouth, and thus influenced by the longitudinal gradient.

Our results allow inferring the preference of *A.* aff. *fasciatus* for certain insect groups, e.g., Hymenoptera, corroborating studies that claim that this species swims at mid-water level and collect particles dragged by the flow (Casatti et al. 2001, Casatti 2002). Therefore, it is expected a higher consumption of organisms that fall into the water body, such as invertebrates, especially some species of ants wingless that walk on the branches and accidentally fall into the water, or the consumption of floating particles. Esteves & Aranha (1999) highlight the importance of the drift material in fish feeding, and the relationship between the drift of invertebrates and feeding as a key aspect to the understanding of factors that regulate the production of fishes in streams. Rezende & Mazzoni (2006) also highlighted the relationship between the consumption of Hymenoptera by a Characidae with its availability in the environment. In this way, the headwaters of the streams in the present study represented the most favorable region because the internal structure was greater, with lower current flow and perimeter-to-area ratio. Even in the headwaters and middle sections, where terrestrial insects dominated, it was possible to verify the influence of the longitudinal gradient when considering the groups of insects; Lepidoptera for example was particularly more consumed in the middle section. In the mouth, these variables presented an opposite trend, leading *A.* aff. *fasciatus* to consume more aquatic insects with benthic habit, such as larvae of Diptera and Trichoptera. In the latter case, it is believed that these macroinvertebrates are abundant in these stretches, and are dragged from the substrate by the current, facilitating the capture by *A.* aff. *fasciatus.* Uieda & Ramos (2007) point out several factors that influence the distribution of aquatic organisms, including the current velocity and substrate type. Meantime, some groups of insects have adaptations to withstand the current flow, such as attachment by adhesive structures, claws and flattened shape (Bennett & Humphries 1974), which permit them to inhabit areas with pronounced current, as verified by Uieda & Ramos (2007) who found twice aquatic insects in areas of higher water velocity compared with sites with lower velocity in a Brazilian stream.

Although *A.* aff. *fasciatus* has flexible feeding habit according to the availability of resources (Vilella et al. 2002, Wolff et al. 2009, Manna et al. 2012), the results demonstrated that it was predominantly sustained by terrestrial food resources, indicating the importance of the riparian vegetation in these aquatic environments. Researches claim that riparian vegetation is of fundamental importance to streams (Pusey & Arthington 2003, Abilhoa et al. 2008), and its removal causes serious impacts on the integrity of fish communities in tropical streams that depend on allochthonous resources (Angermeier & Karr 1983, Bojsen & Barriga 2002), like that of *A.* aff. *fasciatus.* With the expansion of agricultural activities the riparian vegetation of streams in rural areas has been compromised, so in addition to the importance of the longitudinal gradient for the supply of terrestrial resources it is expected that their input and availability for the fish to be more pronounced in streams with greater vegetation cover (Rezende & Mazzoni 2006). Borba et al. (2008) evaluated the diet of *Astyanax asuncionenses* in four streams with different degrees of riparian vegetation preservation and verified that even where vegetation was scarce, the species consumed predominantly allochthonous resources, pointing out that despite the high degradation of the vegetation, the species still depends on these resources.

Although allochthonous resources have been essential in the diet of *A.* aff. *fasciatus* from the headwaters to the mouth, the longitudinal gradient can also be considered important to explain consistent patterns of import, transport, use and storage of organic matter (Uieda & Kikuchi 1995) available for consumption to fish communities (Power 1983). This pattern in the supply of food resources can influence directly the presence of some species that have their optimal distribution coinciding with the degree of preservation of the habitat or specific impacts (Casatti et al. 2006). Thus the response of *A.* aff. *fasciatus* to the longitudinal gradient of the studied streams is positive to the diet, emphasizing the importance of the riparian vegetation for the maintenance of this species in these ecosystems.

Acknowledgments

We thank CS Pavanelli for fish identification; RM Tófoli and GHZ Alves for helping with the stomach contains. MR Silva and N Carniatto acknowledge the Brazilian Council of Research (Capes - CNPq) for providing grants.

References

ABILHOA, V., DUBOC, L.F. & AZEVEDO-FILHO, D.P. 2008. A comunidade de peixes de um riacho de floresta com Araucária, alto rio Iguaçu, sul do Brasil. Rev. Bras. Zool. 25(2): 238-246, doi: http://dx.doi.org/10.1590/S0101-81752008000200012

ANGERMEIER, L.P. & KARR, J.R. 1983. Fish communities along environmental gradients in a system of tropical streams. Environ. Biol. Fish. 9(2):117-135, doi: http://dx.doi.org/10.1007/BF00690857

BAXTER, C.V., FAUSCH, K.D. & SAUNDERS, W.C. 2005. Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. Freshwater Biol. 50: 201–220, doi: http://dx.doi.org/10.1111/j.1365-2427.2004.01328.x

BENNEMANN, S.T., GEALH, A.M., ORSI, M.L. & SOUZA, L.M. 2005. Ocorrência e ecologia trófica de quatro espécies de *Astyanax* (Characidae) em diferentes rios da bacia do rio Tibagi, Paraná, Brasil. Iheringia, Ser. Zool. 95(3): 247-254, doi: http://dx.doi.org/10.1590/S0073-47212005000300004

BENNETT, D.P. & HUMPHRIES, D.A. 1974. Introducción a la ecologia de campo. H. Blume, Madrid.

BOJSEN, B.H. & BARRIGA, R. 2002. Effects of deforestation on fish community structure in Ecuadorian Amazon streams. Freshwater Biol. 47(11): 2246-2260, doi: http://dx.doi.org/10.1046/j.1365-2427.2002.00956.x

BORBA, C.S., FUGI, R., AGOSTINHO, A.A. & NOVAKOWSKI, G.C. 2008. Dieta de *Astyanax asuncionensis* (Characiformes, Characidae), em riachos da bacia do rio Cuiabá, Estado do Mato Grosso. Acta Sci. Biol. Scien. 30(1): 39-45.

CASATTI, L. 2002. Alimentação dos peixes em um riacho do Parque Estadual Morro do Diabo, bacia do Alto Paraná, sudeste do Brasil. Biota Neotrop. 2(2): http://www.biotaneotropica.org.br/v2n2/pt/abstract?article+BN02502022002 (último acesso em 05/07/2013), doi: http://dx.doi.org/10.1590/S1676-06032002000200012

CASATTI, L., LANGEANI, F. & CASTRO, R.M.C. 2001. Peixes de riacho do Parque Estadual Morro do Diabo, bacia do alto rio Paraná, SP. Biota Neotrop. 1(1): http://www.biotaneotropica.org.br/v1n12/pt/abstract?inventory+BN00201122001 (último acesso em 07/07/2013)

CASATTI, L., LANGEANI, F., SILVA, A.M. & CASTRO, R.M.C. 2006. Stream fish, water and habitat quality in a pasture dominated basin, Southeastern Brazil. Braz. J. Biol. 66(2B): 681-696, doi: http://dx.doi.org/10.1590/S1519-69842006000400012

ERÖS T., GUSTAFSSON, P., GREENBERG, L.A. & BERGMAN, E. 2012. Forest-stream linkages: effects of terrestrial invertebrate input and light on diet and growth of brown trout (*Salmo trutta*) in a boreal forest stream. Plos One 7(5):1-11, doi: http://dx.doi.org/10.1371/journal.pone.0036462

ESTEVES, K.E. & ARANHA, M.R. 1999. Ecologia trófica de peixes de riachos. In Ecologia de peixes de riachos (Caramashi, E.P., Mazzoni R. & Peres-Neto, P.R. eds.). Série Oecologia Brasiliensis, Vol. VII, PPGE-UFRJ, Rio de Janeiro, Brasil, p.157-182

FERREIRA, A., GERHARD, P. & CYRINO, J.E.P. 2012. Diet of *Astyanax paranae* (Characidae) in streams with different riparian land covers in the Passa-Cinco River basin, Southeastern Brazil.

Iheringia, Ser. Zool. 102(1): 80-87, doi: http://dx.doi.org/10.1590/S0073-47212012000100011

FERREIRA, A., PAULA, F.R., FERRAZ, S.F.B., GERHARD, P., KASHIWAQUI, E.A.L., CYRINO, J.E.P. & MARTINELLI, L.A. 2011. Riparian coverage affects diets of characids in neotropical streams. Ecol. Freshw. Fish 21(1):12-22, doi: http://dx.doi.org/10.1111/j.1600-0633.2011.00518.x

FERREIRA, C.P. & CASATTI, L. 2006. Influência da estrutura do hábitat sobre a ictiofauna de um riacho em uma micro-bacia de pastagem, São Paulo, Brasil. Rev. Bras. Zool. 23(3): 642-651, doi: http://dx.doi.org/10.1590/S0101-81752006000300006

GARMAN, G.C. 1991. Use of terrestrial arthropod prey by a stream-dwelling cyprinid fish. Environ. Biol. Fish. 30(3): 325-331, doi: http://dx.doi.org/10.1007/BF02028848

HELLAWEL, J.M. & ABEL R.A. 1971. A rapid volumetric method for the analysis of the food of fishes. J. Fish Biol. 3: 29-37, doi: http://dx.doi.org/10.1111/j.1095-8649.1971.tb05903.x

HENRY, R., UIEDA, V.S., AFONSO, A.A.O. & KIKUCHI, R.M. 1994. Input of allochthonous matter and structure of fauna in a Brazilian headstream. SIL 25(3): 1866-1870.

HYSLOP, E.J. 1980. Stomach contents analysis – a review of methods and their applications. J. Fish Biol. 17: 411-429, doi: http://dx.doi.org/10.1111/j.1095-8649.1980.tb02775.x

LANCASTER, J., DOBSON, M., MAGANA, A.M., ARNOLD, M. & MATHOOKO, J.M. 2008. An unusual trophic subsidy and species dominance in a tropical stream. Ecology 89(8): 2325-2334.

LEGENDRE P. & LEGENDRE, L. 1998. Numerical ecology. 2 ed. Amsterdam: Elsevier Science.

LORION, C.M. & KENNEDY, B.P. 2009. Riparian forest buffers mitigate the effects of deforestation on fish assemblages in tropical headwater streams. Ecol. Appl. 19(2): 468-479, doi: http://dx.doi.org/10.1890/08-0050.1

LOWE-McCONNEL, R.H. 1975. Fish communities in tropical freshwaters. Their distribution, ecology and evolution. Longman, London.

LOWE-McCONNELL, R.H. 1999. Estudos ecológicos de comunidades de peixes tropicais. Tradução: A.E.A.M. Vazzoler, A.A. Agostinho. & P.T.M. Cunningham. EDUSP, São Paulo. Título original em inglês: Ecological studies in tropical fish communities.

LYONS, J. 1992. The length of stream to sample with a towed electrofishing unit when fish species richness is estimated. N. Am. J. Fish. Manage. 12(1):198-201, doi: http://dx.doi.org/10.1577/1548-8675(1992)012<0198:TLOSTS>2.3.CO;2

MAIN, M.R. & LYON, G.L. 1988. Contribution of terrestrial prey to the diet of banded kokopu (*Galaxias fasciatus* Gray) (Pisces: Galaxiidae) in South Westland, New Zealand. SIL 23:1785-1789.

MANNA, L.R., REZENDE, C.F. & MAZZONI, R. 2012. Plasticity in the diet of *Astyanax taeniatus* in a coastal stream from South-East Brazil. Braz. J. Biol. 72(4): 919-928, doi: http://dx.doi.org/10.1590/S1519-69842012000500020

MASSON, C.F & MACDONALD, S.M. 1982. The input of terrestrial invertebrates from tree canopies to a stream. Freshwater Biol. 12(4): 305-311, doi: http://dx.doi.org/10.1111/j.1365-2427.1982.tb00624.x

MAZZONI, R. & COSTA, L.D.S. 2007. Feeding ecology of stream-dwelling fishes from a coastal stream in the Southeast of Brazil. Braz. Arch. Biol. Techn. 50(4): 627-635, doi: http://dx.doi.org/10.1590/S1516-89132007000400008

MAZZONI, R., NERY, L.L. & IGLESIAS-RIOS, R. 2010. Ecologia e ontogenia da alimentação de *Astyanax janeiroensis* (Osteichthyes, Characidae) de um riacho costeiro do Sudeste do Brasil. Biota Neotrop. 10(3): http://www.biotaneotropica.org.br/v10n3/en/abstract?article +bn01010032010 (último acesso em 07/07/2013), doi: http://dx.doi.org/10.1590/S1676-06032010000300005

McCUNE, B. & GRACE, J.B. 2002. Analysis of ecological communities. Gleneden Beach, Oregon.

McCUNE, B., MEFFORD, M.J. 2006. PC-ORD, version 5.0, Multivariate analysis of ecological data. MjM Solfware Desing, Gleneden Beach, Oregon.

OLIVEIRA, D.C. & BENNEMANN, S.T. 2005. Ictiofauna, recursos alimentares e relações com as interferências antrópicas em um riacho urbano no sul do Brasil. Biota Neotrop. 1(1): http://www.biotaneotropica.org.br/v5n1/pt/abstract?article+BN02905012005 (último acesso em 07/07/2013)

PERUÇO, J.D. 2004. Identificação das principais fontes poluidoras de afluentes da bacia do alto rio Pirapó. Dissertação Mestrado, Universidade Estadual de Maringá, Maringá.

PACE, M.L., COLE, J.J., CARPENTER, S.R., KITCHELL, J.F., HODGSON, J.R., VAN DE BOGERT, M.C, BADE, D.L, KRITZBERG, E.S & BASTVIKEN, D. 2004. Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. Nature 427: 240-243, doi: http://dx.doi.org/10.1038/nature02227

POLIS, G.A., ANDERSON, W.B. & HOLT, R.D. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annu. Rev. Ecol. Syst. 28: 289-316, doi: http://dx.doi.org/10.1146/annurev.ecolsys.28.1.289

POWER, M.E., RAINEY, W.E., PARKER, M.S., SABO, J.L., SMYTH, A., KHANDWALA, S., FINLAY, J.C., MCNEELY, F.C., MARSEE, K. & ANDERSON, C. 2004. River to watershed subsidies in an old-growth conifer forest. In Food Webs at the Landscape Level. (G.A. Polis M.E. Power& G.R. Huxe eds.). The University of Chicago Press, Chicago, p. 217-240 .

POWER, M.E. 1983. Grazing responses of tropical freshwater fishes to different scales of variation in their food. Environ. Biol. Fish. 9(2):103-115, doi: http://dx.doi.org/10.1007/BF00690856

PUSEY, B.J. & ARTHINGTON, A.H. 2003. Importance of the riparian zone to the conservation and management of freshwater fish: a review. Mar. Freshwater Res. 54:1-16, doi: http://dx.doi.org/10.1071/MF02041

REZENDE, C.F. & MAZZONI, R. 2006. Disponibilidade e uso de recursos alóctones por *Bryconamerius microcephalus* (Miranda-Ribeiro) (Actinopterygii, Characidae), no córrego Andorinha, Ilha Grande, Rio de Janeiro, Brasil. Rev. Bras. Zool. 23(1): 218-222, doi: http://dx.doi.org/10.1590/S0101-81752006000100014

RICHARDSON, J.S., ZHANG, Y. & MARCZAK, L.B. 2009. Resource subsidies across the land-freshwater interface and responses in recipient communities. River Res. Appl. Published online in Wiley InterScience <www.interscience.wiley.com>.

ROSE, M.D. & POLIS, G.E. 1998. The distribution and abundance of coyotes: the effects of allochthonous food subsidies from the sea. Ecology 79(3): 998-1007.

SILVA, J.C., DELARIVA, R.L. & BONATO, K.O. 2012. Food-resource partitioning among fish species from a first-order stream in northwestern Paraná, Brazil. Neotrop. Ichthyol. 10(2): 389-399, doi: http://dx.doi.org/10.1590/S1679-62252012005000008

SMALL, G.E., TORRES, P.J., SCHWEIZER, L.M., DUFF, J.H. & PRINGLE, C.M. 2013. Importance of terrestrial arthropods as subsidies in lowland neotropical rain Forest. Biotropica 45(1): 80-87.

TÓFOLI, R.M., HAHN, N.S., ZAIA, G.A. & NOVAKOWSKI, G.C. 2010. Uso do alimento por duas espécies simpátricas de *Moenkhausia* (Characiformes, Characidae) em um riacho neotropical do Brasil. Iheringia, Ser. Zool. 100: 201-206, doi: http://dx.doi.org/10.1590/S0073-47212010000300003

UIEDA, V.S. & KIKUCHI, R.M. 1995. Entrada de material alóctone (detritos vegetais e invertebrados terrestres) num pequeno curso de água corrente na Cuesta de Botucatu, São Paulo. Acta Limn. Bras. 7: 105-114.

UIEDA, V.S. & RAMOS, L.H.B. 2007. Distribuição espacial da comunidade de macroinvertebrados bentônicos de um riacho tropical (Sudeste do Brasil). Bioikos 21(1): 3-9.

VANNOTE, R.L., MINSHALL, G.W., CUMMINS, K.W., SEDELL, J.R. & CUSHING, C. 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. 37: 130-137, doi: http://dx.doi.org/10.1139/f80-017

VILELLA, F.S., BECKER, F.G. & HARTZ, S.M. 2002. Diet of *Astyanax* species (Teleostei, Characidae) in an Atlantic Foresta River in Southern Brazil. Braz. Arch. Biol. Techn. 45(2): 223-232, doi: http://dx.doi.org/10.1590/S1516-89132002000200015

VITULE, J.R.S., BRAGA, M.R. & ARANHA, J.M.R. 2008 Ontogenetic, spatial and temporal variations in the feeding ecology of *Deuterodon langei* Travassos, 1957 (Teleostei: Characidae) in a Neotropical stream from the Atlantic rainforest, Southern Brazil. Neotrop. Ichthyol. 6(2): 211-222, doi: http://dx.doi.org/10.1590/S1679-62252008000200008

WOHL, D.L., WALLACE, J.B. & MEYER, J.L. 1995. Benthic macroinvertebrate community structure, function and production with respect to habitat type, reach and drainage basin in the southern Appalachian (U.S.A). Freshwater biol. 34(3): 447-464, doi: http://dx.doi.org/10.1111/j.1365-2427.1995.tb00902.x

WOLFF, L.L., ABILHOA, V., RIOS, F.S. & DONATTI, L. 2009. Spatial, seasonal and ontogenetic variation in the diet of *Astyanax* aff. *fasciatus* (Ostariophysi: Characidae) in na Atlantic Forest river, Southern Brazil. Neotrop. Ichthyol. 7(2): 257-266.

Received 06/08/2013
Revised 05/05/2014
Accepted 11/07/2014

Biota Neotropica 14(3): e20130015, 2014
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
article

Sensitivity to fragmentation and spatial distribution of birds in forest fragments of northern paraná

Fernanda Cristina Marques[1,2] *& Luiz dos Anjos*[1]

[1]*Programa de Pós Graduação em Ciências Biológicas, Departamento de Biologia Animal e Vegetal, Universidade Estadual de Londrina, CP 6001, CEP 86051-990, Londrina, PR, Brazil.*
[2]*Corresponding author: Fernanda Cristina Marques, e-mail: fernanda1081@gmail.com*

MARQUES, F.C., ANJOS, L. **Sensitivity to fragmentation and spatial distribution of birds in forest fragments of northern paraná.** Biota Neotropica. 14(3): e20130015. dx.doi.org/10.1590/1676-0603001513

Abstract: In this study we investigated the relationship between the sensitivity to forest fragmentation of bird species and their habitat spatial distribution. We hypothesized that a homogenous spatial distribution is associated to habitat-generalist species, which would tend to be less sensitive to forest fragmentation; in contrast, a heterogeneous spatial distribution would drive to an increase of sensitivity to forest fragmentation. We concentrated on analyses of the suboscine birds, which are known to be closely associated to microhabitats. Field data was obtained in the Parque Estadual Mata dos Godoy (23°27′ S, 51°15′ W), southern Brazilian Atlantic forest. This reserve has high Biotic Integrity levels, with a relatively high number of recorded suboscines. Forty three species of suboscines were recorded, of which 21 species were considered to have high sensitivity and 22 to have low sensitivity to forest fragmentation. There was no association between levels of sensitivity to forest fragmentation and spatial distribution of suboscines. We only found significant correlation with the group of the subcanopy suboscines at the northern portion of Parque Estadual Mata dos Godoy. Therefore, we found a weak relationship between spatial distribution (habitat specialization) and sensitivity to forest fragmentation. The fact that habitat-specialist birds tend to live at the northern portion of Godoy State Park, which has the first type of terrain in a landscape to be converted into agriculture, indicate that that region has special importance for conservation.
Keywords: *microhabitat, habitat-specialization, suboscine birds.*

MARQUES, F.C., ANJOS, L. **Sensibilidade à fragmentação e distribuição espacial de aves em fragmentos florestais do norte do Paraná.** Biota Neotropica. 14(3): e20130015. dx.doi.org/10.1590/1676-0603001513

Resumo: Neste estudo nós investigamos as relações entre sensibilidade à fragmentação florestal de aves e sua distribuição espacial no habitat. Nossa hipótese é que a distribuição espacial homogênea está associada a espécies habitat-generalistas, que tenderiam a ser menos sensíveis à fragmentação florestal, em contrapartida, uma distribuição espacial heterogênea seria resultado de uma maior sensibilidade à fragmentação florestal. As análises foram concentradas em aves suboscines por serem intimamente associada a microhabitats. Dados amostrais foram obtidos no Parque Estadual Mata dos Godoy (23°27′ S, 51°15′ W), sul da Mata Atlântica brasileira. Esta reserva apresenta alto Índice de Integridade Biótica, com um número relativamente elevado de aves suboscines. Quarenta e três espécies de aves suboscines foram registradas, sendo 21 espécies consideradas de alta sensibilidade e 22 de baixa sensibilidade à fragmentação florestal. Não houve associação entre os níveis de sensibilidade à fragmentação florestal e a distribuição espacial de aves suboscines. Só foi observada significância para as aves de sub-bosque da porção norte do Parque Estadual Mata dos Godoy. Portanto, encontramos uma fraca relação entre especialização de habitat e sensibilidade à fragmentação florestal. O fato destas aves habitat-especialistas tenderem a viver na porção norte do Parque Estadual Mata dos Godoy, que é o primeiro tipo de terreno a ser convertido para a agricultura em uma paisagem, indicam que essa região tem uma importância especial para a conservação.
Palavras-chave: *aves suboscines, habitat-especialistas, microhabitat.*

Introduction

Forest fragmentation has been considered a major force in decreasing local biodiversity (e.g. Gardner et al. 2009, Laurance 2010). However, fragmentation does not act homogeneously on different species. In birds, several features have been identified as closely related to species that are more sensitive, such as population size, reproductive performance, annual survival, trophic position, rarity, and biogeographic position (e.g. Henle et al. 2004, Anjos 2006, Devictor et al. 2010). Another feature pointed out by Henle et al. (2004) associating sensitivity of bird species to forest fragmentation is habitat specialization. Species that are more habitat-specialists tend to be locally extinct where forests are fragmented (Ribon et al. 2003, Henle et al. 2004).

Variations in vegetation features, such as vertical and horizontal segregation associated with variations in floristic composition, increase diversity of microhabitats, which in turn drives to a mosaic of resources for the species, even in a continuous forest (Karr 1990). This structural complexity entails, for example, greater diversity of foraging substrates, as occurs in certain groups of birds, like the Furnariidae and Tyrannidae families (e.g. Remsen 2003, Fitzpatrick et al. 2004). Thus, a species could be found in certain habitats within a forest whilst being rare, or even absent, in others. Fragmentation can negatively affect the availability of those habitats for most specialized species, decreasing the chances of their survival in forest fragments (Uezu & Metzger 2011). Therefore, habitat-specialist species may have their survival compromised due to the loss or alteration of vegetation heterogeneity (Cerqueira et al. 2003). However, the chances to persist in forest fragments should be higher in habitat-generalists.

In this study we evaluated the type of spatial distribution of forest suboscine birds in a reserve of southern Brazil, the Parque Estadual Mata dos Godoy (PEMG). The principal microhabitat features of some suboscine species in PEMG have already been described (Poletto et al. 2004, Lopes et al. 2006, Volpato et al. 2006). Here we associated a homogeneous spatial distribution to habitat-generalist species, meaning that they could survive in larger spectrum of biotic and abiotic conditions within the forest. By contrast, species with heterogeneous spatial distribution were considered habitat-specialist, which indicates that they could not support a larger spectrum of environmental variations. We hypothesized that habitat-generalist suboscines are less sensitive to forest fragmentation than the habitat-specialists. The study was carried out in northern Paraná, where the sensitivity of species to forest fragmentation has been previously determined for several species (Anjos 2006). We considered firstly all species of suboscines together and secondly according their general feeding habits (canopy, subcanopy or climber). Additionally, we evaluated variations in the composition of the bird assemblies in the PEMG.

Material and methods

1. *Study area*

The study was conducted in Parque Estadual Mata dos Godoy (PEMG) (23°27′ S, 51°15′ W, 656 ha) located 15 km south of Londrina, Paraná, in southern Brazil. It is characterized as an important conservation area of northern Paraná. According to Anjos et al. (2009), it has a high value of Biotic Integrity (0,85), with a high bird species richness (114 species) compared to other southern Brazil fragments, especially in light of the vegetation heterogeneity resulting from topographical variation between the north and south of the park (the top of the slope to the valley bottom, see Santana & Anjos 2010). In this study, Anjos et al. (2009) determined an Index of Biotic Integrity (IBI) in 39 forest fragments in the north of Paraná State, southern Brazil, based on presence and absence of 30 selected bird species of different sensitivity levels to forest fragmentation.

The northern region of the PEMG, a plateau at about 600 m altitude, has a dense, closed canopy between 12 m to 20 m where the most abundant tree species are *Cabraela canjerana* (Meliaceae), *Euterpes edulis* (Arecaceae), *Ocotea indecora* (Lauraceae) and *Nectandra megapotamica* (Lauraceae) (Torezan & Silveira 2002, Anjos et al. 2007). Because of the dense canopy, the midstory and understory receives little light, and the understory is relatively open, with short trees and bushes such as *Eugenia verrucosa* (Myrtaceae), *Sorocea bonplandii* (Moraceae), *Miconiatritis* (Melastomataceae), *Maranta* sp. (Marantaceae) and *Piper* sp. (Piperaceae) (Torezan & Silveira 2002, Anjos et al. 2007). The southern portion consists of a sloped area (600 – 470 m) ending in a floodplain of the Apertados river, the southern boundary of the Park. The canopy is less compact, with the largest trees sparsely distributed, including *Chrysophyllum gonocarpum* (Sapotaceae), *Campomanesia xanthocarpa* (Myrtaceae) and *Parapiptadenia rigida* (Fabaceae). The midstory, however, has a higher density of smaller tree species, such as *Nectandra megapotamica* (Lauraceae), *Alseis floribunda* (Rubiaceae), *Matayba elaeagnoides* (Sapindaceae), *Lonchocarpus muehlbergianus* (Fabaceae), *Sebastiana commersoniana* (Euphorbiaceae), *Eugenia verrucosa* (Myrtaceae) and *Trichilia cassaretti* (Meliaceae) (Anjos et al. 2007, Santana & Anjos 2010). It is common the fall of the tallest trees in this region, resulting in clearings dominated by the herbaceous *Celtis iguanaea* (Ulmaceae) and bamboo *Chusquea* sp. (Poaceae) (Silveira 2006, Anjos et al. 2007, Santana & Anjos 2010). The edge of the park is characterized by a riparian forest floodplain which has little representation of the coverage in the PEMG; *Bastardiopsis densiflora* (Malvaceae) and *Ocotea puberula* (Lauraceae) are examples of trees that make up the vegetation in this portion of the Park (Silveira 2006).

2. *Field work*

Point counts of limited distance were sampled (always by the same observer) along three trails. Each trail has 1300 m length. Two trails were in the upland forest (TA and TB) and the third on the sloped forest in the southern area (TC). On each trail, 14 points were established every 100 m and numbered in sequence (one to 14, Figure 1). Field sampling was obtained at each point and conducted from October to

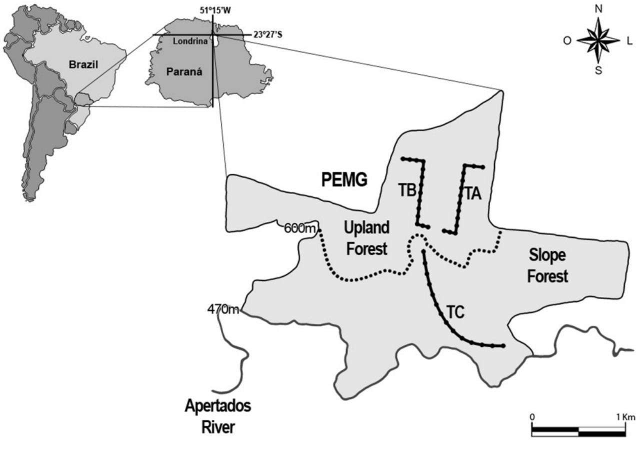


Figure 1. Location of sampling points in each of the three trails sampled in Parque Esta dual Mata dos Godoy, Londrina, Brazil. The dotted line indicates the division by altitude of the two types of forest in the Park.

December 2011, beginning at sunrise. The radius of detection from each point was 50 m. On each day, the trails were walked twice in opposite directions. The point samplings in a day were considered as one sample. The starting point of each trail sample was alternated each day, offering the same odds of detection for all species. The sampling period at each point was 5 minutes, thus it lasted for about four hours. At each point, only the presence of the species was registered.

3. *Vegetation characterization*

In order to evaluate whether the features of vegetation differ between the trails, general data on phyto-physiognomy were obtained at each point (36 points in total), by sampling at 25 m from each point along the trail and at 10 m from each side of the trail. Our sampled area for each point resulted in 1000 m^2 (Figure 2). The vegetation variables that we collected were: number of large trees (height greater than 15 m), number of palmettos (only *Euterpe edulis* occurs in PEMG, height greater than 4 m) and herbaceous, liana and bamboo densities. The densities were estimated according to the proportion of the sample area occupied using the following scale: absent (0); to 40% (1); between 40–60% (2); between 60–80% (3) and up to 100% (4). The sum of the percentages of herbaceous, liana and bamboo did not resulted in 100% because they were estimated independently. Our procedure was to estimate the area occupied for each of these three vegetation types.

4. *Procedure for analysis*

Levels of sensitivity to forest fragmentation of each bird species were based on Anjos (2006), which determined the sensitivity of bird species to fragmentation based on point counts conducted in 14 forest fragments of different sizes and degrees of isolation in northern Paraná. Species were considered highly sensitive if they occurred only in the controls and in large and not-isolated forest remnants; species with low sensitivity were those occurring in all categories of the forest remnants, including those smaller and more isolated (Anjos 2006). We considered the bird list of species of Anjos et al. (1997) to select the suboscines that occur in PEMG. We allocated each species to groups based on foraging habits, according to Remsen 2003, Marantz et al. 2003, Zimmer & Isler 2003, Krabbe & Schulenberg 2003, Whitney 2003, Krabbe & Schulenberg 2003, Snow 2004, Fitzpatrick et al. 2004. The groups were: (1) canopy species, (2) subcanopy species, including species that frequent the ground and (3) climber species. We considered these distinct groups of species because they seem to present different levels of sensitivity to forest fragmentation. Subcanopy species seem to be more sensitive than canopy ones, while climbers seem to have intermediate sensitivity levels (Aleixo & Vielliard 1995, Anjos & Soares 1999, Ribon et al. 2003, Uezu & Metzger 2011).

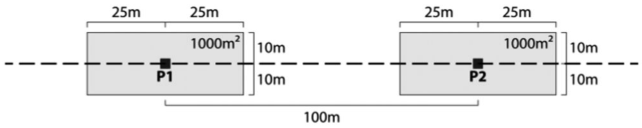


Figure 2. Schematic diagram of the sampling points (P1 and P2) and the sampled area in which the vegetation was characterized.

5. *Statistics and additional comments*

Kruskal Wallis analysis of variance was used to compare the variation in the number of trees and palmettos between trails studied (TA, TB and TC). For the remaining vegetation variables (density of vines, bamboo and herbaceous) it was used a contingency table ($p < 0.05$) to test whether the proportion of variables is similar in the three trails. This analyze was made by comparing the number of sampled areas wherein each vegetation variable was estimated according to the occupancy scale, in other words the number of sampled areas in each percentage range for each trail.

To evaluate whether bird species are habitat-generalist or habitat-specialist in PEMG we calculated the percentage of points that were occupied. To do this, we associated a homogeneous spatial distribution to habitat-generalist species, which would tend to be less sensitive to forest fragmentation. In contrast, we associated a heterogeneous spatial distribution to habitat-specialist species, which would tend to be more sensitive to forest fragmentation. Therefore, if a species was recorded at 4 points out of a total of 32 points from the trails, it would give us a percentage of 13% of occupancy. If another species was recorded at 28 points it would result in a percentage of 88% of occupancy. This way, we determined that species with an occupancy percentage equal and below 30% were habitat-specialists and those above that value were habitat-generalist. In the first example the species would be habitat-specialist and in the second, habitat-generalist. The value of 30% was arbitrary selected. We know that detectability differs between the species, as has already been considered for some birds of the PEMG (Bochio et al. 2012). We countered this by sampling each point 12 times on 12 different days, making the possibility that a species was present but not recorded very low.

We evaluated the association between spatial distribution and the levels of sensitivity in the trails separately. As explained above, the distinction between homogenous and heterogeneous spatial distribution was based in the occupancy of the points; an occupancy equal and below 30% were of heterogeneous spatial distribution (habitat-specialists) and those above that value were of homogenous spatial distribution (habitat-generalist). Contingency table ($p < 0.05$) was used to verify whether the spatial distribution (homogeneous and heterogeneous) was associated with sensitivity levels of suboscine species (sensitive and non-sensitive) or with foraging habits groups. All analyses were carried out using the software R.

Nonmetric multidimensional scaling (NMDS) was also used to examine the distribution of bird assemblies in the PEMG. For data analysis, the data were standardized and square-root transformed to reduce the effect of the most strongly represented species. For this data analysis, the relative frequency of each species at each point was used. Therefore, if a species was recorded 3 times out of 12 times a point was sampled, it had a frequency of occurrence of 40%. This analysis was carried out using the software PRIMER 6.1.13 (Clarke & Gorley 2006).

The sequence of species follows the checklist of the American Ornithologists' Union (2013).

Results

The number of large trees and palmettos and the densities of herbaceous plants were not significantly different among the studied trails (Tables 1, 2). However, higher density of bamboo

Table 1. Mean (*X*) and standard deviation (S) of vegetation variables in each trail. *P* indicates the significance between the values of the variables between trails. (Kruskal Wallis).

Variables	Trails						*P*
	TA		TB		TC		
	X	*S*	*X*	*S*	*X*	*S*	
Trees (> 15m)	8,000	4.11	11,857	4.24	11,929	4.75	0.06
Palmettos (> 4m)	7,500	6.49	9,714	9.33	7,286	6.57	0.79

Table 2. Total sampled areas wherein each vegetation variable was estimated according to the following occupancy scale: absent (0); to 40%; (1) between 40–60% (2); between 60–80% (3) and up to 100% (4). *P* value indicates the significance between trails. (G-test).

Variables	Scale	Trails			*P*
		TA	TB	TC	
Herbaceous stratum	1	1	0	2	0.60
	2	2	3	5	
	3	9	9	6	
	4	2	2	1	
Vine	1	1	0	6	0.01
	2	4	5	5	
	3	4	7	3	
	4	5	2	0	
Bamboo	1	14	14	4	0.001
	2	0	0	2	
	3	0	0	3	
	4	0	0	5	

was found on TC (G = 26.25, p = 0.001), while TA and TB had a higher density of vines (G = 16.28, p = 0.01).

A total of 43 different suboscine birds were recorded; 36 species were seen in the northern and 40 in the southern portion of the PEMG. The numbers of species observed on each trail were: 34 species in TA, 32 species in TB and 40 in TC. For each trail there were (respectively on TA, TB and TC) 15, 13 and 13 species with a heterogeneous spatial distribution (habitat-specialists), and 19, 19 and 27 with a homogeneous spatial distribution (habitat-generalist). Overall, of the 43 species recorded in the present study, 21 were considered to have high sensitivity and 22 to have low sensitivity to forest fragmentation, following the literature cited above (see methods; Table 3). Among the high sensitivity species, 17, 16 and 19 species were found respectively on TA, TB and TC, whereas for species with low sensitivity, 18 were observed on TA, 16 on TB and 21 on TC.

There was no association between levels of sensitivity to forest fragmentation and spatial distribution of suboscine birds when the whole PEMG was considered (G = 0.02, p = 0.87). Significance was only found in the group of subcanopy suboscine birds when TA and TB were considered separately (G = 6.96, p = 0.008 and G = 4.41, p = 0.03 respectively; Table 4). Subcanopy suboscine birds showed a significant association between homogeneous spatial distribution (habitat-generalists) and low sensitivity to forest fragmentation. Canopy and climber suboscine birds did not exhibit significant association in any situation.

The nonmetric multidimensional scaling (NMDS) ordination revealed three distinct groups of bird species. One group is composed of species that preferentially occupy the northern portion, another of species that occupy the southern portion, while the third group consists of species that occupy the transition region between the northern and southern portions of the PEMG (Figure 3).

Discussion

We found an unexpectedly high number of suboscine birds with homogenous spatial distribution, which would suggest the majority of them could be considered habitat-generalists. This could mean that the studied suboscine birds are not closely associated to microhabitats as previously thought. In addition, contrary to our expectations, spatial distribution was not associated with level of sensitivity to forest fragmentation when all species of suboscine birds were considered together. This association was only found for the subcanopy suboscine birds and even then, only in the northern portion of the PEMG. These results should be considered carefully however, since it is possible that the area of forest sampled may not have been large enough to properly detect variations in habitat occupancy.

Subcanopy suboscine birds, or understory birds in general, have been seen as one of the more vulnerable groups to forest fragmentation (Willis 1979, Kattan 1994, Aleixo & Vielliard 1995, Stouffer & Bierregaard 1995, Bierregaard & Stouffer 1997, Goerck 1997, Aleixo 1999, Stratford & Stouffer 1999,

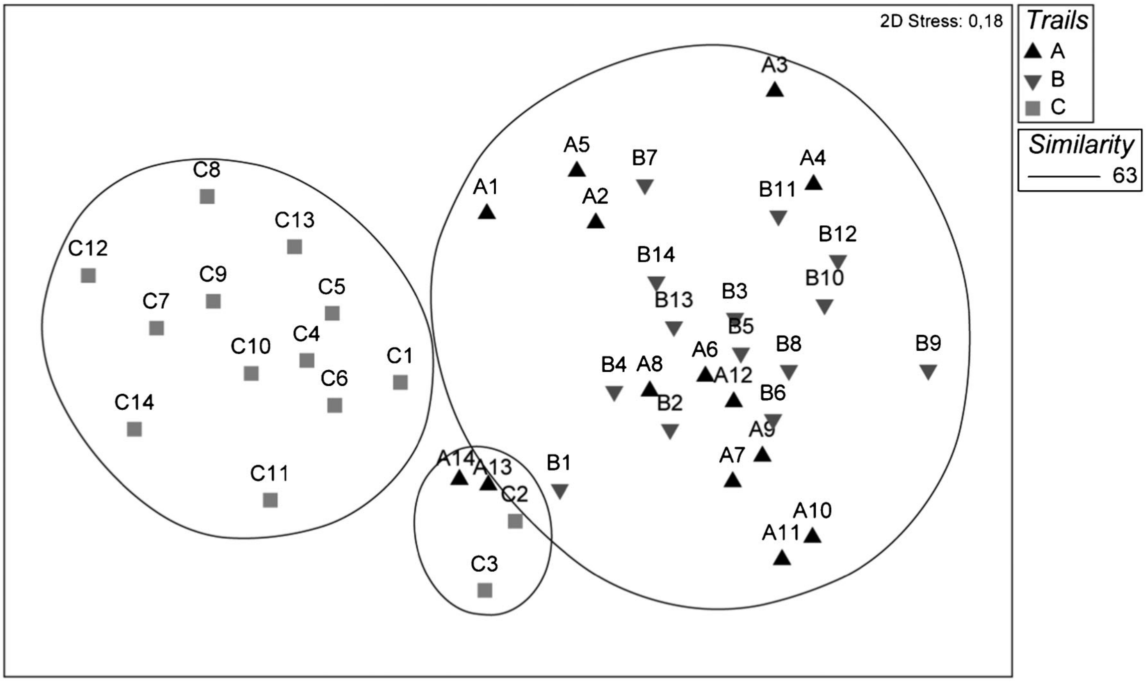


Figure 3. Nonmetric multidimensional scaling (NMDS) representing the profile similarity of sampling points according to the occupancy patterns of suboscine bird species sampled in Parque Estadual Mata dos Godoy.

Ribon et al. 2003, Henle et al. 2004, Lees & Peres 2008, Uezu & Metzger, 2011). Their habitat specialization is thought to be the reason for such higher vulnerability (Stratford & Stouffer 1999, Hansbauer et al. 2008a, 2010, Sodhi et al. 2011), which seems to be corroborated by the data presented here.

Some studies claim that species of suboscine birds found on trunks and twigs, which we referred to as climbers in the present study, can be considered sensitive to even slight changes in the structure of vegetation (Willis 1979, Aleixo & Vielliard 1995, Christiansen & Pitter 1997, Anjos 1998, Poletto et al. 2004). Authors tentatively described the microhabitat of those birds, especially species of Dendrocolaptidae (Cintra et al. 2006, Poletto et al. 2004). However, the results of this study suggest that the reason for climbers being sensitive to forest fragmentation is not closely associated with the microhabitat specialization. Possibly their sensitivity could be the result of variations in vegetation structure at broader scales. According to Poletto et al. (2004), Dendrocolaptidae with stricter ecological requirements may relate with other vegetation features, selecting fairly homogeneous sites at late successional stages, such as *Dendrocincla turdina*, or even areas with tangled vegetation and rough bark large trees as *Xiphocolaptes albicollis*.

Canopy suboscine birds, in turn, do not appear to be associated with microhabitats. The canopy birds are adapted to move long distances seeking resources distributed unevenly in the canopy (Karr & James 1975, Winkler & Preleuthner 2001). Probably the broad potential of habitat occupancy (homogeneous spatial distribution) contributed to their low sensitivity to forest fragmentation (e.g. Karr 1982, Ribon et al. 2003, Uezu & Metzger 2011).

The results found in the analysis of structural variation of the vegetation showed that PEMG can be differentiated according to the structure of understory vegetation, with it being subdivided into areas occupied predominantly by bamboos or vines. Large densities of bamboos were found only in the southern portion of the park (Silveira 2006, Santana & Anjos 2010). The bamboo clusters that make up the southern portion of the Atlantic Forest often occupy gaps and areas of secondary growth, especially in riparian forest or hills, where they have an aggregate distribution, forming a dense environment with a lower stratum of anatomically similar leaves (Kratter 1997, Santana & Anjos 2010). Contrary to clusters of bamboo, vine tangles were found predominantly in the northern portion of the park. According to Kratter (1997), vine tangles are probably the most physiognomic similar microhabitat to clusters of bamboo, providing habitat and food source for many organisms (Schnitzer & Bongers 2002, Thomsen et al. 2010). Data presented here (Table 3 and figure 3) revealed differences between the pattern of occupancy of the suboscine birds species between the areas occupied by bamboos and vines, which seems to disagree with the interpretations of Kratter (1997).

In the present study we found a weak relationship between spatial distribution (habitat specialization) and sensitivity to forest fragmentation. But our data suggested that suboscine birds occupied differently the northern and the southern portions of PEMG. Indeed, higher species numbers that have been considered as habitat-specialists were more common in the bird assembly of the northern portion of PEMG. The northern portion of the PEMG is the first type of terrain in a landscape to be converted into agriculture and should be considered as the most vulnerable area of that reserve. Many studies have evaluated effects of forest fragmentation comparing fragments of different sizes and levels of connectivity (e.g. Uezu et al. 2005, Hansbauer et al. 2008a, b, Gillies & Clair 2010). But variations in the vegetation inside the forest fragment should be also considered in such studies (Hansbauer et al. 2010), as in the case of the PEMG.

Table 3. Species sampled, divided according to levels of sensitivity to forest fragmentation in accordance with Anjos (2006), foraging habits (FH) and frequency of occurrence (FO) in trails (TA, TB and TC). The species frequency of occurrence with heterogeneous distribution is shown in bold. The foraging habits are: subcanopy (SC), climber (CL) and canopy (CP). Species are in systematic and phylogenetic position following nomenclature of the South American Classification Committee of the American Ornithologists' Union (2013).

		FO					FO		
	FH	TA	TB	TC		FH	TA	TB	TC
High Sensitivity					**Low Sensitivity**				
Thamnophilidae					Thamnophilidae				
Drymophila malura (Temminck, 1825)	SC			0.36	*Hypoedaleus guttatus* (Vieillot, 1816)	CP	1.00	1.00	1.00
Rhinocryptidae					*Mackenziaena severa* (Lichtenstein, 1823)	SC	0.64	**0.21**	1.00
Psilorhamphus guttatus (Ménétriès, 1835)	SC	**0.07**	**0.07**	1.00	*Thamnophilus caerulescens* Vieillot, 1816	SC			0.43
Eleoscytalopus indigoticus (Wied, 1831)	SC	**0.29**	**0.14**	0.79	*Dysithamnus mentalis* (Temminck, 1823)	SC	0.79	1.00	0.86
Formicariidae					*Herpsilochmus rufimarginatus* (Temminck, 1822)	CP	**0.29**	**0.29**	**0.07**
Chamaeza campanisona (Lichtenstein, 1823)	SC			**0.07**	*Pyriglena leucoptera* (Vieillot, 1818)	SC	1.00	0.86	1.00
Furnariidae					Conopophagidae				
Sittasomus griseicapillus (Vieillot, 1818)	CL	1.00	1.00	1.00	*Conopophaga lineata* (Wied, 1831)	SC	**0.07**		
Dendrocincla turdina (Lichtenstein, 1820)	CL	0.86	0.79	**0.07**	Furnariidae				
Xiphorhynchus fuscus (Vieillot, 1818)	CL	0.50	0.57	0.57	*Dendrocolaptes platyrostris* Spix, 1825	CL	1.00	0.93	0.57
Campylorhamphus falcularius (Vieillot, 1822)	CL			**0.14**	*Xenops rutilans* Temminck, 1821	CL	**0.07**		0.43
Philydor rufum (Vieillot, 1818)	CP		**0.29**	**0.07**	*Heliobletus contaminatus* Berlepsch, 1885	CL	**0.21**	**0.21**	0.36
Anabacerthia lichtensteini Cabanis & Heine, 1859	CP	1.00	1.00	0.93	*Automolus leucophthalmus* (Wied, 1821)	SC	0.86	0.79	0.93
Cranioleuca obsoleta (Reichenbach, 1853)	CL	0.79	0.57	0.86	*Synallaxis ruficapilla* Vieillot, 1819	SC	0.36	0.43	0.93
Synallaxis cinerascens Temminck, 1823	SC	**0.29**	**0.29**	0.64	Tyrannidae				
Tyrannidae					*Myiopagis caniceps* (Swainson, 1835)	CP	**0.07**		**0.21**
Hemitriccus diops (Temminck, 1822)	SC	**0.14**		0.50	*Leptopogon amaurocephalus* Tschudi, 1846	SC	0.57	0.71	0.64
Poecilotriccus plumbeiceps (Lafresnaye, 1846)	SC			0.79	*Myiornis auricularis* (Vieillot, 1818)	CP	**0.14**	0.43	0.64
Colonia colonus (Vieillot, 1818)	CP	**0.07**			*Todirostrum cinereum* (Linnaeus, 1766)	CP			**0.07**
Sirystes sibilator (Vieillot, 1818)	CP	0.50	0.79	**0.14**	*Tolmomyias sulphurescens* (Spix, 1825)	CP	0.64	0.79	0.93
Oxyruncidae					*Lathrotriccus euleri* (Cabanis, 1868)	SC	**0.29**	**0.21**	0.43
Oxyruncus cristatus Swainson, 1821	CP		**0.07**		*Myiodynastes maculatus* (Statius Muller, 1776)	CP	1.00	1.00	1.00
Pipridae					Tityridae				
Chiroxiphia caudata (Shaw & Nodder, 1793)	SC	**0.21**	0.36		*Pachyramphus viridis* (Vieillot, 1816)	CP	0.36	**0.14**	0.29
Tityridae					*Pachyramphus castaneus* (Jardine & Selby, 1827)	CP		**0.21**	**0.14**
Tityra inquisitor (Lichtenstein, 1823)	CP	0.50	0.50		*Pachyramphus polychopterus* (Vieillot, 1818)	CP	**0.21**	**0.14**	**0.14**
Tityra cayana (Linnaeus, 1766)	CP	0.86	0.93	0.86					
Pachyramphus validus (Lichtenstein, 1823)	CP	**0.07**	**0.29**	**0.14**					

Table 4. The number of species according to sensitivity degree of forest fragmentation and spatial distribution category, homogeneous and heterogeneous, considering the foraging habits and the three sampled trails (TA, TB and TC) in Parque Estadual Mata do Godoy.

	TA			TB			TC	
	High sensitivity	Low sensitivity		High sensitivity	Low sensitivity		High sensitivity	Low sensitivity
Subcanopy ($p<0,05$)			Subcanopy ($p<0,05$)			Subcanopy ($p>0,05$)		
Homogeneous spatial distribution	0	6	Homogeneous spatial distribution	1	5	Homogeneous spatial distribution	6	8
Heterogeneous spatial distribution	5	2	Heterogeneous spatial distribution	4	1	Heterogeneous spatial distribution	2	0
Climber ($p>0,05$)			Climber ($p>0,05$)			Climber ($p>0,05$)		
Homogeneous spatial distribution	4	1	Homogeneous spatial distribution	4	1	Homogeneous spatial distribution	4	3
Heterogeneous spatial distribution	0	2	Heterogeneous spatial distribution	0	1	Heterogeneous spatial distribution	1	0
Canopy ($p>0,05$)			Canopy ($p>0,05$)			Canopy ($p>0,05$)		
Homogeneous spatial distribution	4	4	Homogeneous spatial distribution	4	4	Homogeneous spatial distribution	2	4
Heterogeneous spatial distribution	4	2	Heterogeneous spatial distribution	3	4	Heterogeneous spatial distribution	4	6

Acknowledgments

We thank Erivelto Goulart, Mario Luís Orsi, and Sandra Maria Hartz for revising a previous version of the manuscript, the Universidade Estadual de Londrina for logistical support and the InstitutoAmbiental do Paraná (252/10) for permission to conduct research in the Godoy State Park.

References

ALEIXO, A. 1999. Effects of selective logging on a bird community in the Brazilian Atlantic forest. Condor 101:537-548.

ALEIXO, A. & VIELLIARD, J.M.E.1995. Composição e dinâmica da comunidade de aves da Mata de Santa Genebra, Campinas, SP. Rev. Bras. Zool. 12(3):493-511.

AMERICAN ORNITHOLOGISTS' UNION. 2013. Check-list of South American Birds. [Cited 20 May 2013.] Available from URL: http://www.museum.lsu.edu/~Remsen/SACCBaseline.html (last access on 20/05/2013).

ANJOS, L. 1998. Consequências biológicas da fragmentação no Norte do Paraná. Série. técnica. IPEF 12(32):87-94.

ANJOS, L. 2006. Bird species sensitivity in a fragmented landscape of the Atlantic Forest Southern Brazil. Biotropica 32(2):229-234. http://dx.doi.org/10.1111/j.1744-7429.2006.00122.x

ANJOS, L., SCHUCHMANN, K.L., BERNDT, R. 1997. Avifaunal composition, species richness, and status in the Tibagi river basin, Paraná State, southern Brazil. Ornitol. Neotrop. 8(2): 145-173.

ANJOS, L., SOARES, E.S. 1999. Efeito da fragmentacão florestal sobre aves escaladoras de tronco e galho na região de Londrina, norte do Estado do Paraná, Brasil. Ornitol. Neotrop. 10:61-68.

ANJOS, L., VOLPATO, G.H., LOPES, E.V., SERAFINI, P.P., POLETTO, F. & ALEIXO, A. 2007. The importance of riparian forest for the maintenance of bird species richness in an Atlantic Forest remnant, southern Brazil. Rev. Bras. Zool. 24(4):1078-1086. http://dx.doi.org/10.1590/S0101-81752007000400027

ANJOS, L., BOCHIO, G.M., CAMPOS, J.V., MCCRATE, G.B. & PALOMINO, F. 2009. Sobre o uso de níveis de sensibilidade de aves à fragmentação florestal na avaliação da Integridade Biótica: um estudo de caso no norte do Estado do Paraná, sul do Brasil. Rev. Bras. Ornitol. 17(1):28-36.

BIERREGAARD, R.O. & STOUFFER, P C. 1997. Understory birds and dynamic habitat mosaics in Amazoniam rainforests. In Tropical forest remnants: ecology, management, and conservation of fragmented communities (Laurance,W.F. & Bierregaard, R.O. eds). The University of Chicago Press, Chicago.

BOCHIO, G.M., ANJOS, L. 2012. The Importance of Considering Bird Detectability for Assessing Biological Integrity. Nat. Conservação 10:72-76. http://dx.doi.org/10.4322/natcon.2012.012

CERQUEIRA, R., BRANT, A., NASCIMENTO, M.T. & PARDINI, R. 2003. Fragmentação: alguns conceitos. In Fragmentação de ecossistemas: causas efeitos sobre a biodiversidade e recomendações de políticas públicas (Rambalsi, D.M. & Oliveira, D.A.S. eds). MMA/SBF, Brasília.

CHRISTIANSEN, M.M. & PITTER, E. 1997. Species loss in a forest bird community near Lagoa Santa in Southeastern Brazil. Biol. Conserv 80(1):23-32. http://dx.doi.org/10.1016/S0006-3207(96)00073-0

CINTRA, R., MARUOKA, A.E. & NAKA, L.N. 2006. Abundance of two Dendrocincla woodcreepers in relation to forest structure in Central Amazonia. Acta. Amazônica. 36(2):209-219. http://dx.doi.org/10.1590/S0044-59672006000200011

CLARKE, K.R. & GORLEY, R.N. 2006. Primer v6: user manual/tutorial. PRIMER-E. Plymouth.

DEVICTOR, V., CLAVEL, J., JULLIARD, R., LAVERGNE, S., MOUILLOT, D., THUILLER, W., VENAIL, P., VILLEGER, S. & MOUQUET, N. 2010. Defining and measuring ecological specialization. J. Appl. Ecol. 47(1):15-25. http://dx.doi.org/10.1111/j.1365-2664.2009.01744.x

FITZPATRICK, J., BATES, J., BOSTWICK, K., CABALLERO, I., CLOCK, B., FARNSWORTH, A., HOSNER, P., JOSEPH, L., LANGHAM, G., LEBBIN, D., MOBLEY, J., ROBBINS, M., SCHOLES, E., TELLO, J., WALTHER, B. & ZIMMER, K. 2004. Family Tyrannidae (Tyrant-flycatchers). In Handbook of birds of the world (del Hoyo, J., Elliot, A. & Sargatal, J. eds). Cotingas to pipits and wagtails. Lynx Edicions, Barcelona, v.9, p. 170-463.

GARDNER, T.A., BARLOW, J., CHAZDON, R.L., EWERS, R.M., HARVEY, C.A., PERES, C.A. & SODHI, N.S. 2009. Prospects for tropical forest biodiversity in a human-modified world. Ecol. Lett. 12(6):561-582. http://dx.doi.org/10.1111/j.1461-0248.2009.01294.x

GILLIES, C.S. & ST CL.A.I.R., C.C. 2010. Functional responses in habitat selection by tropical birds moving through fragmented forest. J. App. Ecol. 47(1):182-190. http://dx.doi.org/10.1111/j.1365-2664.2009.01756.x

GOERCK, J.M. 1997. Patterns of Rarity in the Birds of the Atlantic Forest of Brazil. Conserv. Biol. 11(1):112-118. http://dx.doi.org/10.1046/j.1523-1739.1997.95314.x

HANSBAUER, M.M., STORCH, I., PIMENTEL, R.G. & METZGER, J.P. 2008a. Comparative range use by three Atlantic Forest understorey bird species in relation to forest fragmentation. J. Trop. Ecol. 24(3):291-299. http://dx.doi.org/10.1017/S0266467408005002

HANSBAUER, M.M., STORCH, I., LEU, S., NIETO-HOLGUIN, J.P., PIMENTEL, R.G., KNAUER, F. & METZGER, J.P. 2008b. Movements of neotropical understory passerines affected by anthropogenic forest edges in the Brazilian Atlantic rainforest. Biol. Conserv. 141:782-791. http://dx.doi.org/10.1016/j.biocon.2008.01.002

HANSBAUER, M.M., VEGVÁRI, Z., STORCH, I., BORNTRAEGER, R., HETTICH, U., PIMENTEL, R.G. & METZGER, J.P. 2010. Microhabitat Selection of three Forest Understory Birds in the Brazilian Atlantic Rainforest. Biotropica 42(3):355-362. http://dx.doi.org/10.1111/j.1744-7429.2009.00593.x

HENLE, K., DAVIES, K.F., KLEYER, M., MARGULES, C. & SETTELE, J. 2004. Predictors of species sensitivity to fragmentation. Bio. Divers. Conserv. 13:207-251. http://dx.doi.org/10.1023/B:BIOC.0000004319.91643.9e

KARR, JR. 1982. Avian Extinction on Barro Colorado Island, Panama: A Reassessment. Am. Nat. 119(2):220-239. http://dx.doi.org/10.1086/283904

KARR,JR. 1990. Interactions between forest birds and their habitats: A comparative synthesis. In Biogeography and ecology of forest bird communities. (Keast, A. ed). SPB Academic Publishing. Ann Arbor.

KARR, R. & JAMES, F.C. 1975. Eco-morphological configurations and convergent evolution in species and communities. In Ecology and Evolution of Communities. (Cody, M.L. & Diamond, J.M. eds). Harvard University Press. Cambridge.

KATTAN, G.H., ALVAREZ-LÓPEZ, H. & GIRALDO, M. 1994. Forest fragmentation and Bird extinctions: San Antonio eighty years later. Conserv. Bio. l8(1):138-146. http://dx.doi.org/10.1046/j.1523-1739.1994.08010138.x

KRABBE, N. & SCHULENBERG, T. 2003. Family Formicariidae (Ground-antbirds). In Handbook of birds of the world (del Hoyo, J., Elliot, A. & Sargatal, J. eds). Broadbills to tapaculos. Lynx Edicions. Barcelona, v.8, p. 682-731.

KRATTER, A.W. 1997. Bamboo specialization by Amazonian birds. Biotropica 29(1):100-110.

LAURANCE, W.F. 2010. Understanding habitat fragmentation in the real world. In The Theory of Island Biogeography Revisited. (Losos, J.B. & Ricklefs, R.E. eds). Princeton University Press, New Jersey.

LEES, A.C. & PERES, C.A. 2008. Avian life-history determinants of local extinction risk in a hyper-fragmented neotropical forest landscape. Anim. Conserv. 11(2):128-137. http://dx.doi.org/10.1111/j.1469-1795.2008.00162.x

LOPES, E.V., VOLPATO, G.H., MENDONÇA, L.B., FÁVARO, F.L. & ANJOS, L. 2006. Abundância, microhabitat e repartição ecológica de papa-formigas (Passeriformes, Thamnophilidae) na bacia hidrográfica do rio Tibagi, Paraná, Brasil. Rev. Bras. Zool. 23(2):395–403. http://dx.doi.org/10.1590/S0101-81752006000200013

MARANTZ, C., ALEIXO, A., BEVIER, L. & PATTEN, M. 2003. Family Dendrocolaptidae (Woodcreepers). In Handbook of birds of the world (del Hoyo, J., Elliot, A. & Sargatal, J. eds). Broadbills to tapaculos. Lynx Edicions, Barcelona, v.8, p. 358–447.

POLETTO, F., ANJOS, L., LOPES, E.V., VOLPATO, G.H., SERAFINI, P.P. & FAVARO, F.L. 2004. Caracterização do microhabitat e vulnerabilidade de cinco espécies de arapaçus (Aves: Dendrocolaptidae) em um fragmento florestal do norte do estado do Paraná, sul do Brasil. Ararajuba. 12(2):89-96.

REMSEN, JR.J.V. 2003. Family Furnariidae (Ovenbirds). In Handbook of birds of the world (del Hoyo, J., Elliot, A. & Sargatal, J. eds). Broadbills to tapaculos. Lynx Edicions, Barcelona, v.8, p. 162–357.

RIBON, R., SIMON, J.E. & MATTOS, G.T. 2003. Bird extinctions in Atlantic forest fragments of Viçosa region, southeastern Brazil. Conserv. Biol. 17(6):1827-1839. http://dx.doi.org/10.1111/j.1523-1739.2003.00377.x

SANTANA, C.R. & ANJOS, L. 2010. Associação de aves a agrupamentos de bambu na porção sul da Mata Atlântica, Londrina, Estado do Paraná, Brasil. Biota. Neotrop. 10(2): http://www.biotaneotropica.org.br/v10n2/en/abstract?article+bn00510022010 ISSN 1676-0603 (last access on 21/05/2013).

SCHNITZER, S.A. & BONGERS, F. 2002. The ecology of lianas and their role in forests. Trends. Ecol. Evol. 17(1):223-230. http://dx.doi.org/10.1016/S0169-5347(02)02491-6

SILVEIRA, M. 2006. A vegetação do Parque Estadual Mata dos Godoy. In Ecologia do Parque Estadual Mata dos Godoy. (Torezan, J.M.D. Org). Itedes, Londrina, p. 19-27.

SNOW, D. 2004. Family Pipridae (Manakins). In Handbook of birds of the world (del Hoyo, J., Elliot, A. & Sargatal, J.). Cotingas to pipits and wagtails. Lynx Edicions, Barcelona, v.9, p. 110-169.

SODHI, N.S., SEKERCIOGLU, C.H., BARLOW, J. & ROBINSON, S.K. 2011. Conservation of Tropical Birds. Wiley-Blackwell, Oxford.

STOUFFER, P.C. & BIERREGAARD, R.O. 1995. Use of Amazonian forest fragments by understory insectivorous birds. Ecology 76(8):2429-2445.

STRATFORD, J.A. & STOUFFER, P.C. 1999. Local extinctions of terrestrial insectivorous birds in a fragmented landscape near Manaus, Brazil. Conserv. Biol. 13(6):1416-1423. http://dx.doi.org/10.1046/j.1523-1739.1999.98494.x

THOMSEN, M.S., WERNBERG, T., ALTIERI, A., TUYA, F., GULBRANSEN, D., MCGLATHERY, K.J., HOLMER, M. & SILLIMAN, B.R. 2010. Habitat Cascades: The Conceptual Context and Global Relevance of Facilitation Cascades via Habitat Formation and Modification. Integr. Compe. Biol. 50(2):158-175. http://dx.doi.org/10.1093/icb/icq042

TOREZAN, J.M.D. & SILVEIRA, M. 2002. Fatores ambientais, diversidade e similaridade em florestas da bacia do rio Tibagi. In A bacia do rio Tibagi. (Medri, M.E., Bianchini, E., Shibata, O.A. & Pimenta, J.A. eds). Edição dos Autores, Londrina.

UEZU, A., METZGER, J.P. & VIELLIARD, J.M.E. 2005. Effects of structural and functional connectivity and patch size on the abundance of seven Atlantic Forest bird species. Biol. Conserv. 123:507-519. http://dx.doi.org/10.1016/j.biocon.2005.01.001

UEZU, A. & METZGER, J.P. 2011. Vanishing bird species in the Atlantic Forest: relative importance of landscape configuration, forest structure and species characteristics. Biodivers. Conserv. 20:3627-3643. http://dx.doi.org/10.1007/s10531-011-0154-5

VOLPATO, G.H., ANJOS, L., POLETTO, F., SERAFINI, P.P., LOPES, E.V. & FÁVARO, F.L. 2006. Terrestrial passerines in an Atlantic Forest remnant of Southern Brazil. Braz. J. Biol. 66(2A):473-478. http://dx.doi.org/10.1590/S1519-69842006000300013

WHITNEY, B. 2003. Family Conopophagidae (Gnateaters). In Handbook of birds of the world (del Hoyo, J., Elliot, A. & Sargatal, J. eds). Broadbills to tapaculos. Lynx Edicions. Barcelona, v.8, p. 732-745.

WILLIS, E.O. 1979. The composition of avian communities in remanescent woodlots in southern Brazil. Papéis Avulsos de Zoologia 33:1-25.

WINKLER, H. & PRELEUTHNER, M. 2001. Behaviour and ecology of birds in tropical rain forest canopies. Plant. Ecol. 153:193-202. http://dx.doi.org/10.1023/A:1017595321548

ZIMMER, M. & ISLER, M. 2003. Family Thamnophilidae (Typical Antbirds). In Handbook of birds of the world (del Hoyo, J., Elliot, A. & Sargatal, J. eds). Broadbills to tapaculos. Lynx Edicions, Barcelona, v.8, p. 448-681.

Received 05/08/2013
Revised 03/02/2014
Accepted 13/08/2014

Biota Neotropica 14(3): e20140004, 2014
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
article

Population dynamics and reproductive biology of *Achatina fulica* Bowdich, 1822 (Mollusca, Gastropoda) in Salvador - Bahia

***Eder Carvalho da Silva*[1,3] *& Elianne Pessoa Omena*[2]**

[1]Núcleo Integrado de Estudos em Zoologia – NIEZ, Instituto de Ciência Biológicas da Universidade Católica do Salvador – UCSal, Av. Prof. Pinto de Aguiar, 2589, 41.740-090, Salvador, BA, Brasil.
[2]Departamento de Biologia Marinha, Instituto de Biologia da Universidade Federal do Rio de Janeiro – UFRJ, Avenida Carlos Chagas Filho, 373, Bloco G, Cidade Universitária, 21.941-902, Rio de Janeiro, RJ, Brasil.
[3]Corresponding author: Eder Carvalho da Silva, e-mail: edercarvalho514@oi.com.br

SILVA, E.C., OMENA, E.P. **Population dynamics and reproductive biology of *Achatina fulica* Bowdich, 1822 (Mollusca, Gastropoda) in Salvador - Bahia.** Biota Neotropica. 14(3): e20140004. http://dx.doi.org/10.1590/1676-0603000414

Abstract: The risks following introduction of invasive species are enormous and incalculable. *Achatina fulica* is considered one of the 100 species of greatest invasive potential, stands out among the land pulmonate snails, mostly for its high reproductive potential that accelerates the process of dispersion, increasing the damages related to health (disease transmission), to economy (crops destruction) and to environment (biodiversity loss). In order to investigate the relationship of the African snail with the environment, trying to relate sexual activity with climatic variables (temperature, rainfall and humidity), a study was conducted on aspects of population dynamics and reproductive biology in Salvador city. The snails were collected by hand, monthly. Morphometric data were obtained in the field and dissections to analyze the reproductive system at laboratory. To test if there was a greater sexual activity in wet periods was performed first a Principal Component Analysis (PCA) with climatic variables, followed by a regression between precipitation (the most influential environmental variable) and the variable of interest (sexual activity). The results showed that there is an apparent annual cycle for *A. fulica*, with a recruitment period covering the end of rainy season and the dry season (August to December 2006 and February 2007). Increase of shell size (height) and of sexual activity were observed during the rainy season, although snails were found in full sexual activity during the whole period of study. The von Bertalanffy curve showed that the oldest specimen collected would have three years and eleven months. The regression between precipitation and sexual activity was significant ($P = 0.002$) showing that the higher rainfall increased sexual activity. In addition, it was observed that there is an increase in the thickness of peristome as the specimens reaches sexual maturity however this relationship isn't precise (it were found individuals with sexual structures not consistent with the reproductive stage given by the thickness of peristome) and should be set for each region studied. As the eradication becomes impossible because of high level of invasion found in Salvador, it is recommended to control the population of African snails by continuously removing specimens, especially in the rainy periods.
Keywords: *bioinvasion, invasive species, reproductive cycle, growth, giant African snail.*

SILVA, E.C., OMENA, E.P. **Dinâmica populacional e biologia reprodutiva de *Achatina fulica* Bowdich, 1822 (Mollusca, Gastropoda) na cidade de Salvador - Bahia.** Biota Neotropica. 14(3): e20140004. http://dx.doi.org/10.1590/1676-0603000414

Resumo: Os riscos com a introdução de espécies invasoras são enormes e incalculáveis. Constando como uma das 100 espécies de maior potencial invasor, *Achatina fulica* se destaca dos demais gastrópodes pulmonados terrestres, principalmente, pelo seu elevado potencial reprodutivo que acelera o processo de dispersão, aumentando os danos relacionados à saúde (transmissão de doenças), à economia (destruição de cultivos) e ao meio ambiente (perda da biodiversidade). Com o intuito de investigar as relações do caramujo africano com o ambiente, tentando relacionar atividade sexual com variáveis climáticas (temperatura, precipitação e umidade), foi realizado um estudo sobre aspectos da sua dinâmica populacional e biologia reprodutiva na cidade de Salvador. Os caramujos foram coletados manualmente, mensalmente. Dados morfométricos foram obtidos em campo e dissecações para estudo do sistema reprodutivo em laboratório. Para testar se havia uma maior atividade sexual em períodos úmidos foi realizada, inicialmente, uma Análise de Componentes Principais (PCA) com as variáveis climáticas, seguida de uma regressão entre a precipitação (variável ambiental com maior influencia) e a variável de interesse (atividade sexual). Os resultados mostraram que há um aparente ciclo anual para *A. fulica*, com um período de recrutamento abrangendo o final da estação chuvosa e a estação seca (agosto a dezembro de 2006 e fevereiro de 2007). O aumento do tamanho da concha (altura) e da atividade sexual foi observado durante a estação chuvosa, embora tenham sido encontrados caramujos em plena atividade sexual em todo o período de estudo. A curva de von Bertalanffy mostrou que o exemplar mais velho teria três anos e onze meses. A regressão entre a precipitação e a atividade sexual foi significativa (P = 0,002) mostrando que precipitações mais elevadas aumentam a atividade sexual. Além disso, observou-se que a espessura do perístoma aumenta à medida que o individuo atinge a maturidade sexual, porém esta relação não é precisa (havia indivíduos com estruturas sexuais não consistentes com o estagio reprodutivo dado pela espessura do perístoma) e deve ser ajustada para cada região estudada. Como a erradicação se torna impossível pelos níveis de invasão encontrados em Salvador, é recomendada, para controlar a população de caramujos africanos, a remoção continua de espécimes, especialmente nos períodos de chuva.

Palavras-chave: *bioinvasão, espécies invasoras, ciclo reprodutivo, crescimento, caramujo gigante africano.*

Introduction

The introduction of species in a new habitat represents environmental and economic risks; free of predators, parasites and natural competing, in positive environmental conditions, these organisms can reach high population densities. Once established, they are rarely eliminated resulting generally in losses of local biodiversity (Carlton 1996, Dajoz 2005, Towsend et al. 2006). The introduction of invasive species is considered the second greatest cause of biological diversity loss in many ecosystems, also, can cause a change in its structure and function, increasing the homogenization of biota (USC 2001, Alowe et al. 2004, Fischer & Colley 2004).

Known as giant African snail, the land pulmonate mollusc *Achatina fulica* Bowdich, 1822, reaches considerable dimensions, nearly 20 cm shell length and weigh up 200 g (Teles et al. 1997, Vasconcellos & Pile 2001), but in Brazil the maximum average records vary in about 11 cm and little over 100 g (Vasconcellos & Pile 2001, Carvalho et al. 2003, Simião & Fischer 2004, Fischer & Colley 2005, Fischer et al. 2006).

The species is distinguished from other pulmonate molluscs due the high invasion potential (Teles et al. 2004). The characteristics that makes it one of the 100 species of greatest invasive potential at the List of the International Union for Conservation of Nature (IUCN) (Alowe et al. 2004) are: high reproductive capability, being a protandric hermaphrodite species with reciprocal copulation (Tomiyama 1993), the ability to stock sperm for long periods (Raut & Barker 2002), and a high annual eggs postures (5 to 6) and eggs per posture (Tomiyama & Miyashita 1992). These aspects of African snail reproductive biology is an increment to the growth and population explosion process, affecting the environment (potential predator and competitor of native mollusks leading to extinction of species), economy (agricultural pests) and local health (a possible intermediate host for nematodes that cause eosinophilic, meningoencephalitis and abdominal angiostrongyliasis in humans and other zoonosis in domestic animals) (Cowie 1998, 2001, Fischer & Colley 2005, Thiengo et al. 2008, Caldeira et al. 2007, Graeff-Teixeira 2007, Neuhauss et al. 2007).

Studies in the state of Paraná conducted by Fisher & Colley (2005) led the supposition that *A. fulica* has a seasonal cycle of one or two generations per year with copulas occurring mainly in spring and autumn. The evidence of seasonality of *A. fulica* was also registered by several authors (Berry & Chan 1968, Lai et al. 1982, Raut & Barker 2002, Fisher & Colley 2005) which showed that there are high sexual activity points in favorable environmental conditions such as high humidity, moderate temperatures and abundant rainfall.

Studies show that the economic losses caused by the introduction of invasive species are estimated at US$ 42.6 billion per year, the environmental costs, US$ 6.7 billion per year and the costs with human health, despite more hard to estimate, are also huge (Pimentel et al. 2001). Although the trend described by Simberloff & Gibbons (2004) of population collapse after some time of invasion, researches about population dynamics and reproductive biology of *A. fulica* is extremely important to be able to understand their ecological relationships and the search for better handling and control strategy, reducing the problems caused by the species.

Therefore, this study intend to present data about population dynamics of *A. fulica* in Salvador, describing aspects of life cycle of the species such as growth rate, size, recruitment season, age (life time) and reproductive season, as well as characterize the reproductive biology, evaluating the existing relationship between the thickness of peristome and stage of sexual maturity, and among weather effects, such as rainfall, temperature and humidity with sexual activity periods.

Considering the explained data, our hypothesis of interest is that exists a relation between sexual activity and weather variables studied (temperature, rainfall and humidity), being waited a largest sexual activity in humid periods in Salvador - Bahia.

Material and methods

1. *Study site*

The studied site is located at Salvador metropolitan area, which is situated at 12°57'13''S and 38°27'24''W (between the Tropic of Capricorn and the Ecuador line); its climate is hot and humid - sunny, with an average temperature of 25.5°C with low variation during the year. The annual rainfall rate of the city is around 2000 mm, the average humidity is 81% (maximum 83% in May and minimum 79% in February). It has historically been a wet season which is usually April to September and a dry season from October to March. (INMET 2008). These characteristics make Salvador propitious to the establishment and expansion of invasive gastropod *Achatina fulica*.

2. *Sampling & experimental design*

According to previous studies (Silva 2005), there are 15 districts infested by *A. fulica* in Salvador (Amaralina, Barra, Barris, Caminho das Árvores, Costa Azul, Itaigara, Itapuã, Jardim Encantamento, Ondina, Piatã, Pituba, Praia do Flamengo, Rio Vermelho, Stella Maris and STIEP). From this data, three districts were randomly chosen for each monthly

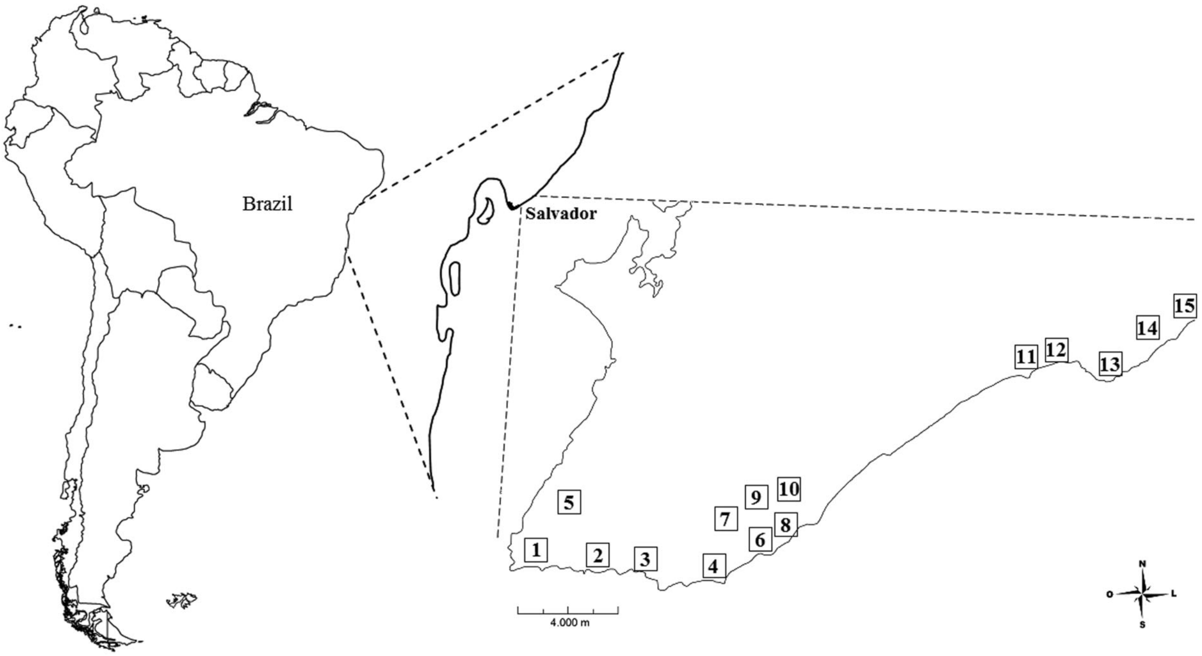


Figure 1. Map of localization of the city of Salvador, Bahia, showing the sites of the sampling areas visited during the study period. Areas (visits). 1. Barra (2); 2. Ondina (1); 3. Rio Vermelho (3); 4. Amaralina (1); 5. Barris (3); 6. Pituba (3); 7. Itaigara (3); 8. Costa Azul (2); 9. Caminho das Árvores (2); 10. Stiep (2); 11. Piatã (3); 12. Itapuã (3); 13. Jardim Encantamento (3); 14. Stella Maris (2); 15. Pr. do Flamengo (3).

sampling campaign. In each district a 1 km^2 area was demarcated (Figure 1). Some districts were chosen more than once, for a maximum of three times, when it happened, the limited 1 km^2 areas were moved to new areas that could be visited, but remaining inside the chosen districts. The collects were designed to sample, representatively, the study area, avoiding the spatial variability as a confounding variable affecting the temporal variation that was searched in the present study. Maps with the delimited sample areas were used, as well as the route to be followed during the gathering, with the intent to assist the moving of collectors.

The gathering procedure consisted on hand capture of specimens at streets, squares, gardens and some other public places. All gathering were made by two collectors, early in the morning, activity time of snails end (Tomiyama 1993, Raut & Barker 2002, Albuquerque 2003), and lasted about one hour. Collectors wore surgical gloves to protect them against snails' and/or possible environmental pathogens where they were found.

Gathering were made monthly from September 2006 to August 2007, in the areas of sampling. All the specimens of *A. fulica* found in one hour were gathered, within each sampling area (a total of 884 in 12 months). All then were submitted to morphologic analysis in the field to population dynamic data. Of the total collected, 45 snails per campaign (540 in total) were taken, in plastic containers to Universidade Católica do Salvador's laboratory, to macro-anatomical analysis of reproductive system.

The monthly amounts of accumulated rainfall (mm), temperature average (°C) and humidity (%) were collected from the Center of Weather Forecasting and Climatic Studies – National Institute for Space Research (CPTEC/INPE), through the database available on the World Wide Web at the site: http://www.cptec.inpe.br/. The amounts corresponded to 30 days preceding gathering.

3. *Morphometric analysis*

The animals collected in September 2006 were measured with the assistance of a pachymeter (0.05 to 150 mm) and weighed with Titan's field digital weighing-machine with accuracy of 0.1 g. The measured dimensions were: large diameter (ld), opening height (oh), opening width (ow), spire height (sh), shell height (h), thickness of peristome (pe), number of whorls (Figure 2) and weight (p). After found the best measure that represents the growth of the animal, to enhance the sampling, only the shell height (h) and total weight (p) were measured in remaining collects.

4. *Laboratorial analysis*

To examine the anatomical characteristics of reproductive system, specimens of different sizes were sacrificed by heating

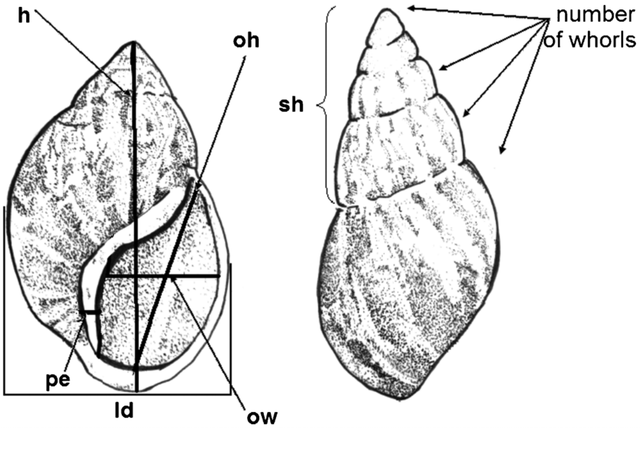


Figure 2. Shell scheme of *A. fulica* Bowdich, 1822 indicating the morphometrical measurements (ld, large diameter; h, shell height; oh, opening height; sh, spire height; ow, opening width; pe = thickness of peristome). Illustration from Fischer (2005).

(100°C for a minute) and dissected under stereo-microscope. The structures of reproductive system were observed following the illustrations and descriptions by Tomiyama (1993, 2002), Caetano (2005), Fisher & Colley (2005) and Teixeira et al. (2008), to *A. fulica*.

The weight of protein gland was obtained using a digital analytical weight-machine with accuracy of 10mg (0.01g) to verify if the snail was sexually active or not.

The number of eggs inside the uterus of the snails was also quantified.

5. *Statistical analysis*

Initially it was applied a Principal Components Analysis (PCA) using MVSP (Multi-Variate Statistical Package) version 3.13l for Windows, with the purpose of extract the first two axis of variation (PC1 e PC2). Subsequently PC1 x PC2 were plotted to observe the clustering.

Because of small variation in temperature, humidity and, consequently, small influence on sexual activity, also correlated to the species biology, just the rainfall was used in the regression with the variable of interest (sexual activity). The regression was done using SPSS (Statistical Package for the Social Sciences) 13.0 for Windows, being 0.05 the deemed value to α. The variable of interest (sexual activity) represented the proportion between number of sexually active individuals (protein gland > 650 mg) and the sexually inactive (gland < 650 mg).

To establish the morphometric measurement that best represents the growth of the snail, it was made Pearson correlations among morphometric variables (h, dm, ha, la, he, number of turns) and the weight of each individual using SPSS 13.0 for Windows (Statistical Package for the Social Sciences). From this determination was possible to create a linear equation and define, more accurately, the frequency distribution of the population of *A. fulica*. It was stipulated that the value of α would be 0.05, however, this value was fixed by Bonferroni correction (α/n, where $\alpha = 0.05$ and n is the number of hypothesis testing). This procedure was adopted because they were conducted several hypothesis testing for the same data set. 0.008 is the deemed value of α in this present study.

The growth curve was obtained using the von Bertalanffy model, which is:

$$Lt = L\infty\ [1-\exp(-k(t-t_0)]$$

where:

Lt = length at age t;
L∞ = maximum asymptotic length;
k = constant of growth;
t_0 = "age" in zero length (Lt = 0)

The growth parameters, as the growth curve were obtained using Microsoft Excel 2002.

The value of the constant of growth (k) was estimated from three pairs of known literature values of length and age or found in this study. Initially it was estimated the asymptotic length (L∞) from the biggest taken specimen (Lmax), being: $L\infty = Lmax/0.95$ (Pauly 1983). The average longevity to *A. fulica* found in literature is three to five years, being able to live until nine years in favorable conditions (laboratory for instance) (Tomiyama 1993; Raut & Barker 2002). Thus, with the value of asymptotic length (L∞) found, the snail would have, nearly, six years.

Another characteristic found in literature is the period since fecundation until the birth (egg hatching), that varies around 13 days (0.036 years) (Raut & Barker 2002, Rao & Singh 2000). As it is improbable that a species grows following this equation since the moment of birth until it reaches the senility, the curve frequently cuts the x-axis, that refers to the age, on a point less than zero (King 1996). Knowing that with length zero (Lt = 0 mm) the snail has -0.036 years (-13 days) and that at birth (t = 0 years) presents nearly 3 mm shell length (E.C. Silva, unpublished data) it was able to estimate the steady growth (k). From that, it was just replace the value of t to get the Lt.

The longevity to the present study, established as how long the specimen takes to reach 95% of asymptotic length, was estimated based on formula proposed by Taylor (1958): $tmax = to + 2.996/k$.

Results

1. *Aspects of population dynamics of* Achatina fulica

The shell of *A. fulica* is reddish brown with strips of variable colors, from slightly brown until slightly purplish. The number of turns ranges between 5 and 8 and increase in diameter quick and progressively. The general form is bulimuloid (h/dm=1.58), with an elongated spire (he/h=0.41) and opening oval slant (ha/la=1.63; ha/dm=0.87; la/dm=0.54).

A total of 884 snails were collected in 12 sample campaign. The average of all gathering period was 50.17 mm height and 17.20 g weight. The remaining variables measured are described in tables 1 and 2.

All values of correlation among morphometric measurements and total weight found were positive and significative. The shell height was the best descriptor of size, presenting higher values of correlation with weight. (Table 3).

Table 1. Descriptive statistics of morphometric variable of 60 snails *Achatina fulica* Bowdich, 1822 collected in the city of Salvador, Bahia in September 2006. h = Shell height (mm); ld = large diameter (mm); oh = opening height (mm); ow = opening width (mm); sh = spire height (mm); Perist. = thickness of peristome (mm).

	Weight (g)	H	Ld	ow	ow	Sh	Turns	Perist.
Average	14.96	47.70	30.27	26.37	16.21	19.68	6.25	1.01
Median	14.60	50.50	32.45	27.75	16.90	20.45	6.00	0.95
Modal	23.0	54.6	30.7	33.6	20.8	25.7	6.0	1.9
Standard dev.	9.748	14.403	7.666	7.052	4.245	6.772	0.875	0.637
Standard err.	1.258	1.859	0.989	0.910	0.548	0.874	0.113	0.095
Variance	95.037	207.449	58.772	49.737	18.021	45.863	0.766	0.406
Minimum	2.1	23.6	17.4	14	9.4	9.4	5	0.2
Maximum	38.5	76.2	43.9	41.1	25.4	35	8	2.4

Table 2. Descriptive statistic of weight and shell height of 884 snails *Achatina fulica* Bowdich, 1822 collected in 12 samples from Salvador, Bahia, from September 2006 to August 2007.

	Weight (g)	Height (mm)
Average	17.20	50.17
Median	15.6	50.7
Modal	11.2	56.8
Standard dev.	11.750	12.760
Standard err.	0.395	0.429
Variance	138.078	162.833
Minimum	1.9	23.6
Maximum	104.2	107.6

Set shell height (h) as morphometric variable which better represent the growth of *A. fulica*, histograms of height rate were made monthly (Figure 3).

By analyzing the Figure 3 we can verify that there is, in almost every month, two modals featuring different cohorts, these two cohorts are more evident in August 2006. From March 2007, what is seen is a gradual average increase of shell height and, consequently, a shifting of the modals to right, featuring the aging of the population that is soon balanced by the rising of younger cohorts.

From August to December 2006 and February 2007 it was registered a higher number of young individuals in the population, unlike what occurred from March to July 2007 which there is a predominance of older individuals. The month of January 2007 had its frequency histogram different from other months due to the scarce amount of animals collected.

The growth study of *A. fulica* resulted in values of Lmax = 107.6 mm; L∞ = 113.3 mm; k = 0.75 and to = -0.036mm.

After replacement of these values, the expression was in the following way:

$$Lt = 113.3 \text{ x } [1\text{-}\exp(-0.75(t-(-0.036)].$$

The figure 4 shows the growth curve following the von Bertalanffy formula, with emphasis on the largest animal collected which measured 107.6 mm of shell height and was

Table 3. Correlations among weight and other morphometric variables for *A. fulica* Bowdich, 1822 from the city of Salvador, Bahia, in September 2006. ld = large diameter; oh = opening height; ow = opening width; sh = spire height. *n* = 60.

		Weight	Height	DM	ha	La	he	Turns
Weight	**Correlation of Pearson**	1	**.978(**)**	.962(**)	.960(**)	.933(**)	.953(**)	.767(**)
	Sig.(1-tailed)		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

**Correlation significant < 0,01 (1-tailed).

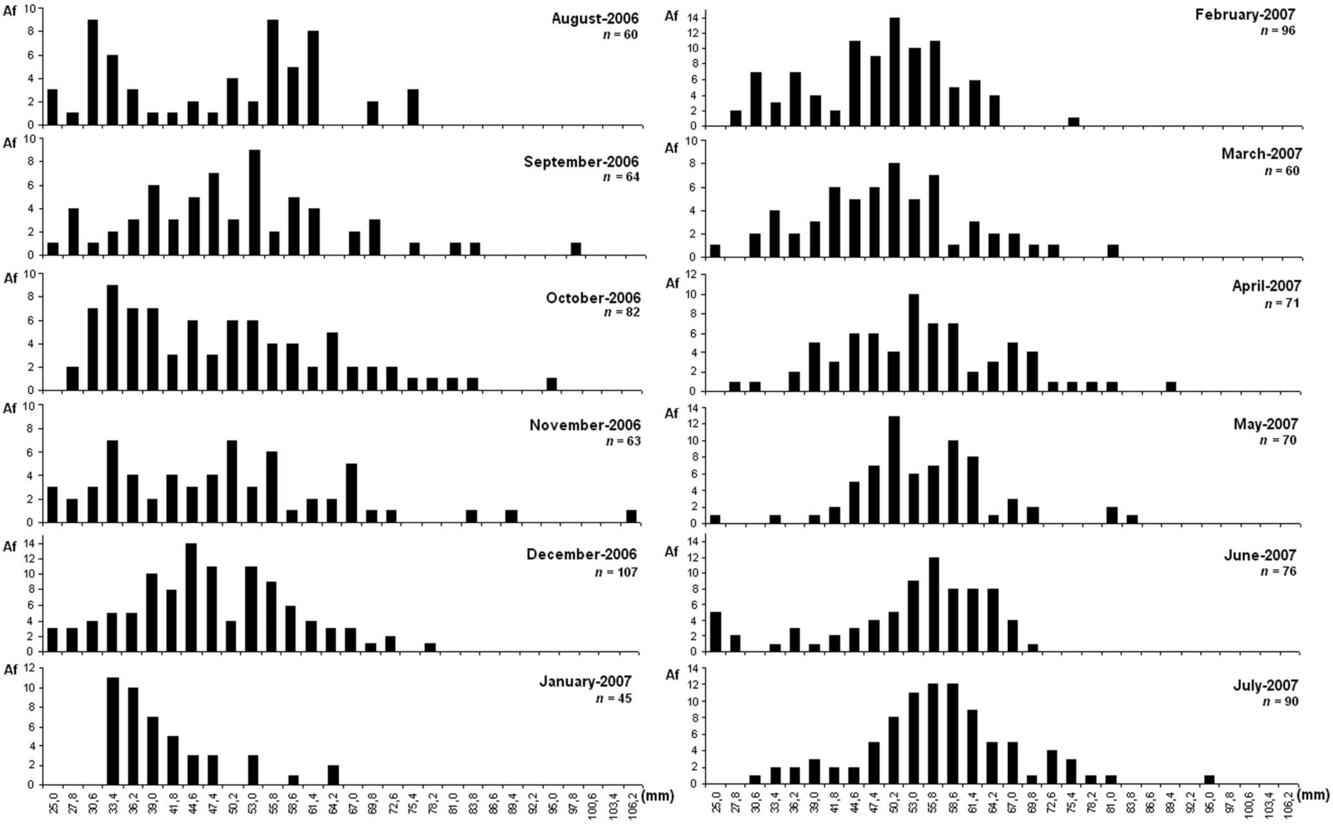


Figure 3. Monthly histograms of frequency of shell height of *A. fulica* Bowdich, 1822 from Salvador, Bahia in 12 sampling campaign from September 2006 to August 2007. Af = Absolute frequency.

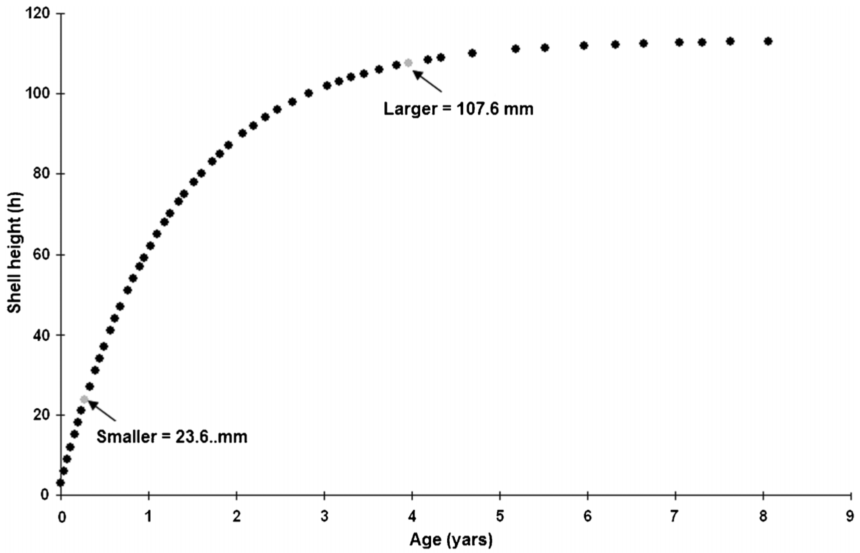


Figure 4. Growth curve to *A. fulica* Bowdich, 1822 from Salvador city, Bahia, obtained through the mathematic expression by von Bertalanffy - $L_t = L_\infty [1-\exp(-k(t-t_0)]$ where: $L_\infty = 113.3$ mm; $k = 0.75$ and $t_0 = -0.036$). Highlights for the highest and lowest snail collected.

nearly 3 years and 11 months old, and the smallest with 23 mm and 3 months old.

The longevity was estimated in 3 years and 11 months old; in this age the snail would reach 107.6 mm of shell height, the same value of the largest animal collected.

2. *Climatic factors and sexual activity*

The average values of temperature and rainfall to the study period were close to historical values, however, humidity presented, nearly, 14% higher than the historical average, that did not affect the results, since that continues to be able to visualize a division between a humid or rainy season (April to September) and a less humid or dry season (October to March). Also is noteworthy the low average of temperature and humidity, as for historical values as for the study (Figures 5 and 6).

We collected 540 snails at 12 gathering campaign, 100 out of them were sexually active, 318 inactive and 122 could not be determined because did not present protein gland.

The results of this study show that there is a greater relation between rainfall and the sexual activity of *A. fulica* (Figure 6), once that temperature and humidity had low variation and low influence.

In months of higher rainfall, there were a higher percentage of animals sexually active, for example, in May and June 2007, with 180 and 202 mm, and 41% and 43% sexually active snails, respectively. In months with lower rainfall, January and February, with 19 and 37 mm, were found only 5% and 9% sexually active snails, respectively. We can still emphasize the period of March to July 2007, which had accumulated rainfall 916 mm - 56.5% of rainfall on full study period - and average per month 31% of sexually active animal while in the rest of the year the average of sexually active animals was 18%.

December 2006 and April 2007 were exceptions. During these months the precipitation was high, and sexual activity was low.

The Principal Components Analysis drew the two first axis of variation (PC1 and PC2) data. PC1 represented 59.7% of variation and PC2, 32.5% (92.2% total). Among the variables,

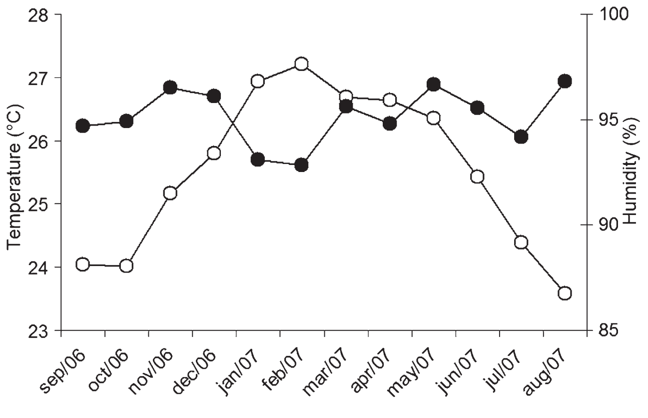


Figure 5. Temperature monthly average (○) and humidity (●) for Salvador, Bahia (September 2006 to August 2007). Source: CPTEC/INPE, 2008.

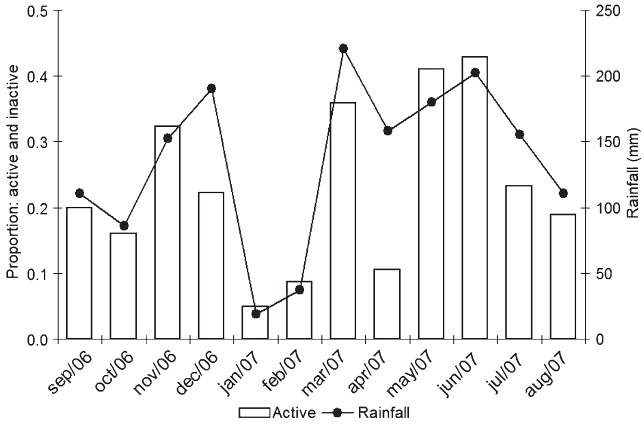


Figure 6. Proportion among active snails *A. fulica* Bowdich, 1822 and the relation with rainfall from September 2006 to August 2007 for Salvador, Bahia.

Table 4. Representativeness of each variable in each axis, extracted by Principal Component Analysis (PCA) to datasets of *Achatina fulica* Bowdich, 1822 from Salvador city, Bahia, collected from September 2006 to August 2007.

	PC1	PC2
Temperature	0.348	**0.883**
Humidity	**-0.701**	0.023
Rainfall	**-0.623**	0.468

humidity and rainfall were more inversely related to PC1 (as the PC1 grows humidity and rainfall decrease) and temperature, to PC2. The rainfall was also related to PC2, almost in the same intensity as to PC1 (Table 4).

When plotting PC1 x PC2 (Figure 7) the spatial distribution of points shows the generation of three clusters.

The first group, formed by the January and February contained the lowest values of sexual activity and was related to the lowest values of humidity and rainfall, in addition to high temperatures, characteristically of dry season. Inversely, November, December 2006 and March, May and June 2007 presented the highest values of sexual activity, related to higher values of humidity and rainfall and mild temperatures (humid season).

The group formed by September, October 2006 and July 2007 (transition between seasons), showed intermediate values of humidity, rainfall and temperature, related to average values of sexual activity.

Only April 2007, which had the highest temperature if compared to rainfall and humidity values showed, and August 2007, with higher humidity, that came out a little of what was expected to average values to sexual activity.

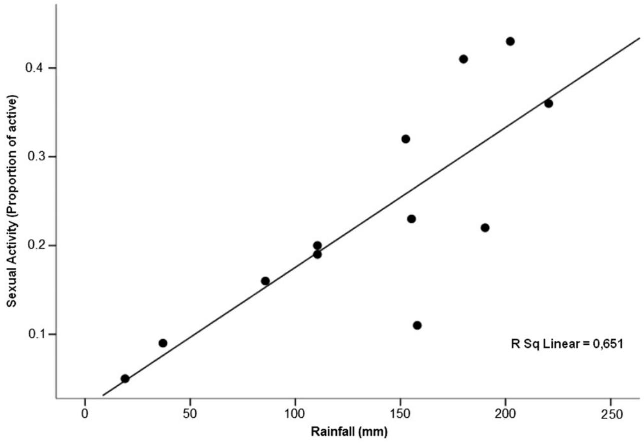


Figure 8. Linear regression between rainfall and the sexual activity (proportion of active) of *Achatina fulica* Bowdich, 1822 form Salvador city, Bahia, from September 2006 to August 2007. F = 18,617; *P* = 0,002; R^2 = 0,651

The regression between rainfall and sexual activity was significant (*P* = 0.002; *F* = 18.617) (Figure 8). So, the higher rainfall the higher the sexual activity is, that is, higher the number of specimen sexually active.

3. *Relationship between peristome and sexual maturity*

A total of 540 snails were collected, being 54% "Old-Adults", 34% "Intermediate" and only 12% "Young-Adults". Observing the monthly rate among "Old-Adults", "Intermediate" and "Young-Adults", shown on figure 9, it is possible to note increase of "Old-Adults" on rainy months.

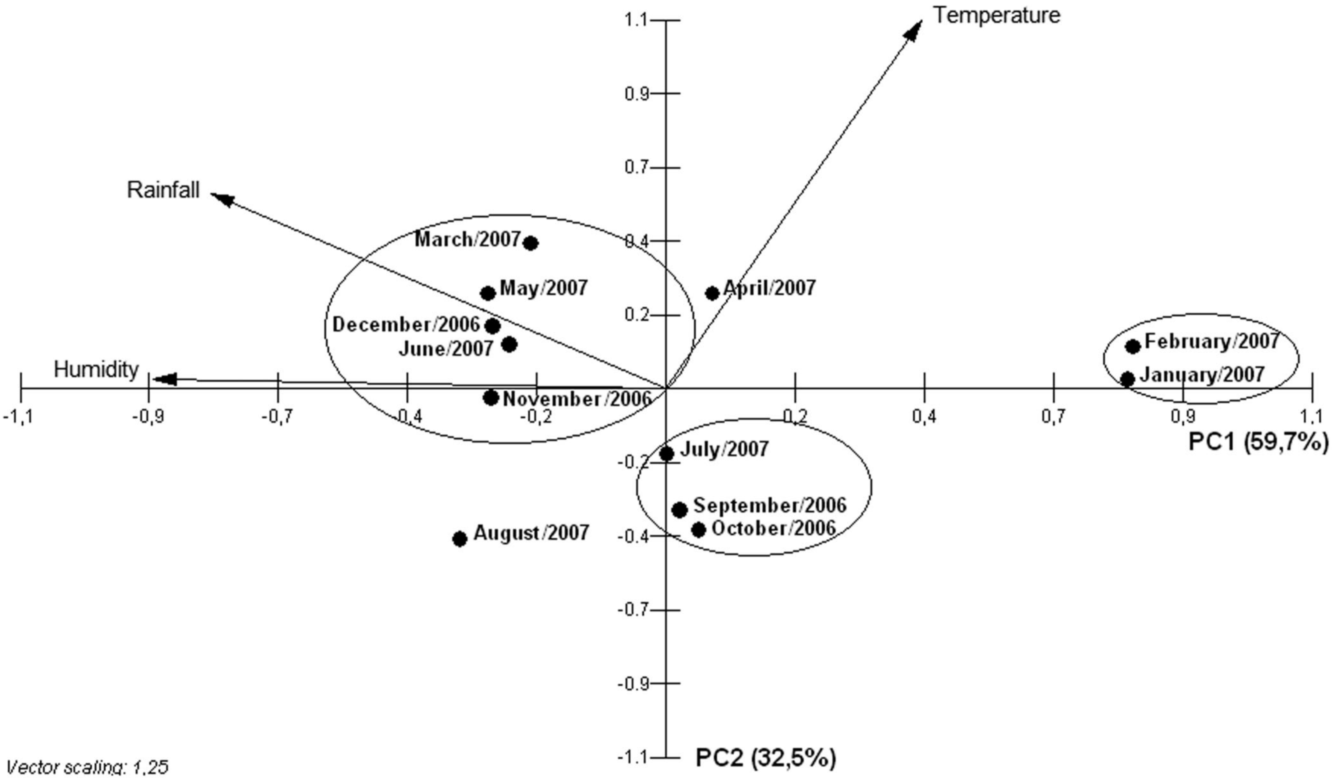


Figure 7. Plotting of the two first axis of variation (PC1 x PC2) of Principal Component Analysis to datasets of *Achatina fulica* Bowdich, 1822 from Salvador city, Bahia, collected from September 2006 to August 2007, shows the generation of three clusters showing three groups.

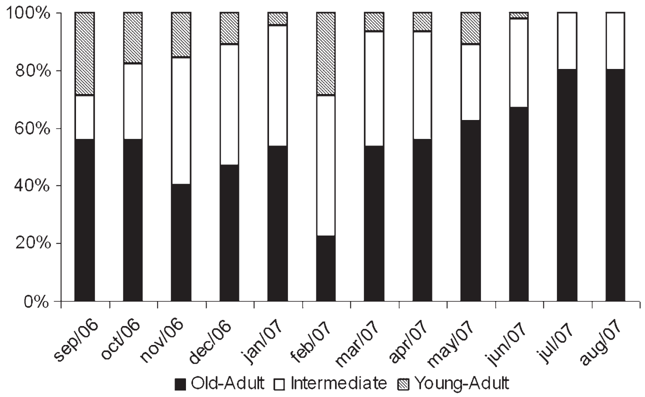


Figure 9. Monthly proportion of "Old-Adults", "Intermediates" and "Young-Adults" of *A. fulica* Bowdich, 1822 in Salvador, Bahia (n = 45 monthly) from September 2006 to August 2007.

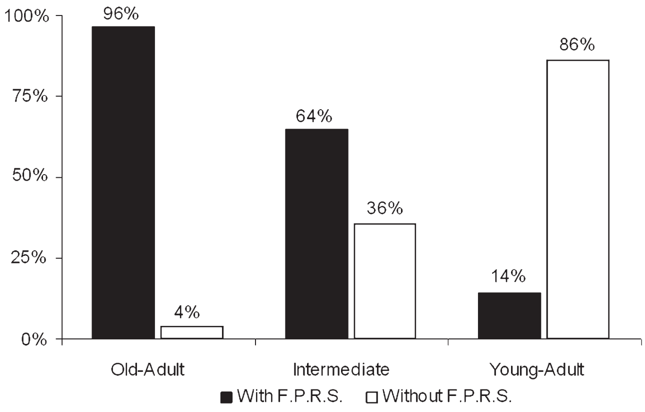


Figure 10. Proportion of *A. fulica* Bowdich, 1822 with and without F.P.R.S. (Female Portion of Reproductive System) per stages of sexual maturity in Salvador city, Bahia.

The thickness of peristome ranged from 0.1 mm to 2.95 mm, with average 0.93 mm, although the most frequent thickness was 0.85 mm.

Almost all snails belonging to stage "Old-Adult" sexual development showed portions of female and male reproductive systems (96%). Among "Intermediates", 65% presented both portions of reproductive systems and, among "Young-Adults" 86% had only the male portion (Figure 10).

Snails with eggs were observed in November 2006, January, March, May, June and July 2007, being found more than one snail carrying eggs only in March 2007. In seven *A. fulica* were recorded 560 eggs, that is, in average 80 eggs per snail. From these, five were "Old-Adults" with an average of 78 eggs per snail and two "Intermediates" with an average of 86 eggs per snail. "Young-Adults", despite the developed female portion of reproductive system, showed no eggs.

Discussion

1. *Aspects of population dynamics of* Achatina fulica

As observed in Paraná by Fischer & Colley (2005), the *A. fulica* population in Salvador is composed of animals of medium to large size and in full sexual activity. Comparing to the data obtained by Caetano (2005) and Ohbayashi & Takeuchi (2007), the weight and height average shell were lower than those obtained by this, as compared to Albuquerque (2003) the average obtained were higher. The specimen large and strong presence, probably with sexual development complete reflects the process of invasion of the species in Salvador, with the occupation of urban ecosystems, not only causing serious ecological and economical problems, but also possible damages to human and domestic animals health (Teles et al. 1997, Vasconcellos & Pile 2001, Raut & Barker 2002, Bender et al. 2003, Silva et al. 2003, Thiengo et al. 2006, Thiengo et al. 2007, Thiengo et al. 2008).

Despite of having young individuals (recruits) throughout the year, it was evident that recruitment was more frequent from August to December 2006 and February 2007 which characterize the end of wet season and the beginning of the dry season. This result corroborates the information obtained on items related to species reproduction. The period of greater sexual activity, measured by the weight of the protein gland, and the largest proportion of "Old-Adults" (present both parts of reproductive system complete) occurs, mostly, during the wet season. So the animals breed in the rainy season, investing their energy in production of gametes and eggs, and the recruitment occurs during the dry season.

In most animals, the body size is closed related to age, but this increase in size is not constant throughout life and usually describes an exponential curve with a rapid growth in the beginning (young animal) that is decelerated as the animal becomes older. As observed in the results, the growth curve of *A. fulica* follows this pattern, with a rapid growth until two years old (≅ 90 mm) becoming slower as the animal becomes older.

Gomes et al. (2004), in studies with land pulmonates gastropods in Rio Grande do Sul, came to the conclusion that the cycle of life of population of *Simpulopsis ovata* (Sowerby, 1822) is annual and the species is semelparous (the species has only one reproductive event, that is, adult individuals die after the reproductive period), also, affirm that annual cycles have been commonly found among land pulmonate gastropods as *Helicella (Xerothracia) pappi* (Schütt, 1962), *Salinator takii* Kuroda, 1928) (Lazaridou-Dimitriadou 1995, Kosuge 2000). Unlike these land pulmonates with short life cycle, *A. fulica* can live, in the wild life, more than four years and reproduce from 15 to 25 times in its life, this fact, combined to series of other makes the species an excellent invader. Another species with long life cycle and because of time of life and fast reproduction may becomes invasive is *Helix aspersa* (Müller, 1774) (Madec et al 2000).

The fact that snails have been found with the same age and close to the value obtained to longevity and living almost four years, suggests how well adapted they are to the environmental conditions in Salvador. Being so, the eradication of the species would be very difficult, being most suitable the population control, since the eradication was only achieved in incipient populations of *A. fulica* in California (EUA), Florida (EUA), Queensland (Australia), Fiji, Samoa and Vanuatu (Raut & Barker 2002, Thiengo et al 2007).

2. *Climatic factors and sexual activity*

Sexual activity at *A. fulica* could be evaluated through albumen gland weight. The albumen gland, also called protein gland, is responsible for producing and storaging of nutrient substances that will "provide" the eggs (Nieland & Goudsmit 1969). Runham & Laryea (1968) showed this gland fluctuates substantially in size during the different phases of reproductive cycle, becoming larger before the egg posture and withering immediately after the egg posture that, according to Tompa (1984) may be the original size of this gland once all fluid contained within it was moved to the eggs. The study of Tomiyama (1993) showed that the maximum size of protein gland in "Young-Adults" (not able to produce eggs) was 650 mg, so, glands heaviest than this would characterize sexual activity at that moment.

By taking in mind the low temperature range (3.6°C, Min. 23.6°C and Max. 27.2°C) and humidity (4.0%, Min. 92.8% e Max. 96.8%), it is believed that these variables had little influence on the activities of the snail, once this is a species resistant to environmental variations, probably because they evolved in forests edge (Raut & Ghose 1981). Ruat & Ghose (1984) confirm this sentence showing that the activities of the African snails are only affected by a long time under 10°C or over 30°C and humidity under 80%, when they stow. These limit values of temperature and humidity did not occur during the study period and rarely occur.

The evidence of seasonality of *A. fulica* was recorded by Lai et al (1982) which report the spread of the species, that is, the occupation of new areas by new individuals added to population occurs throughout the year, but is particularly evident during or after winter. Raut & Barker (2002) also consider the seasonality, with cycles related to favorable periods and there may be two pronounced peaks in each season, being the first after the resumption of activity and completion of stowing phase and the second, 2 to 3 months later. In Malaysia, Berry & Chan (1968) also consider the existence of an apparent annual cycle of *A. fulica*, but related to the dry and rainy seasons.

The results of the study showed that there is a clear relationship among the environmental conditions, particularly the rainfall range, and sexual activity of *A. fulica* in Salvador. In addition, we can also say that for the studied period, the sexual activity was more manifested in the rainy period and in March, when it rained more than expected, confirming the hypothesis of interest. Albuquerque (2003) reached similar results; observing the sexual behavior of the snail in Lauro de Freitas - Bahia found that the copulations occurred more frequently from April to August and in rainy days.

Apart from environmental conditions, sexually active individuals were found in all sampled months, which suggest that *A. fulica* is able to reproduce throughout the year.

3. *Relationship between peristome and sexual maturity*

Studies made by Tomiyama & Miyashita (1992) and Tomiyama (1993, 2002) describe that the thickness of peristome has a close relation with sexual maturity, appearing in the beginning of maturity and developing as the specimen matures, being so, able to feature three stages: "Young-Adult" (peristome < 0.5 mm): section of male reproductive system developing or complete, no female section detected; "Intermediate" (0.5 to 0.8 mm): section of male reproductive system fully developed, may or not presents female section; e "Old-Adult" (peristome > 0.8 mm) presents both parts of reproductive system well developed (hermaphrodites).

Different from presented by Tomiyama (1993), there were found some "Old-Adults" without the female portion of reproductive system. This fact combined to the presence of snails presenting hermaphroditic reproductive system complete with peristome of only 0.35 mm (Young-Adult), being the trend suggested by Tomiyama (1993; 2002) that the species completes its sexual development after the thickness of peristome exceed the 0.5 mm ("Intermediate"), suggests an early sexual maturity of the population of *A. fulica* in Salvador. This precocious maturation of the population may have occurred due to abundance of resources (food, shelter) and/or favorable climatic conditions found in the city. Fisher & Colley (2005) also found snails with reproductive system complete (performing posture, inclusive) with peristome smaller than 0.5 mm (0.4 mm).

The presence of nearly 75% of the analyzed population with sexual development complete, that is, "Old-Adults" and some "Intermediates" and characterized by large and vigorous specimen are indications of the first phase of population establishment suggested by Civeyrel & Simberloff (1996) where there is an exponential increase of population of the species. Despite not having been observed in this study, the increase of the population of *A. fulica* has been reported for some time in Salvador.

The increase in the number of "Old-Adults" on rainy months, found in this study, also was noticed by Albuquerque (2003) at Lauro de Freitas. This increase is related to a larger observation of sexual activity in the rainy period, once that these animals ("Old-Adults") are larger and present sexual development complete, making them more capable to reproduction.

Tomiyama & Miyashita (1992) found in their studies that "Old-Adults" present a higher number of eggs per posture when compared with "Intermediates". These data are conflicting with those found in this present study becouse the values obtained to "Old-Adults" (77.6 eggs per individuals) are lower than the values obtained to "Intermediates" (86.0 eggs per individuals). The answer to this observation may be in the insufficient number of animals with eggs collected (seven).

Despite not having been found "Young-Adults" producing eggs - a result also found by Tomiyama & Miyashita (1992) - the absence of the female portion of reproductive system as a justification for the fact cannot be used in this case. The most plausible is to consider that these animals, despite of having the female portion of reproductive system, it is not completely developed, not being able to produce eggs. We cannot rule out the possibility that these animals were not sexually active only when collected.

By not obtain a significant number of snails with eggs to perform posture, we cannot make any kind of inference related to period when it is more frequent or the amount of eggs that each individual can stock, requiring further studies to elucidate these points. Specimen with eggs were found as in the top of dry season (November to January) as in the rainy season (March, May, June and July), possibly, because of the low annual climate variation in Salvador, which presents a humid tropical climate, conducive to the development of *A. fulica*. In addition, Raut & Barker (2002) affirm that the storage capacity of sperm provides to the species of Achatinidae ability to produce eggs in any time of year.

4. *Conclusions*

The favorable climate found in Salvador provides to the African snail optimal conditions for survival and development, reproducing earlier. In addition, the low variation of temperature and humidity requires very few of the ability to resist great environmental variations that the species has, being thus, able to reproduce throughout the year, increasing its activity as rainfall increases.

The relationship between thickness of peristome and the stage of sexual maturity suggested by Tomiyama & Miyashita (1992) and Tomiyama (1993, 2002) is real, but should be adjusted to each specific region. Although there is a relationship between the thickness of peristome and sexual maturity, the limits among stages are not well determined and, depending on where the study is performed, the deemed values of thickness of peristome per each sexual stage may vary. In the case of Salvador ''Young-Adults'' would have peristome smaller than 0.35 mm, ''Intermediate'', from 0.35 mm to 0.90 mm and ''Old-Adults'', larger than 0.90 mm.

As the eradication becomes impossible because of high levels of invasion found in Salvador, it is recommended to control the species. This control, in spite of occur continuously, should be intensified in the rainy periods, since these are the periods when the animals are seen more frequently, just because they are sexually active, looking for sexual partners or for reproduction areas. It is suggested the implement of management and control of *A. fulica* created by IBAMA in 2004 and is already proving successful in another cities where the snail is a pest (Brasil 2007).

Studies about the population biology of the species are essential to the implementation of control programs and eradicate invasive species. The knowledge about population dynamic of *A. fulica* in Salvador can provide further actions to minimize the impacts caused by this species.

Acknowledgments

The Post-Graduation Program in Ecology and Biomonitoring of Universidade Federal da Bahia for the host during the time of development of the study and for the teachings given by all professors; to Universidade Católica do Salvador which gave the physical space of laboratories to perform the analysis and, specially, to all those who helped me during the gatherings.

References

ALBUQUERQUE, F.S. 2003. Ocorrência, aspectos da dinâmica populacional e comportamental de *Achatina fulica* Bowdich, 1822 (Gastropoda: Pulmonata) em Lauro de Freitas. Dissertação de Mestrado, Programa de Pós-Graduação em Ecologia e Biomonitoramento, Universidade Federal da Bahia, Bahia.

ALOWE, S., BROWNE, M. & BOUDJELAS, S. 2004. 100 of the world's worst invasive alien species. A selection from the global invasive species database. www.issg.org/database. (último acesso em: 04/05/2008).

BENDER, A.L., MAURER, R.L., SILVA, M.C.F., BEM, R., TERRACIANO, P.B., SILVA, A.C.A., & GRAEFF-TEIXEIRA, C. 2003. Ovos e órgãos reprodutores de fêmeas de *Angiostrongylus costaricensis* são reconhecidos mais intensamente por soros humanos de fase aguda na angiostrongilíase abdominal. Rev. Soc. Bras. Med. Trop., 36(4): 449–454, http://dx.doi.org/10.1590/S0037-86822003000400003

BERRY, A.J & CHAN, L.C. 1968. Reproductive condition and tentacle extirpation in Malayan *Achatina fulica* (Pulmonata). Aust J Zool, 16: 849-855.

BRASIL. Ministério da Saúde. Sec. de Vigilância em Saúde. Dep. de Vigilância Epidemiolůgica. 2007. Vigilância e controle de moluscos de importância epidemiológica: diretrizes técnicas: Programa de Vigilância e Controle da Esquistossomose (PCE). 2 ed. Editora do Ministério da Saúde, Brasília.

CAETANO, F.A.M. 2005. Estudo comparativo do aparelho reprodutor do molusco *Achatina fulica* criado em cativeiro e asselvajado. Dissertação de Mestrado. Faculdade de Medicina Veterinária e Zootecnia. Universidade de São Paulo, São Paulo.

CALDEIRA, R.L., MENDONÇA, C.L.G.F., GOVEIA, C.O., LENZI, H.L., GRAEFF-TEIXEIRA, C., LIMA, W.S., MOTA, E.M, PECORA, I.L, MEDEIROS, A.M.Z, CARVALHO, O.S. 2007. First record of molluscs naturally infected with *Angiostrongylus cantonensis* (Chen, 1935) (Nematoda: Metastrongylidae) in Brazil. Mem. Inst. Oswaldo Cruz, 102(7): 887–889, http://dx.doi.org/10.1590/S0074-02762007000700018

CARLTON, J.T. 1996. Biological Invasion and Cryptogenic Species. Ecology, 77(6): 1653-1655.

CARVALHO, O.S., TELES, H.M.S., MOTA, E.M., MENDONÇA, C.L.G.F. & LENZI, H.L. 2003. Potentiality of *Achatina fulica* Bowdich, 1822 (Mollusca: Gastropoda) as intermediate host of the *Angiostrongylus costaricensis* Morera & Céspedes 1971. Rev. Soc. Bras. Med. Trop., 36: 743–745.

CPTEC/INPE. Centro de Previsão de Tempo e Estudos Climáticos - Instituto Nacional de Pesquisas Espaciais . PCDA - Plataforma de Coleta de Dado. http://www.cptec.inpe.br (último acesso em 04/04/2008).

CIVEYREL, L. & SIMBERLOFF, D. 1996. A tale of two snails: is the cure worse than the disease? Biodivers. Conserv., 5: 1231–1252, http://dx.doi.org/10.1007/BF00051574

COWIE, R.H. 1998. Patterns of introduction of non-indigenous non-marine snails and slugs in the Hawaiian Islands. Biodivers. Conserv., 7: 349-368, http://dx.doi.org/10.1023/A:1008881712635

COWIE, R.H. 2001. Can snail ever be effective and safe biocontrol agent? Int J Pest Manag., 47: 23–40, http://dx.doi.org/10.1080/09670870150215577

DAJOZ, R. 2005. Princípios de Ecologia, 7 ed. Editora Artmed. Porto Alegre.

FISCHER, M.L & COLLEY, E. 2004. Diagnóstico da ocorrência do caramujo gigante africano *Achatina fulica* Bowdich, 1822 na APA de Guaraqueçaba, Paraná, Brasil. Estud. Biol., 26(54): 43–50.

FISCHER, M.L & COLLEY, E. 2005. Espécie invasora em reservas naturais: caracterização da população de *Achatina fulica* Bowdich, 1822 (Mollusca - Achatinidae) na Ilha Rasa, Guaraqueçaba, Paraná. Brasil. Biota neotrop., 5 (1): http://www.biotaneotropica.org.br/v5n1/pt/download?article+BN03305012005+it. (último acesso em 22/06/2008)

FISCHER, M.L., SIMIÃO, M., COLLEY, E., ZENNI, R.D., SILVA, D.A.T. and LATOSKI, N.. 2006 O caramujo exótico invasor na vegetação nativa em Morretes, PR: diagnóstico da população de *Achatina fulica* Bowdich, 1822 em um fragmento de Floresta Ombrófila Densa aluvial. Biota Neotrop. 6(2): http://www.biotaneotropica.org.br/v6n2/pt/abstract?short-communication+bn03306022006. (último acesso em 22/06/2008)

GRAEFF-TEIXEIRA, C. 2007. Expansion of *Achatina fulica* in Brazil and potential increased risk for angiostrongyliasis. Trans. Roy. Soc. Trop. Med. Hyg., 101: 743–744, http://dx.doi.org/10.1016/j.trstmh.2007.03.012

GOMES, S.R., SILVA, R.S., GIL, G.M. & THOMÉ, J.W. 2004. Ciclo biológico de *Simpulopsis ovata* (Gastropoda, Bulimulidae) em São Francisco de Paula, Rio Grande do Sul, Brasil. Iheringia. Sér. Zool., 94(3):253–259, http://dx.doi.org/10.1590/S0073-47212004000300005

INMET. Instituto Nacional de Meteorologia. INMET - Clima. Disponível em: http://www.inmet.gov.br/html/clima.php. (último acesso em 04/04/2008)

KING, M. 1996. Fisheries biology, assessment and management. Ed. Blackwell Science, Oxford. UK.

KOSUGE, T. 2000. Seasonal aspects in the life history and ecology of the intertidal pulmonate *Salinator takii* Kuroda (Gastropoda: Amphibolidae). Venus: Jap. Jour. Malac., 59(1): 19–28.

KUMPRATAUNG, W., KRUATRACHUE, M, SUCHART, E.U., CHITRAMVONG, Y., SETARUGSA, P., CHAVADEJ, J. &

SOBHON, P. 1989. Comparative Studies on reproductive system of *Achatina fulica, Hemiplecta distincta* and *Cyclophorus aurantiacus*. J. Sci. Soc. Thailand, 15(2): 71–107, http://dx.doi.org/10.2306/scienceasia1513-1874.1989.15.071

LAI, P.Y., FUNASAKY, S.Y. & HIGA, S.Y. 1982. Introduction for biological control in Hawaii: 1979 and 1980. Proc. Hawaii. Entomol. Soc., 24: 109–113.

LAZARIDOU-DIMITRIADOU, M. 1995. The life cycle, demographic analysis, growth and secondary production of the snail *Helicella (Xerothracia) pappi* (Schütt, 1962) (Gastropoda Pulmonata) in E. Macedonia (Greece). Malacologia, 37(1): 1–11.

MADEC, L., DESBUQUOIS, C. & COUTELLEC-VRETO, M.A. 2000. Phenotypic plasticity in reproductive traits: importance in the life history of *Helix aspersa* (Mollusca: Helicidae) in a recently colonized habitat. Biol. J. Linn. Soc., 69: 25–39, http://dx.doi.org/10.1111/j.1095-8312.2000.tb01667.x

NEUHAUSS, E., FITARELLI, M., ROMANZINI, J. & GRAEFF-TEIXEIRA, C. 2007. Low susceptibility of *Achatina fulica* from Brazil to infection with *Angiostrongylus costaricensis* and *A. cantonensis*. Mem. Inst. Oswaldo Cruz, 102(1): 49–52, http://dx.doi.org/10.1590/S0074-02762007000100007

NIELAND, M & GOLDSMIT, E. 1969. Ultrastructure of galactogen in the albumen gland of *Helix pomatia*. J. Ultra. Res., 29: 119–140, http://dx.doi.org/10.1016/S0022-5320(69)80059-6

OHBAYASHI, T. & TAKEUCHI, K. 2007. Fluctuation of the distribution and population of the giant African snail, *Achatina fulica* Bowdich (Pulmonata Achatinidae) in Chichijima and Hahajima Islands, of the Ogasawara (Bonin) Islands, from the Years 1995 to 2001. Jpn. J. Appl. Entomol. Z., 51: 221–230, http://dx.doi.org/10.1303/jjaez.2007.221

PAULY, D. 1983. Some simple methods for the assessment of tropical fish stocks. FAO Fisheries Technical Paper, 234: 52p.

PIMENTEL, D., MCNAIR, S., JANECKA, J., WIGHTMAN, J., SIMMONDS, C., CO'CONNELL, C., WONG, E., RUSSEL, L., ZERN, J., AQUINO, T., & TSOMONDO, T. 2001. Economic and environmental threats of alien plant, animal, and microbe invasion. Agr. Ecosyst. Environ., 84: 1–20, http://dx.doi.org/10.1016/S0167-8809(00)00178-X

RAO, I.G. & SINGH, D.K. 2000. Effect of single and binary combinations of plant-derived molluscicides on reproduction and survival of the snail *Achatina fulica*. Arch. Environ. Con. Tox., 39: 486–493, http://dx.doi.org/10.1007/s002440010131

RAUT, S .K. & BARKER, G. 2002. *Achatina fulica* Bowdich and others Achatinidae pest in tropical agriculture. In Mollusks as Croup Pest (Barker, G. eds). CAB Publishing. New Zealand, Hamilton. p.55–114.

RAUT, S.K & GHOSE, K.C. 1981. Viability of sperms in aestivating *Achatina fulica* Bowdich and *Macrochlamys indica* Godwin-Austen. J. Mollus. Stud., 48: 87–94.

RAUT, S.K & GHOSE, K.C. 1984. Pestiferous Land Snails of India. Zoological Survey of India n° 11, Bani Press, Calcutta.

RUNHAM, N.W. & LARYEA, A.A. 1968. Studies on the maturation of the reproductive system of *Agriolimax reticulatus* (Pulmonata: Limacidae). Malacologia, 7: 93–108.

SILVA, E.C. 2005. Ocorrência de *Achatina fulica* (Bowdich, 1822) (Mollusca, Gastropoda), caramujo hospedeiro intermediário de *Angiostrongylus sp* (Nematoda: Metastrongyloidae), em Salvador - Bahia. Monografia de Graduação. Instituto de Ciências Biológicas. Universidade Católica do Salvador. Salvador, Bahia.

SILVA, A.C.A., GRAEFF-TEIXEIRA, C. & ZAHA, A. 2003. Diagnóstico da angiostrongilíase abdominal utilizando PCR em soro de pacientes. Rev. I. Med. Trop., 45(5) 295–297.

SIMBERLOFF, D. & GIBBONS, L. 2004. Now you see them, now you don't! - population crashes of established introduced species. Biol. Invasions, 6: 161–172, http://dx.doi.org/10.1023/B:BINV.0000022133.49752.46

SIMIÃO, MS. & FISCHER, M.L. 2004. Estimativa e inferências do método de controle do molusco exótico *Achatina fulica* Bowdich 1822 (Stylommatophora; Achatinidae) em Pontal do Paraná, litoral do Estado do Paraná. Cad. biodivers, 4: 74–82.

TAYLOR, C.C. 1958. Cod growth and temperature. J. Cons. int. Explor. Mer. 23(3): 366–370, http://dx.doi.org/10.1093/icesjms/23.3.366

TEIXEIRA, D.G., MARTINS, M.F., GUERRA, J.L, BLAZQUEZ, F.J.H. & SINHORINI, I.L. 2008. Descrição histológica da via genital masculina e hermafrodita de escargots das espécies *Achatina fulica* e *Achatina monochromatica*. Braz. J. vet. Res. anim. Sci., 45(6): 413–420.

TELES, H.M.S., VAZ, J.F., FONTES, L.R. & DOMINGOS, M.D. 1997. Registro de *Achatina fulica* Bowdich, 1822 (Mollusca, Gastropoda) no Brasil: Caramujo hospedeiro intermediário da angiostrongilíase. Rev. Saúde. Publ., 31: 310–312, http://dx.doi.org/10.1590/S0034-89101997000300014

TELES, H.M.S., FONTES, L.R., AMARAL, W. 2004. Pesquisa nacional de opinião pública sobre a espécie do caramujo *Achatina fulica*. Instituto Brasileiro de Helicicultura – IBH/CEDIC; 01-24.

THIENGO, S.C., BARBOSA, A.F., COELHO, P.M. & FERNANDEZ, M.A. 2006. Moluscos exóticos com importância médica no Brasil. Available in: http://www.mma.gov.br/invasoras/capa/docs/co/silvana_carvalho.pdf. (último acesso em 15/04/2008).

THIENGO, S.C., FARACO, F.A., SALGADO, N.C., COWIE, R.H. & FERNANDEZ, M.A. 2007. Rapid spread of an invasive snail in South America: the giant African snail, *Achatina fulica*, in Brazil. Biol. Invasions, 9: 693–702, http://dx.doi.org/10.1007/s10530-006-9069-6

THIENGO, S.C., FERNANDEZ, M..A., TORRES, E.J.L., COELHO, P.M. & LANFREDI, R.M. 2008. First record of a nematode Metastrongyloidea (*Aelurostrongylus abstrusus* larvae) in *Achatina (Lissachatina) fulica* (Mollusca, Achatinidae) in Brazil. J. Invertebr. Pathol., 98(1) 34–39, http://dx.doi.org/10.1016/j.jip.2007.10.010

TOMIYAMA, K. & MIYASHITA, K. 1992. Variation of egg clutches in the Giant African snail, *Achatina fulica* (Ferussac) (Stylomatophpra; Achatinidae). Venus: Jap. Jour. Malac., 51(4): 293–301.

TOMIYAMA, K. 1993. Growth and maturation pattern in the African giant snail, *Achatina fulica* Férussac) (Stylommatophora: Achatinidae). Venus: Jap. Jour. Malac., 52: 87-100.

TOMIYAMA, K. 2002. Age dependency of sexual role and reproductive ecology in a simultaneously hermaphroditic land snail, *Achatina fulica* (Stylommatophora: Achatinidae). Venus: Jap. Jour. Malac., 60: 273–283.

TOMPA, A.S. 1984. Land snails (Stylommatophora). In The Mollusca Reproduction, (Tompa, A.S., Verdonk, N.H. and van den Biggelaar, J.A.M. eds). Academic Press. London, v.7, p. 47–140.

TOWNSEND, C.R., BEGON, M. & HARPER, J.L. 2006. Fundamentos em Ecologia, 2 ed. Artmed, Porto Alegre.

USC - Union Concerned Scientists. 2001. The science of invasive species. http://www.ucsusa.org/global_environment/invasive_species/index.cfm. (último acesso em 02/05/2008)

VASCONCELLOS, M.C. & PILE, E. 2001. Ocorrência de *Achatina fulica* no Vale do Paraíba, Estado do Rio de Janeiro, Brasil. Rev. Saúde. Publ., 35(6): 582–584.

Received 07/01/2014
Revised 24/06/2014
Accepted 05/08/2014