

Activity and habitat use of two species of stingrays (Myliobatiformes: Potamotrygonidae) in the upper Paraná River basin, Southeastern Brazil

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The life history of freshwater stingrays (Potamotrygonidae) under natural conditions has been poorly documented. In this study, we investigated the period of activity and the habitat use of two species of the genus *Potamotrygon* in the upper Paraná River basin, Southeastern Brazil. *Potamotrygon falkneri* and *P. motoro* are similar to each other as far as the analyzed behavior is concerned. Individuals of both species segregate according to their size, and in function of the depth and period of the day. Younger individuals inhabit mostly sandy beaches and places that are no deeper than four meters throughout the whole day. Bigger stingrays realize bathymetric migrations, alternating their position between places deeper than eight meters during the day, and shallow areas at night. Individuals of intermediate size inhabit transition environments that have greater habitat diversity. Both species presented mostly nocturnal habits, especially regarding their feeding behavior. The behavioral patterns observed seem to go through ontogenetic variations and probably change throughout the year, between dry and wet seasons.

Informações sobre o modo de vida das raias de água doce (Potamotrygonidae) sob condições naturais são escassas. Neste trabalho, estudamos o período de atividade e as formas de uso do habitat de duas espécies do gênero *Potamotrygon* na bacia do alto rio Paraná, no Sudeste do Brasil. *Potamotrygon falkneri* e *P. motoro* apresentaram comportamento muito semelhante, com nítida segregação espacial dos indivíduos em função do seu tamanho, da profundidade e do período do dia. Juvenis estiveram associados a praias arenosas e locais com profundidades abaixo de quatro metros ao longo de todo o dia. Raias de grande porte realizaram migrações batimétricas, alternando sua posição entre locais com profundidade superior a oito metros durante o dia e áreas mais rasas à noite. Indivíduos com tamanho intermediário ocuparam ambientes de transição, com maior heterogeneidade ambiental. Ambas as espécies apresentaram atividade, especialmente alimentar, predominantemente noturna. Os padrões comportamentais estudados parecem sofrer variações ontogenéticas e é provável que se alterem ao longo do ano, entre períodos de seca e cheia.

Key words: Bathymetric migration, Dial movements, *Potamotrygon*, Scientific diving, Spatial ecology.

Introduction

Studies on patterns of movement, spatial distribution, activity and habitat use of fishes, associated with the description of the characteristics of the occupied areas, have been used as central topics of ecological research, including resources sharing, organization of communities, ecomorphology, and optimum foraging (Anderson *et al.*, 1989;

Rincón, 1999). Knowledge of fishes' way of life in nature and their preference for particular types of environments have also been used to identify and protect specific habitats and to support actions of management and conservation of species in different parts of the world (Steimle & Zetlin, 2000; Simpfendorfer & Heupel, 2004; Aguiar *et al.*, 2009).

In Brazil, research into the behavior of fish in the natural environment is recent but has been steadily growing from

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year to year, normally focusing on species of teleostean fish (Sazima, 1986; Casatti & Castro, 1998; Sabino, 1999; Teresa *et al.*, 2011). Information of this kind is scarce when dealing with elasmobranchs, particularly with stingrays of the family Potamotrygonidae (Castex, 1963; Achenbach & Achenbach, 1976; Rosa, 1985). Only in recent years, the surveys of Brazilian fish species, and particularly of freshwater stingrays, were intensified. In the last years, the potamotrygonids have rekindled the interest of researchers, enabling the generation of data on the biology, ecology, taxonomy, and toxicology of some species (see Garrone Neto & Haddad Jr., 2010; Rosa *et al.*, 2010; Carvalho & Lovejoy, 2011 and references therein).

However, the life history of the family Potamotrygonidae under natural conditions has been poorly studied (Garrone Neto & Sazima, 2009a, b), since the majority of existing data are based on indirect observations, with this methodology's inherent limitations. In this sense, we have aimed to present information on patterns of activity and forms of habitat use by two co-occurring species of potamotrygonids, thereby contributing to the better understanding of the way of life of these animals in their natural environment.

Material and Methods

Study area. The present study was carried out in the upper course of the Paraná River, Southeastern Brazil, between 2005 and 2008. The investigations were concentrated in the municipalities of Castilho, SP and Três Lagoas, MS (about 20°47'S 51°37'W), on the border of the states of São Paulo and Mato Grosso do Sul. Additional information was obtained from expeditions conducted in the municipality of Panorama, SP (21°22'S 51°54'W), in the region comprising the lower reaches of the Tietê (20°40'S 51°25'W) and Paranapanema (22°39'S 53°05'W) rivers, and in the Ilha Grande National Park (24°00'S 54°07'W). These expeditions comprised a stretch of approximately 350 kilometers which are under the influence of two hydroelectric dams: Engenheiro Sérgio Motta (Porto Primavera) and Engenheiro Souza Dias (Jupiá) (Fig. 1).

According to the Köppen-Geiger classification (Peel *et al.*, 2007), the region has a *Cfa* or humid sub-tropical climate, comprising two clearly defined seasons: one hot and rainy, which runs from the beginning of November through April, and the other dry and cooler, beginning in May and ending around October. In the dry period, when the underwater observations were concentrated, the water from the rivers and marginal lakes has excellent transparency, which may reach depths of 6 to 8 meters, mainly in October.

The region comprising the upper Paraná River has a long history of human occupation, with the deployment of large hydroelectric projects and few locations which have maintained the original environmental characteristics (*e. g.* rapids and waterfalls). The substrate and the composition of the banks range from muddy bottom with pasture present at the water's edge, to sandy substrate with the presence of

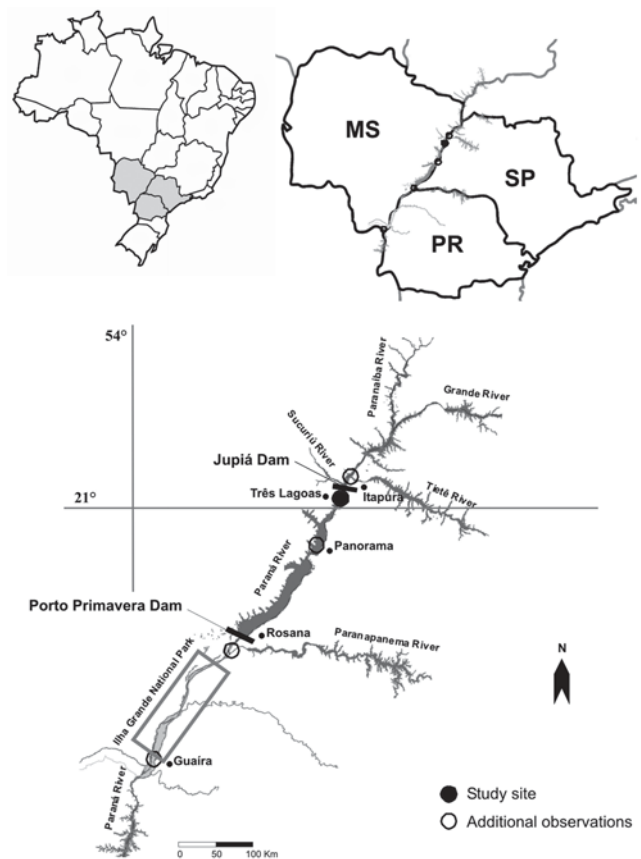


Fig. 1. Illustrative map of the upper Paraná River in the Southeastern Brazil, showing the main rivers in the basin, the study location, and the places where expeditions were conducted to obtain additional data.

rocks, aquatic macrophytes and riparian vegetation. In order to represent a large number of these features, different locations for observational studies were explored, with depths ranging from 0.5 to 18 meters.

Potamotrygonids species in the studied area. Of the three species of stingrays recorded in the upper Paraná River basin (Garrone Neto & Haddad Jr., 2010), two were selected for this study: *Potamotrygon falkneri* Castex & Maciel, 1963 and *P. motoro* (Müller & Henle, 1841) (Fig. 2). *Potamotrygon falkneri* is endemic to the Paraná-Paraguay River basin and its specific identity is well corroborated (Rosa, 1985; Silva & Carvalho, 2011). *Potamotrygon motoro* integrate a set of morphotypes widely distributed through the rivers of South America and at least one new form was identified in the Paraná River basin, requiring further investigation for its determination (Rosa, 1985; Loboda, 2010). Therefore, two specimens of each studied species were collected and stored in the fish collection of the Museu de Zoologia Prof. Dr. Adão José Cardoso - ZUEC/UNICAMP (voucher-specimens: ZUEC 6331 - *P. falkneri*, and ZUEC 6332 - *P. motoro*), in a

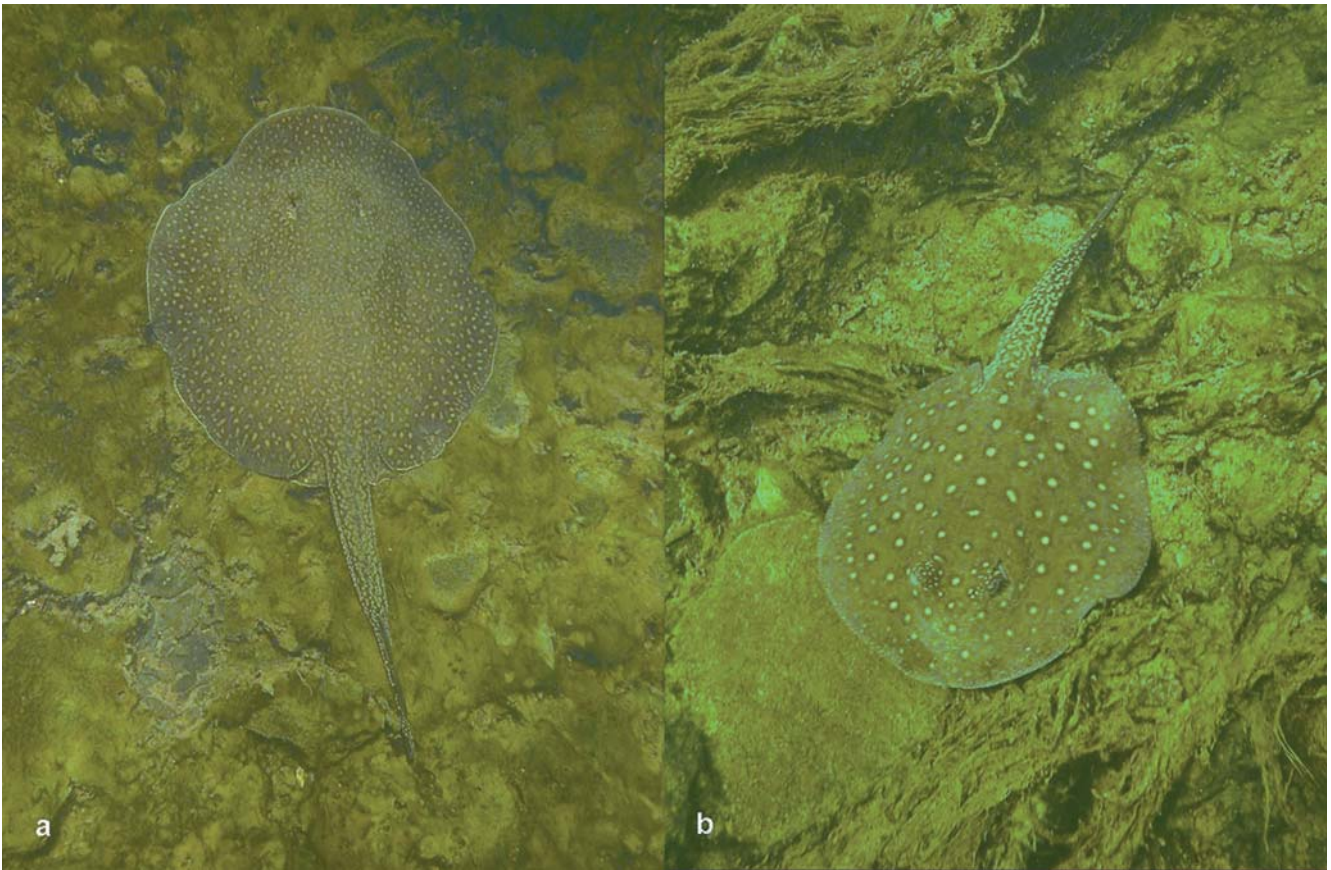


Fig. 2. *Potamotrygon falkneri* Castex & Maciel, 1963 (female; DW = 43 cm) (a), and *P. motoro* (Müller & Henle, 1841) (male; DW = 32 cm) (b).

way to enable future verification and the potential revision of their identification.

Underwater observations. Data concerning activity and habitat use were obtained during 112 hours of underwater observations, comprised through random transects with the use of self-contained diving technique (scuba diving) and free diving (snorkeling) (Sabino, 1999). In order to detect possible temporal variations, we divided the daily cycle into three time periods: day - from the total sunrise to the beginning of sunset; dusk - from the period in which the sun is near to and below the horizon; night - from the total sunset until the beginning of sunrise. Data on the water temperature and depth of the observation sites were obtained during the dives using mercury thermometer and a graduate lead line, respectively, in order to verify possible correlations of these environmental variables with the behavior of the rays. In addition, data on the habitats occupied by the animals at the time of the sightings were collected, aiming to investigate the use of the different types of environments (beach, flooded forest, stone, or grass), substrate type (sand, muddy, or gravel), and places with presence or absence of horizontal and/or vertical restrictions (*i.e.* structures used as shelters, like rocks, trunks, and macrophyte banks).

The scuba diving totaled 15 hours (day = 08 hours; night = 05 hours; dusk = 02 hours) and was performed in locations where the depth of the water was between eight and 18 meters. The free diving totaled 97 hours (day = 52 hours; night = 29 hours; dusk = 16 hours) and was carried out in locations with depth less than 12 meters. Observations were described according to a standard protocol built for this study and recorded using digital photography and video, and notes were made on PVC boards, based on methodology presented by Sazima (1986) and Sabino (1999). During the dives carried out at dusk and at night, we used indirect lighting and lamps with red cellophane filter in order to reduce the disturbance by the observer (Helfman, 1992; Sabino, 1999).

Species identification (based on the coloration of the animals' dorsum) and sex identification (based on the presence of claspers in the males - easily observed dorsally) were done *in situ* during the underwater observations, without the necessity of catching the animals. The size of the observed individuals was estimated using a hand net of known dimensions placed close to the animals in a way to determine the disc width (DW, *i.e.* the distance between the extremities of the stingray's pectoral fins). A similar method has been successfully used to estimate the disc length of marine rays (*e.g.* Aguiar *et al.*, 2009).

Data analysis. To ascertain potential relationship between the observed individuals and environmental variables as time period, depth, type of environment, substrate, and local with presence or absent of horizontal and/or vertical restrictions, a Factorial Correspondence Analysis (FCA) was conducted using the software Statistica 7.0®. Data about temperature were not included in this analysis, but were used to correlate this variable to the others analyzed. During underwater observations it was observed a similarity among species - but a difference between individuals of different sizes - in relation to distribution and habitat use. In function of these observations, the FCA analysis was applied to data grouped by size, without differentiation by species. According to disc width (DW) and sexual maturity (Garrone Neto, 2010), the individuals were divided into three size classes: f1 (DW 15-25 cm), f2 (DW 26-45 cm) and f3 (DW 46-65 cm).

The FCA is a graphical procedure which aims to represent the associations of a contingency table; therefore it does not take absolute values into consideration, but rather the correspondence between characters. This method analyses the data frequency table via the chi-square distance, thereby permitting the object of the study to be linked to different variables.

Results

In total, 132 animals were observed, 62 of which were identified as *P. falkneri* (males = 14; females = 48) and 70 as *P. motoro* (males = 17; females = 51; unidentified = 02). Of these 132 sightings, 87 corresponded to daytime observations (65.9%), 34 at night time (25.8%), and 11 at dusk (8.3%). From the daytime observations, 42 were related to stationary animals (48.3%), 23 to roaming animals (26.4%), 18 foraging (20.7%), and four were buried (4.6%). At night, 24 stingrays were foraging (70.5%), five were roaming (14.7%), four were stationary (11.8%), and one was buried (3.0%). At dusk, eight individuals were involved in foraging activity (73%), two were roaming (18%), one was stationary (9%), and none was buried.

The two studied species presented very similar patterns of number of individuals observed per size class, sex and time of the day. They also presented very similar behavior. For both species there was a variation in the spatial distribution of the individuals and the ways in which they use the habitat related to the size (disc width, DW), as shown in a schematic diagram produced with the results of underwater observation (Fig. 3) and in the graphic representation of FCA analysis (Fig. 4).

In the main river channel, stingrays belonging to the smaller size class (f1) were associated with the riverbanks, at depths between zero and four meters, in waters with about 24°C ($x = 23.8^\circ\text{C}$; $sd = 0.64$) and over a predominantly sandy substrate, throughout the day cycle. For this size class, it was common to observe intra-specific aggregations of up to five stingrays resting, sometimes close to some kind of shelter, particularly tree trunks. Otherwise, in marginal lakes

and in some stretches of the impoundment areas, these small stingrays showed behavioral variation related to time period, depth and temperature. In these lentic environments, where the temperature of the water approached 27°C in depths up to three meters ($x = 26.5^\circ\text{C}$; $sd = 0.53$), the individuals remained in deeper places during the day, in waters of about 24°C ($x = 24.2^\circ\text{C}$; $sd = 0.70$) and over muddy substrate. However, these small-sized stingrays were more active at night and were observed approaching to shallow banks, when the temperature of the water was about 24°C ($x = 23.7^\circ\text{C}$; $sd = 0.48$).

Intermediate-sized individuals (size class f2) were also found in the river channel and in marginal lakes, frequently between zero and four meters depth and temperatures near 23°C ($x = 23.3^\circ\text{C}$; $sd = 0.52$), and showed greater activity during the night. These individuals were associated to different types of environments, which ranged from rocky banks and stony bottom to places with low-growing riverbank vegetation, sandy substrates, tree trunks and macrophyte banks. In this group, many individuals were observed moving or foraging and, in some cases ($n = 07$), interacting with species of Cichlidae during their feeding activity.

Large individuals (size class f3) were observed only in the river channel. These stingrays were usually solitary and were rarely seen totally or partially buried in the substrate. For this size class, the spatial distribution showed variation related to depth and time period, with individuals occurring in deeper locations (more than eight meters) during the day and in shallower sites (between 3-8 meters) during the night. During the day they were observed over muddy or sandy substrates at the bottom of the river channel, resting in deeper waters at temperatures around 22°C ($x = 21.6^\circ\text{C}$; $sd = 0.74$), sometimes near some type of vertical restriction (*e. g.* shelters like rocks and macrophyte banks). These large stingrays were more active at night, when they forage in shallow marginal places and temperatures near 24°C ($x = 24.2^\circ\text{C}$; $sd = 0.70$).

Discussion

The use of underwater observation was shown to be of great relevance for the study of freshwater stingrays in their natural environment, enabling the pioneering collection of data related to their way of life. Excepting for difficulties during the peak of the wet period (December to March), when there is a natural tendency for river visibility to be drastically reduced, the use of scuba and free diving techniques provided valuable information about the periods of activity and the ways of habitat use of *P. falkneri* and *P. motoro*, and made possible a preliminary understanding of their spatial distribution in lentic and lotic environments.

Both species presented very similar patterns of spatial distribution and of ways in which they use the habitat. *Potamotrygon falkneri* and *P. motoro* were considered predominantly nocturnal animals, particularly regarding feeding activity. Studies suggested that various species of elasmobranchs are active during different periods of

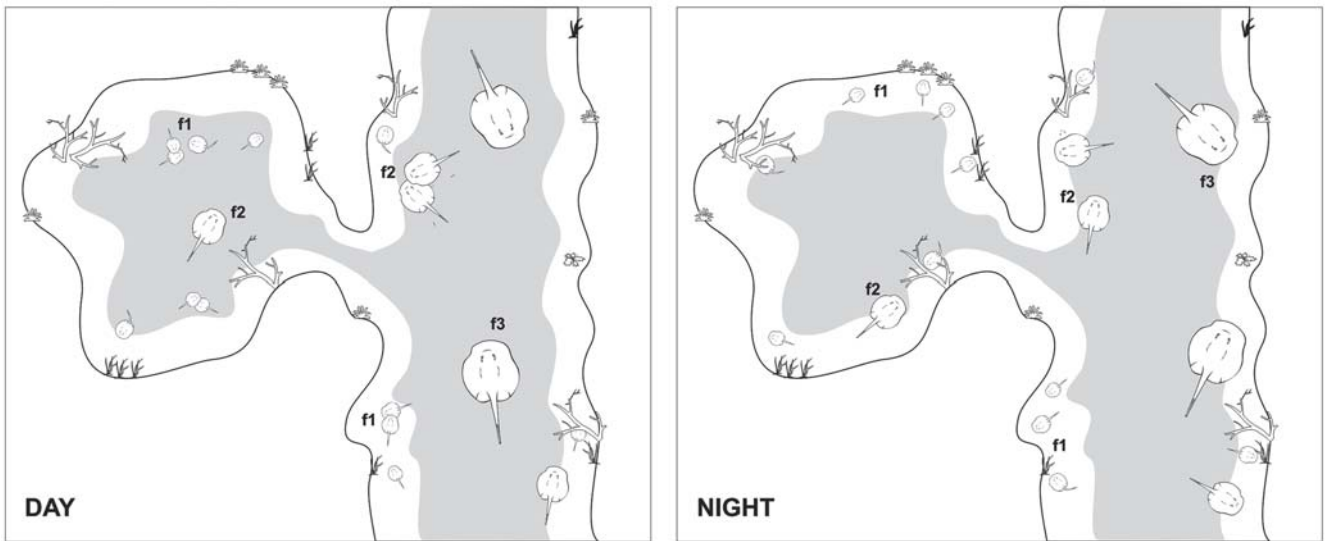


Fig. 3. Distribution of individuals of *P. falkneri* and *P. motoro* of three size classes (f1 = DW 15-25 cm, f2 = DW 26-45 cm, and f3 = DW 46-65 cm) in the upper Paraná River, illustrating the daily variation (day and night) in the occupation of shallower marginal areas (in white) and deeper central areas (in gray), both in marginal lakes and in the main river channel.

the day, notably at night (Ackerman *et al.*, 2000; Simpfendorfer & Heupel, 2004). In many cases, this pattern of movement seems to be associated with different factors, such as foraging, thermoregulation, and anti-predation strategies (Silliman & Gruber, 1999; Matern *et al.*, 2000; Aguiar *et al.*, 2009), or having a close relationship with environmental factors, such as the lunar phases or tide movements (Smith & Merriner, 1985; Cartamil *et al.*, 2003; Simpfendorfer & Heupel, 2004).

The behavior of *P. falkneri* and *P. motoro* showed ontogenetic differences and a clear spatial segregation of individuals, mainly in relation to water depth and period of day. The spatial distribution and period of activity of the stingrays also presented an interesting relationship with the diet and the hunting tactics employed by *P. falkneri* and *P. motoro* in the study area. Class f1 stingrays are mainly insectivorous (Silva & Uieda, 2007) and use the hunting tactic namely “undulate disc and stir substrate” to uncover hidden prey in unconsolidated substrata (Garrone-Neto & Sazima, 2009a), both in lentic and lotic environments. As a result, these individuals probably prefer to inhabit the riverbanks and shallow areas with predominantly sandy or muddy substrate due to their feeding behavior during this stage of maturity.

Aguiar (2005) and Aguiar *et al.* (2009), studying the behavior of the ray *Dasyatis americana* (Dasyatidae) on an oceanic island in Northeastern Brazil, observed that the spatial distribution of young individuals (disc length < 35 cm) was closely related to sandy beaches and shallow waters, and that this size class was commonly observed in aggregations in these sites. These authors assigned this fact primarily to anti-predatory strategies and secondarily

to feeding strategies, and suggested the use of sandy beaches by young individuals as nursery areas (Aguiar, 2005; Aguiar *et al.*, 2009). Despite *P. falkneri* and *P. motoro* not being native to the upper Paraná River basin and possessing a relatively recent history of occurrence in the study area (Vazzoler *et al.*, 1997; Garrone Neto *et al.*, 2007), it is possible that this behavior is also recurrent in these taxa and among other species of potamotrygonids, with f1

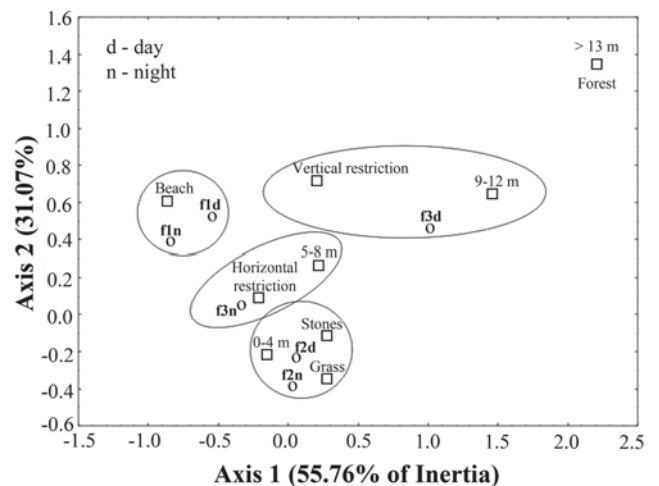


Fig. 4. Graphical representation of the results of a Factorial Correspondence Analysis (FCA): projection in factorial axes 1 and 2 of frequencies of size classes (f1 = DW 15-25 cm, f2 = DW 26-45 cm, and f3 = DW 46-65 cm) of the individuals according to habitat use, regarding period of the day, water depth, type of environment, and vertical and horizontal restrictions.

individuals normally forming aggregations or looking for shelter in shallow waters in lotic environments, associated with sandy beaches, or in deeper waters in lentic environments, associated with muddy substrates.

The large stingrays realized daily bathymetric migrations, occupying locations with different depths during the day and at night, especially for feeding purposes. At night, they were observed searching for preys in shallow waters, mainly freshwater shrimps (Palaemonidae) and small fishes (mostly Characiformes), using predominantly the hunting tactic known as “charging in the shallows” (Garrone Neto & Sazima, 2009a). During the day, those individuals were observed resting in deeper places, eventually moving and using the hunting tactic known as “undulate disc and stir substrate” to prey upon aquatic insects and mollusks (Ampullariidae) (Garrone Neto & Sazima, 2009a).

Matern *et al.* (2000), in a study of movement patterns of the bat ray *Myliobatis californica* (Myliobatidae) in Tomales Bay (California/USA), based on information obtained by means of ultrasonic telemetry, suggested that the individuals of this species carried out daily movements with the aim of making the capture and digestion of food more efficient. In this case, one of the factors which apparently exert a strong influence on the animals' patterns of movement is the water temperature, with stingrays alternating their behavior between foraging in shallow warmer waters, located in the bay, and digestion in the open sea, with greater depth and colder waters (Matern *et al.*, 2000). This search for optimum feeding temperatures seems to fit the model originally described by McLaren (1963), where the post-foraging relocation to cooler places reduces the energy demands of the metabolism, by providing additional energy on account of the reduction in the rate of gastric evacuation (Parrish & Margraf, 1990; Cortes & Gruber, 1992), while food assimilation efficiency is maintained (Brett, 1971) or possibly intensified (Wetherbee & Gruber, 1990). To the size class f3 the temperature variation was not significant in this study, although large individuals of *P. falkneri* and *P. motoro* showed a strong association of feeding activity with depths under four meters and at temperatures close to 24°C at night, and resting activity with places where the depth is over eight meters and the temperatures are near 22°C at day.

The intermediate-sized stingrays (class f2) occupied a great diversity of environments and comprised the most active of the three studied size classes. Individuals of this size class are known to eat a wide variety of items in the study area, including aquatic insects, mollusks, crustaceans, small fishes, and even residues of artisanal and sport fisheries (Silva & Uieda, 2007; Garrone Neto & Uieda, 2009), using the two hunting tactics mentioned previously and another one, least common, known as “picking up prey on vertical and inclined substrata above water surface” (Garrone Neto & Sazima, 2009a). Those behaviors demonstrated the great flexibility of these individuals in the exploitation of resources (habitat and food), while f1 individuals were basically

restricted to shallow areas and to a diet composed mainly of insects, and f3 individuals consumed snails, shrimps and small fishes. The versatility of the hunting behavior of *Potamotrygon* spp. (Garrone Neto & Sazima, 2009a) also contributed to the interspecific interactions observed between *P. falkneri* and *P. motoro* and some species of cichlids (Garrone Neto & Sazima, 2009b), and to their recent and successful colonization of new areas and habitats in the upper Paraná River basin (Garrone Neto *et al.*, 2007; Garrone Neto & Haddad Jr., 2010).

Ontogenetic variations in diet and in feeding behavior are known for some species of elasmobranchs (*e.g.* Ebert, 2002; Sisneros & Tricas, 2002; Wetherbee & Cortés, 2004). The distribution of individuals in the environment may also have a close relationship with their stage of development, as indicated by the data obtained for *P. falkneri* and *P. motoro* in this study. The field observations and the FCA analysis (see Fig. 4) showed that f3 individuals alternate their position throughout the day cycle (bathymetric migration), approaching the banks during the night, whereas f1 stingrays are strongly associated with beach and sandy substrate environments, and the f2 class is present in different environments and depths, associated with rocks and vegetation. Seasonal variations in activity, spatial distribution and habitat use of the species of stingrays here studied may likely occur, but as the peak of the rainy period impairs the use of underwater observations in the upper Paraná River basin, only the use of biotelemetry or other similar technology (see Simpfendorfer & Heupel, 2004 for overviews) may enable the acquisition of these information and expand our knowledge of the behavior of potamotrygonids in their natural environment.

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