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Silversides in South Brazil: Morphological and ecological aspects

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The coastal plain of the Rio Grande do Sul state (RS) in southern Brazil, has an enormous lagoon complex in its southern portion formed by Patos Lagoon, Mirim Lagoon, Mangueira Lagoon and several other smaller lakes. This system was originated from successive transgression and regression cycles, which took place since the upper Pleistocene (Vilwoock, 1984). Patos Lagoon is a huge chocked lagoon (10.360 area km^2), with an estuarine area comprising 10% of the total area in its southern portion, where one can find small, oligohaline shallow water embayment less than 3m maximum depth (Delaney, 1965).

Mirim Lagoon has an area of 3.749 km^2, but only 2.382 km^2 is located on Brazilian's territory because the remaining belongs to Uruguay. Mirim Lagoon communicates with Patos Lagoon through the São Gonçalo channel (Delaney, 1965).

Other small coastal lagoons are located along the coastal line, and are locally known as "lakes in rosary". The larger one is the Mangueira Lake, a closed lake with an area of approximately 800 km^2. Its northern portion is connected with the Taim wetland, which is formed by small lakes (Jacaré, Nicola, Flores). The other lakes are shallow (from 2 to 3 m) and are situated in the north portion of the Rio Grande do Sul state (Fig. 1).

Ten species of silversides, comprising the genus *Odontesthes*, occur along the RS coastal plain (Bemvenuti, 2002; Malabarba and Dyer, 2002). In Patos Lagoon estuary and its adjacent marine coastal area occurs *O. argentinensis* (Valenciennes) and *O. incisa* (Jenyns), whereas in the freshwater habitats of Patos-Mirim lagoon system can be found *O. bonariensis* (Valenciennes)*, O. humensis* De Buen, *O. retropinnis* (De Buen)*, O. aff. perugiae* Evermann and Kendall and *O. mirinensis* Bemvenuti. In contrast, *O. bicudo* Malabarba and Dyer, *O. piquava* Malabarba and Dyer and *O. ledae* Malabarba and Dyer are endemic of a chain of small shallow lakes spread along the RS north coastline. Most of *Odontesthes* species co-occur in the same habitats and they are characterized by morphological similarities, which hinder their taxonomic identification.

Comparisons among multivariate morphometric techniques

There has been a lot of controversy regarding morphological differences among organisms due to the lack of agreement about which morphometric variables are more adequate and which kind of mathematic transfor-

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mations of these variables are better to distinguish different forms.

Morphometric variables obtained by traditional measurement techniques do not take into account allometric variation or the different growth stages, which occur naturally in the organisms. Consequently, these data usually bring error to the analyses, leading to misleading interpretation and, ultimately, do not represent the real form of the organisms Bookstein *et al.* (1985). According to these authors, the use of traditional morphometric measurements can lead to more difficulties when differentiating among forms due to: (1) longitudinal measurements along the main body axis produces repetitive information in the same direction, which results in a unequal coverage of the body form, (2) utilization of maximum and minimum distances, which are not homologous from one individual to another, (3) utilization of long measurements, crossing several growth units (e.g., bones) and short ones, which contain local information.

Currently, the selection of characters that represent the whole body utilize short measure distances that connect anatomical points (homologous among individuals) (e.g., truss network) (Strauss and Bookstein, 1982). The different forms are distinguished in all directions, which compensate for random errors of measurements. Beside the description of growth and allometric patterns inside populations, the technique can discriminate among groups of organism that change in size and shape.

A comparison between traditional measurements and truss network (Fig. 2A, B) was investigated between two silverside species *Odontesthes bonariensis and O. humensis* through a principal component analysis. The first main component have been interpreted as variation in size, which results from the different growth stages in fishes, whereas the second component represents changes in shape of the organisms (Bookstein *et al*., 1985).

In the comparative study of the different forms between two species it is interesting to separate the infor-
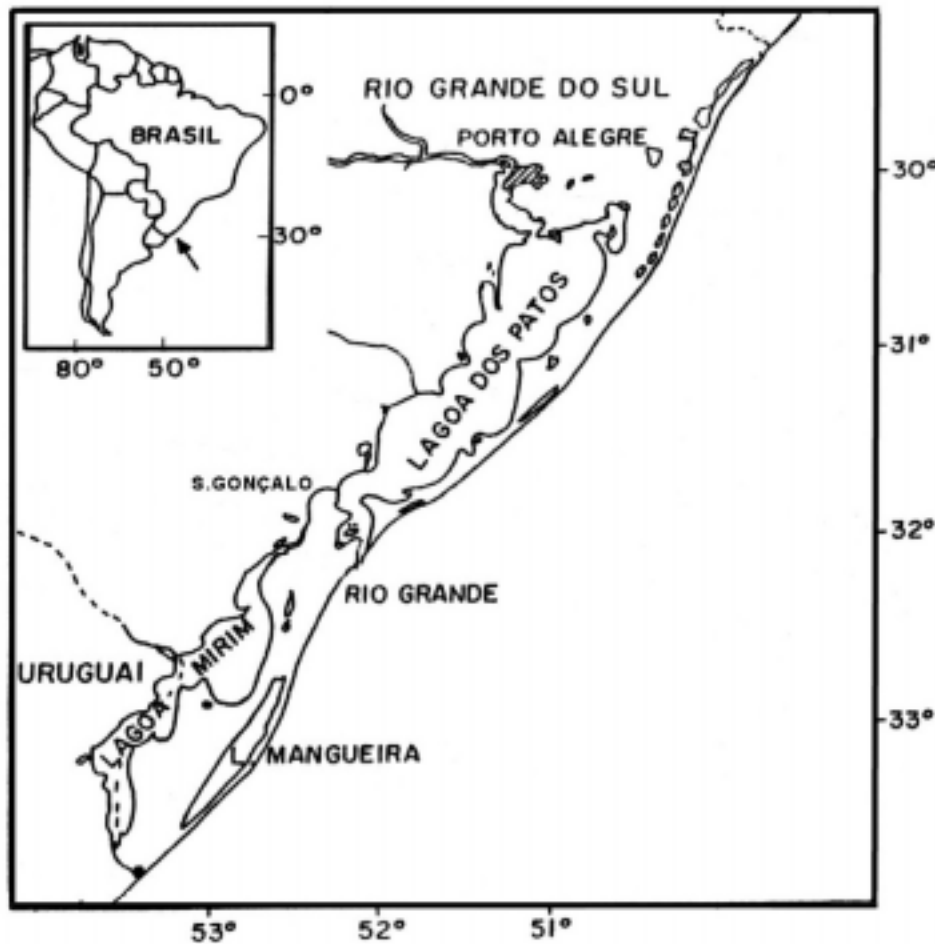

FIGURE 1. Area of distribution of silverside species in southern Brazil.

mation obtained with size due to the differential growth showed by the organisms. Therefore, the data were log transform and also adjusted to remove the size effect factor (Burnaby, 1966). The two series of measurements used during adjust and no-adjust analyzes showed the formation of two groups. However, truss network analysis results in a better differentiation between *O. bonariensis* and *O. humensis*, probably because the method enhances the discrimination of the forms with the utilization of cross measurements not considered in the traditional method (Fig. 3A, 3B). The differential pattern between both groups was also observed by the

position of values in the graph, which was located nearer one another in the truss network when compared with the traditional method (Fig. 3B; Bemvenuti and Rodrigues, 2002).

Morphological differentiation among species of the *perugiae* complex[1]

Due to the large variation in the abiotic factors in the estuarine area of the Patos Lagoon it is possible to find *O. argentinensis* and *O. mirinensis* in the same location inside the estuary, but in distinct conditions of

FIGURE 2. (A) Conventional set of distances measures used in fish morphometrics of *O. humensis;* (B) a truss networks of distances measures connect ten homologous landmarks of *O. bonariensis* (for explanation of this data set see Bemvenuti and Rodrigues, 2002).
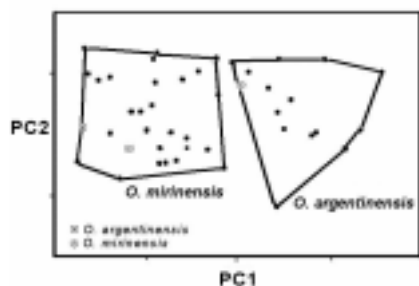

FIGURE 3. Principal components analysis of *O. bonariensis* (n=26) and *O. humensis* (n=25)*,* in the space of the first two adjusted principal components (PC1, PC2), for (A) 25 traditional measures and (B) 21 distances measures with truss network.

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[1] In the lagoons located in the south portion of the Rio Grande do Sul, the *perugiae* complex is comprised by the freshwater silversides *O. perugiae, O. mirinensis*, and the estuarine *O. argentinensis*. These three species are morphologically much closed and the morphometric and meristic characters are no sufficient tools to distinguish among them (Bemvenuti, 2002). According to Beheregaray *et al*. (2002), these silverside species were originated from the marine ancestor *O. argentinensis*.

salinity. *Odontesthes argentinensis* shows preference for the lower reaches of the estuary, whereas *O. mirinensis* can be found in the northern reaches of the estuary, near the São Gonçalo Canal and Mirim Lagoon. This pattern brings difficult to the taxonomic identification of these species in the estuarine region. With the principal component analysis it was possible to distinguish *O. argentinensis and O. mirinensis* groups, but differences were not observed in body proportion between both species (Fig. 4). Only the meristic character of number of lower gill rakers shows differentiation: *O. argentinensis* 20 and 24 gill rakers, and *O. mirinensis* - 24 and 29 gill rakers (Bemvenuti, 1995, 2000, 2002).

In the freshwater sites, the silversides *O. aff. perugiae* e *O. mirinensis*[2] also were discriminated through the form (Fig. 5), being possible to observe a superficial variation in snout length, head and jaw (Fig. 6). In *O. perugiae*, the head is prominent (24.3% cp), the snout is salient (9,1% cp) and the maxilla is located ahead of the mandible. These proportions are relatively larger in *O. peruagiae* when compared with *O. mirinensis*, where the head is smaller (22.5% cp), the snout is shorter (6.9% cp) and the maxilla and mandible are in the same anterior projection (Bemvenuti, 1995, 1997, 2002).

Description of the bone structure for the *perugiae* complex

A compared description of some bone structures was investigated to incorporate new data for the differentiation of the following species *O. mirinensis, O. perugiae* and *O. argentinensis*[3]. The teeth in the vomer are placed in three large plates in *O. perugiae*, in a medium size plate in *O. argentinensis*, and absents in *O. mirinensis*. The accessory canal of the infraorbital 1 (IO1) has two external pores in *O. mirinensis*, one anterior and intern pore and other posterior and external pore in *O. perugiae*, and it is absent in *O. argentinensis*. The infraorbital 2 (IO2) has a main dorsal canal and four long accessory canals with six pores in *O. argentinensis,* three long canals with five pores in *O. mirinensis*, and two short canals in *O. perugiae*.
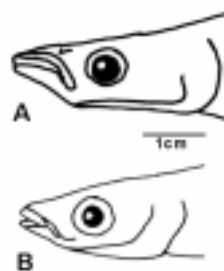

FIGURE 4. Principal components analysis of *O. mirinensis* (n=41) and *O. argentinensis* (n=35), in the space of the first two adjusted main components (PC1, PC2), for 21 measures with truss network.
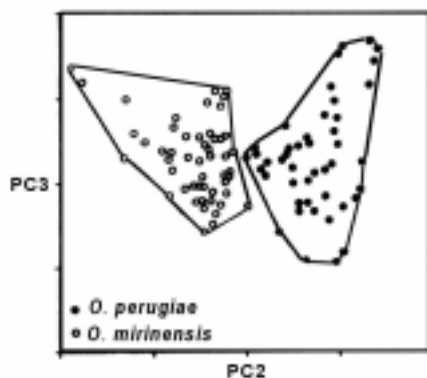
FIGURE 5. Principal components analysis of *O. aff. perugiae* (n=52) and *O. mirinensis* (n=44), in the space of the second and third components (PC2, PC3), for 25 traditional measures.

FIGURE 6. Lateral view of the head of *O. perugiae* (A) e *O. mirinensis* (B).

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[2] *Odontesthes perugiae is a specie restrict to rivers. Its occurrence is cited for Rio da Prata and Rio Paraná floodplain, Argentina (Marrero 1950), and including Uruguai and Negro rivers. Beheregaray et al. (2002) believed that the individuals from Uruguai river, RS are O. perugiae, and the individuals from the coastal lagoons as a new specie, O. aff. perugiae. Odontesthes mirinensis is restricted to coastal lagoons in the southern RS, Mirim, Mangueira e Lagoa dos Patos (Tapes, Camaquã, Guaíba river). Its high abundance is recorded in the Mirim Lagoon, with many juvenile individuals being captured in the Canal São Gonçalo Channel, Pelotas, which makes the connection between Mirim lagoon and Lagoa dos Patos (Bemvenuti, 1995).*

[3] The complete bone description for all *Odontesthes* species can be found in Bemvenuti (2005).

The premaxilla shows posterior process upward, lengthier than higher in *O. mirinensis*, slim, large and straight in *O. perugiae* positioned upward in *O. argentinensis*. The mandibular sensory canal of the dentary shows seven pores in *O. mirinensis* and *O. perugiae*, and six in *O. argentinensis*. The tooth plate of the endopterygoid is numerous in *O. mirinensis* and *O. argentinensis*, whereas it is reduced a few and scattered tooth in *O. perugiae*. The preopercle sensory canal of the horizontal shaft has four large pores in *O. mirinensis,* three large pores in *O. argentinensis*, and no pore in *O. perugiae* due to the absence of bridges among them. The vertical shaft is totally open in *O. mirinensis,* with a wide opening in *O. perugiae* and with two openings in *O. argentinensis*. The hemal funnel, expansion of the hemal arch, start at the 24a vertebra in *O. argentinensis,* whereas in *O. mirinensis, O. perugiae*, and in the other freshwater silversides, the hemal funnel start at the 27a vertebra. The estuarine species has the same number of precaudal and caudal vertebrae, whereas the freshwater species has a larger number of pre-caudal vertebrates (Dyer, 1997; Bemvenuti, 2005).

Geographical differentiation in *O. argentinensis*

Odontesthes argentinensis is an estuarine-resident (Chao *et al.*, 1985) showing a complete life-cycle inside the estuary. Morphological variations were observed among specimens occurring in the estuarine area and in the marine adjacent region (Fig. 7; Bemvenuti, 1995; 1997).

Based on isoenzyme electrophoresis analysis, Beheregaray and Levy (2000) also observed similar patterns of genetic divergence between both populations. These authors suggested that the colonization of the estuary occurred when a segment of the marine silverside group managed to stay in the estuarine area, leading to the generation of differences among these groups. Based on molecular analysis, Beheregaray and Sunnucks (2001) suggested that all species of the genus Odontesthes are derived from the marine group. Therefore, this geographical area supports two resident silverside populations: one inside the estuarine area of Patos Lagoon and other in the adjacent marine area (Bemvenuti, 2000; Beheregaray, 2000; Beheregaray and Levy, 2000).

Bio-ecological aspects of the silversides

Feeding

The occurrence of several silverside species along the coastal lagoons of southern Brazil suggest changes in the feeding modes and prey selection to avoid diet overlap and, therefore, allowing their co-existence in the same system. The feeding behaviour is a fundamental step in understanding the ecology of one species and its place in the food web.
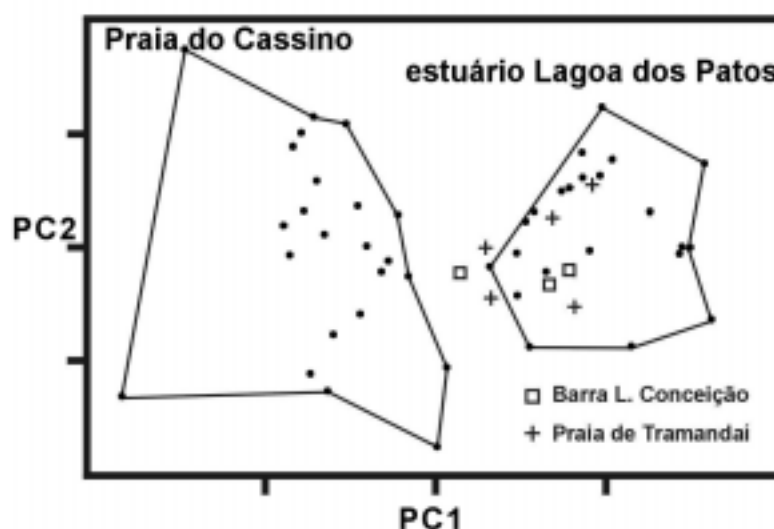

FIGURE 7. Principal components analysis of *O. argentinensis,* obtained in the marine coastal (n=26 - Praia do Cassino) and estuarine area (n=26 - Lagoa dos Patos; n=5 - Tramandai; n=3 - Lagoa da Conceição), in the space of the first two adjusted components (PC1, PC2), for 25 traditional measures.

Species occurring in the same location probably feed upon different kinds of food, or occupy different habitats or utilize distinct resources in different time frames. Such species usually show diets with low overlap, which can be attributed to differences in mouth morphology and/or feeding behaviour. Diet composition must be related with morphological structures linked to feeding, such as the form, position and mouth size, and form and number of gill rakers (Wootton, 1990).

The feeding habit of *O. humensis* is benthos carnivore, preying upon mainly on mollusks *Heleobia* sp. (61.4% frequency of occurrence FO), *Corbicula fluminea* (58%), *Neocorbicula limosa* (17.4%) and coleoptera insects (18,8%). In lower frequency can be found diptera insects, crustacean *Paleomonetes argentinus* and fishes (Rodrigues and Bemvenuti, 2001). In spite of being a pelagic organism, the species browse for food in the bottom as well, making it different from other silverside species. The predominance of *Heleobia sp*, in comparison of other mollusks found in the diet, can be related with the high abundance of this prey in the study area and, probably, due to its low size. The majority of the shells of adults from *C. fluminea* and *N. limosa* were found crushed, whereas the shells of the young individuals were found as whole shells.

The form and number of the short and thick gill rakers (20-24= 4-6+15-19), as well the form and type of the tooth in the pharyngeal plates[4] (molariforms in the center and conic in the edges), can be related with the zoo-benthos diet. The pharyngeal plates play a role in crushing mollusk shells and/or arthropod carapaces when they are too large. Shells of young *C. fluminea, N. limosa* and, in part *Heleobia* sp, are not crushed by the pharyngeal plates but are swallowed as entire pieces.
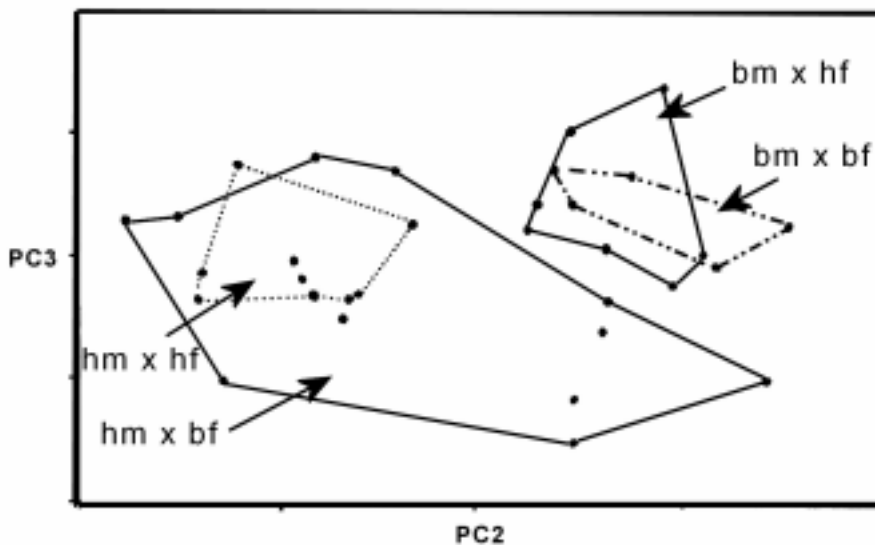

FIGURE 8. Principal components analysis of *O. bonariensis* (n=6 — bm x bf), *O. humensis* (n=5 – hm x hf), hybrids of *O. bonariensis* male and *O. humensis* female (n=8 - bm x hf), hybrids of *O. humensis* male and *O. bonariensis* female (n=14 - hm x bf) in the space of the second and third components (PC2, PC3), for 21 distances measures with truss network.

[4] The pharyngeal plates are distributed in four pairs, being three located in the upper portion of the mouth and the other in the lower portion. The three plates of the upper portion have different size; one is large and oval shape with molariform tooth showing different size and the other two are smaller, external with small, slim and conic tooth. The lower plate is large, has a triangular shape, showing in the center several tooth of medium and small size, with a molariform shape, and in its edges show few small, slim and conic teeth.

The occurrence of coleopteran insects, as a prey item, can be explained by the high temperatures during the sample collection. This item also showed larger size them mollusk preys.

In the same environment, *O. bonariensis* shows zoobenthos habit preying upon mollusks, isopods, shrimps, tanaidacea, insects and small fishes. Among mollusk items, showed preference in frequency of occurrence by the gastropod *Heleobia sp.* (31%), bivalves *Corbicula fluminea* (16.7%) and *Neocorbicula limosa* (7.1%). *Odonthestes* aff. *perugiae* shows preference by mollusks, isopods, insect larvae, followed by tanaidacea and copepods. The food items with higher weight in the stomachs were the mollusk *Corbicula fluminea* (48% of the total weight) and isopods (31%), followed by insect larvae (8%), Hymenoptera insects (2%) and fishes remaining (5%) showing lower weight.

In the estuarine region, *O. argentinensis*'s juvenile shows preference for zooplankton food items, whereas adults show preference for benthic preys. Juveniles prey upon copepods (63,8%) and diptera insects (35.5%), whereas adults feed on tanaidacea *Kalliapseudes schubartii* (35.6%), amphipod (22%) and polychaete *Laeonereis acuta* (25.7%) (Bemvenuti, 1990).

Reproduction

The estuarine specie *O. argentinensis* shows spawning season between late winter and early spring (June and November with a peak in September). The size of first maturation (L_{50}) is 230mm TL (Bemvenuti, 1987). Recent information suggests a reduction of the maturation size in females (162mm TL in the estuary and 179mm TL in the marine area).

The freshwater species *O. humensis* and *O. bonariensis* spawn in the coastal lagoons during the early winter and spring. *Odonthesthes bonariensis* spawns between May and July, whereas *O. humensis* between July and September (Kleerekoper, 1945; Moraes, 1991). The overlap in the spawning season increases the chances of combination among theses species, which favors hybridization. Preliminary karyotype studies[5] suggest there is chromosomal affinity between both species, with chromosomes showing high similarity in number and morphology.

In spite of the extent literature about *O. bonariensis* and, in a less extent, about *O. humensis*, there is no record regarding hybridization with these species or others South American silversides. Recently it was developed its hybridization in laboratory with the help of natural populations of *O. humensis* e *O. bonariensis*[6]. The sampled individuals were analyzed by principal component. Two groups were observed (Fig. 8); one group showing a relationship between the natural population of *O. humensis* with hybrids which male progenitors were *O. humensis*, and the other group showed a relationship between the natural populations of *O. bonariensis* with hybrids which male progenitors were *O. bonariensis*. Such affinity shows the morphological similarity among the hybrids and the species of the male progenitor.

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[5] Personal communication of G. Born (DCMB-FURG, Rio Grande, Rio Grande do Sul, Brasil).

[6] Hybridization developed in laboratory in partnership with J. Pouey (UFPel, Pelotas, Rio Grande do Sul, Brazil).

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