

## Parasites of the Brazilian sandperch *Pinguipes brasiliensis* Cuvier: a tool for stock discrimination in the Argentine Sea

J. T. TIMI\*†, A. L. LANFRANCHI\*, J. A. ETCHEGOIN\*  
AND F. CREMONTE‡

\*Laboratorio de Parasitología, Departamento de Biología, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata (CONICET), Funes 3350, (7600) Mar del Plata, Argentina and ‡Centro Nacional Patagónico (CONICET), Boulevard Brown s/n, 9120 Puerto Madryn, Argentina

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The use of parasites as biological tags allowed the identification of three stocks of Brazilian sandperch *Pinguipes brasiliensis* in the Argentine Sea. A total of 156 specimens caught during spring 2006, off the coasts of Buenos Aires Province (38°27' S; 57°90' W,  $n = 54$ ), San Matías Gulf (42° S; 65°10' W,  $n = 52$ ) and Nuevo Gulf (42°09' S; 64°05' W,  $n = 50$ ), were examined for parasites and 21 species were found, including monogeneans, digeneans, cestodes, nematodes, acanthocephalans and crustaceans. Diet analyses showed that Brazilian sandperch feed mainly on benthic macroinvertebrates, with differences in relative importance of main items between zones. Univariate analysis on parasite population descriptors, as well as a discriminant analysis, allowed the identification of discrete stocks in each zone, although a higher degree of similarity was observed between both Patagonian Gulfs. Northern samples were characterized by higher values of prevalence and abundance of *Grillotia* sp., *Corynosoma australe* and *Scolex polymorphus*. On the other hand, *Trifur tortuosus* and Opecoelidae gen. sp. were important in determining the position of Patagonian samples and were specially related to those from Nuevo Gulf, whereas none of the species was clearly related only to fish from San Matías Gulf. The size of the hosts is likely to influence the similarity among zones, with larger fish as better tools to discriminate stocks, probably because they harbour 'mature' assemblages shaped longer by differential environmental conditions and diet in each zone. Parasite species characteristic of sandperch from Buenos Aires Province, which display very low specificity among fish species in this area, have been previously identified as biological tags for other host species in these region, confirming their value as tags for stock delineation.

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Key words: biological tags; fish populations; south-west Atlantic.

### INTRODUCTION

The implementation of policies that ensure an efficient and sustainable management of any exploited fish population requires a correct delineation of its

†Author to whom correspondence should be addressed. Tel.: +54 223 4752426; fax: +54 223 4753150; email: [jtimi@mdp.edu.ar](mailto:jtimi@mdp.edu.ar)

boundaries with neighbouring populations of conspecifics. The Brazilian sandperch *Pinguipes brasilianus* Cuvier, 1829, inhabits coastal waters from Rio de Janeiro, Brazil to Nuevo Gulf, Argentina (Rosa & Rosa, 1997). Despite having little importance in regional fisheries (Froese & Pauly, 2007), *P. brasilianus* is caught as an incidental catch over its distribution range (Cousseau & Perrotta, 2004), and therefore is being indirectly exploited.

The biology of *P. brasilianus* is poorly known. Some systematic studies of their parasites have been carried out (Ringuelet, 1945; Vicente & Fernandes, 1973; Etchegoin *et al.*, 2006; Timi *et al.*, 2007), but there is no information on diet, migration and population sizes (Cousseau & Perrotta, 2004). Consequently, the number and distribution of stock units is unknown and requires further investigation.

Parasites have been widely used as biological tags to provide information on the stock discreteness of their fish hosts (Lester, 1990; Williams *et al.*, 1992; MacKenzie & Abaunza, 1998; MacKenzie, 2002). In the south-western Atlantic, in particular, their use is thought to be a promising tool, due to the presence of a group of parasite species with low host specificity, suitable as biological tags and therefore applicable to all host species harbouring them (Timi, 2007).

The aim of this study was therefore three-fold: (1) to characterize the parasite fauna of *P. brasilianus* in the Argentine Sea, (2) to identify the existence of different stocks in the study area and (3) to test the hypothesis that suitable tags for other fish species in the region are applicable to population studies of *P. brasilianus*.

## MATERIALS AND METHODS

A total of 156 specimens of *P. brasilianus* were examined for parasites. Only adult fish caught during spring were included in the analysis in order to minimize the possible influence of seasonality on the parasite burden. Fish were caught by trawling along the coast of Buenos Aires Province (BAP; 38°27' S; 57°90' W;  $n = 54$ ; October to November, 2006) and by scuba diving in the Patagonian gulfs: Puerto Lobos, San Matías Gulf (SMG; 42° S; 65°10' W;  $n = 52$ ; December, 2006) and Craker Bay, Nuevo Gulf (NG; 42°09' S; 64°05' W;  $n = 50$ ; December 2006). Fish were either kept fresh or deep frozen in plastic bags at  $-18^{\circ}$  C until examination. After thawing each Brazilian sandperch was measured for total length ( $L_T$ ; mm). Parasites were recovered from the body surface, gills, branchial and body cavities and viscera (stomach, intestine, liver, gonads and mesenteries) by stereoscopic microscopy.

Prevalence, mean abundance and mean intensity were calculated for each parasite species in each area following Bush *et al.* (1997). For those species with prevalences  $>10\%$  in a given zone (component species; Bush *et al.*, 1990), the relationships between host size and abundances were analysed by Spearman's rank correlation coefficients ( $r_s$ ).  $\chi^2$  analyses and *a posteriori* multiple comparisons for proportions, previous angular transformation of each proportion, were used to test for significant differences of prevalence between zones for those component species in at least one of the zones. ANOVA and *a posteriori* Tukey tests for unequal samples on  $\log_{10}(x + 1)$  transformed data were used to analyse the effects of locality on abundance of each parasite species (Zar, 1984).

Two measures of similarity, the Jaccard qualitative and Sørensen quantitative indices (Magurran, 1988), were calculated among infracommunities within and between zones, after eliminating non-parasitized fish.

A discriminant analysis, based on Mahalanobis distances, was used to find differences between zones and to identify which parasite species were responsible for these differences. Analyses were computed on  $\log_{10}(x + 1)$  transformed data and performed using the Brodgar 1.8 package (Brodgar, 2000).

## RESULTS

Mean  $\pm$  s.d. host  $L_T$  from BAP ( $333 \pm 31$  mm), SMG ( $327 \pm 34$  mm) and NG ( $371 \pm 31$  mm) were significantly different between zones ( $F_{3,153}$ ,  $P < 0.01$ ), fish from NG being significantly larger than those from both BAP ( $P < 0.01$ ) and SMG ( $P < 0.01$ ); Brazilian sandperch from both northern areas did not differ significantly in size ( $P > 0.05$ ).

Analyses of stomach contents showed that fish fed mainly on benthic macro-invertebrates, with differences in relative importance of main items between zones. The most prevalent food items in all zones were brachyuran crabs and polychaetes, although sea urchins occurred more frequently in both Patagonian gulfs, where amphipods and gastropods were also quite common. A common feature of diet was the scarce representation of fishes among food items across all zones.

Most of Brazilian sandperch (98.7%) were parasitized by at least one of 21 parasite species (Table I); only two fish, both from SMG, were free of parasites. A total of 4932 metazoan parasites were found in the overall sample. *Grillotia* sp., *Corynosoma australe* and *Scolex polymorphus* were the most prevalent and abundant species in BAP, representing 96.0% of all parasites found in this zone (46.3, 37.5 and 12.2%, respectively). In SMG *Neolebouria georgenascimentoi*, *C. australe*, *Paracapillaria argentinensis* and *Trifur tortuosus* accounted for 91.3% of all parasites (53.8, 20.2, 8.7 and 8.5%, respectively) whereas in NG *N. georgenascimentoi*, Opecoelidae gen. sp., *T. tortuosus* and *Neobrachiella spinicephala* represented 89.3% of all parasites (43.6, 29.6, 9.7 and 6.3%, respectively).

Most correlation analyses showed no relationships between parasite abundance and host size; no repeatability in the significant relationships was found across localities, with *T. tortuosus* as the unique species displaying a higher abundance in larger fish in two localities (Table II).

Comparisons of prevalence between areas (Table III) showed that most parasite species varied significantly, with a higher and lower number of differences occurring between BAP and NG and between BAP and SMG, respectively. Considering prevalence, *Grillotia* sp., *S. polymorphus* and *C. australe* showed higher values in BAP than in the other two zones, therefore BAP has no indicator species in common with any of the two Patagonian areas. On the other hand *N. georgenascimentoi*, Opecoelidae gen. sp., *Terranova* sp., *Pseudoterranova* sp. and *T. tortuosus* were characteristic of NG. Despite *N. georgenascimentoi* and *T. tortuosus* showing higher prevalence in SMG than in BAP, their values were significantly lower than in NG. None of the species was, therefore, more prevalent in SMG than in the other localities. Abundances yielded similar results to those for prevalences.

A high degree of variability was observed in the analysis of both Jaccard and Sørensen similarity indices (Fig. 1) (see s.d.); qualitative comparisons within zones showed that BAP was the most homogeneous group, whereas the lowest similarity was observed among fish from SMG [Fig. 1(a)]. Analyses between zones showed that similarity values between Patagonian infracommunities were higher than those involving sandperches from BAP and also higher than those within SMG [Fig. 1(a)]. The same trend, although more evident, was observed for quantitative data [Fig. 1(b)].

The first two discriminant variables explained 100% of the variance, contributing 95.56% (eigenvalue = 9.335) and 4.44% (eigenvalue = 0.434), respectively. A significant overall group effect was observed (Wilks' lambda = 0.067,  $F_{42,262}$ ,  $P < 0.01$ ).

TABLE 1. Taxonomic composition, microhabitat, prevalence and mean intensity of parasites of *Pinguipes brasiliensis* in three zones of the Argentine Sea

	Site	Prevalence			Mean $\pm$ s.d. intensity		
		BAP	SMG	NG	BAP	SMG	GN
<i>Microcotyle pseudoperis</i> Amato & Cezar, 1994 (MO)	GI	13.0	11.5	16.0	1.4 $\pm$ 0.8	1.5 $\pm$ 0.5	1.2 $\pm$ 0.5
<i>Neolebouria georgenascimentoi</i> Bray, 2002 (DI)	IN	3.7	51.9	72.0	2.0 $\pm$ 1.4	9.8 $\pm$ 12.3	10.5 $\pm$ 10.5
Opecoelidae gen. sp. (DI)	IN	0	5.8	36.0	0	2.7 $\pm$ 2.1	14.6 $\pm$ 18.8
<i>Derogenes varicus</i> Müller, 1784 (DI)	IN	1.8	0	0	1.0 $\pm$ 0.0	0	0
<i>Aponurus laguncula</i> Looss, 1907 (DI)	IN	1.8	0	0	1.0 $\pm$ 0.0	0	0
<i>Grillotia</i> sp., plerocercoid (CE)	ME	100.0	9.6	0	30.5 $\pm$ 32.4	1.6 $\pm$ 0.9	0
<i>Scolex polymorphus</i> Müller, 1784, plerocercoid (CE)	IN	59.3	1.9	4.0	13.6 $\pm$ 13.8	1.0 $\pm$ 0.0	1.0 $\pm$ 0.0
<i>Anisakis simplex</i> (Rudolphi, 1809), third stage larvae (NE)	ME	1.8	1.9	4.0	1.0 $\pm$ 0.0	1.0 $\pm$ 0.0	1.0 $\pm$ 0.0
<i>Terranova</i> sp., third stage larvae (NE)	ME	0	0	10.0	0	0	1.2 $\pm$ 0.4
<i>Pseudoterranova</i> sp., third stage larvae (NE)	ME	0	0	10.0	0	0	1.4 $\pm$ 0.5
<i>Hysterothylacium</i> sp., third stage larvae (NE)	ME	3.7	0	0	1.0 $\pm$ 0.0	0	0
<i>Hysterothylacium aduncum</i> (Rudolphi, 1802) (NE)	ST	1.8	0	0	1.0 $\pm$ 0.0	0	0
<i>Paracapillaria argentinensis</i> , Timi, Rossin, Lanfranchi & Etchegoin, 2007 (NE)	ST	22.2	26.9	30.0	4.4 $\pm$ 5.2	3.1 $\pm$ 2.05	1.7 $\pm$ 0.9
<i>Cucullianus carioca</i> Vicente & Fernandez, 1973 (NE)	IN	9.3	0	0	1.0 $\pm$ 0.0	0	0
<i>Ascarophis marina</i> (Szidat, 1961), third stage larvae (NE)	IN	5.6	0	0	2.0 $\pm$ 1.0	0	0
<i>Corynosoma australe</i> Johnston, 1937, juvenile (AC)	ME	92.6	61.5	50.0	26.7 $\pm$ 33.8	3.2 $\pm$ 7.1	1.6 $\pm$ 0.9
<i>Corynosoma cetaceum</i> Johnston & Best, 1942, juvenile (AC)	ME	5.6	0	0	1.3 $\pm$ 0.6	0	0
Unidentified acanthocephalan, juvenile (AC)	ME	3.7	0	0	1.5 $\pm$ 0.7	0	0
<i>Neobrachiella spinicephala</i> (Ringuelet, 1945) (CO)	BS	31.5	25.0	42.0	1.8 $\pm$ 0.9	1.3 $\pm$ 0.6	2.6 $\pm$ 2.4
<i>Trifur tortuosus</i> Wilson, 1917 (CO)	GI	3.7	57.7	86.0	1.0 $\pm$ 0.0	1.4 $\pm$ 0.7	2.0 $\pm$ 1.3
Gnathiidae gen. sp. (IS)	GI	9.3	0	2.0	3.4 $\pm$ 2.3	0	1.0 $\pm$ 0.0

AC, Acanthocephala; BAP, Buenos Aires Province; BS, body surface; CE, Cestoda; CO, Copepoda; DI, Digenea; GI, gills; IN, intestine; IS, Isopoda; ME, mesenteries; MO, Monogenea; NE, Nematoda; NG, Nuevo Gulf; SMG, San Matías Gulf; ST, stomach.

TABLE II. Relationship between abundance and fish size of selected parasite species (those with prevalence >10%) of *Pinguipes brasiliianus* in three zones of the Argentine Sea

	BAP		SMG		NG	
	$r_s$	$P$	$r_s$	$P$	$r^2$	$P$
<i>Microcotyle pseudopercis</i>	-0.02	0.84	0.24	0.08	0.12	0.39
<i>Neolebouria georgenascimentoi</i>	—	—	-0.03	0.80	0.59	<0.01
Opecoelidae gen. sp.	—	—	—	—	-0.48	<0.01
<i>Grillotia</i> sp. plerocercoid	0.43	<0.01	—	—	—	—
<i>Scolex polymorphus</i>	0.31	0.02	—	—	—	—
<i>Terranova</i> sp.	—	—	—	—	0.03	0.85
<i>Pseudoterranova</i> sp.	—	—	—	—	-0.02	0.90
<i>Paracapillaria argentinensis</i>	0.02	0.86	0.12	0.37	0.29	0.04
<i>Corynosoma australe</i>	0.24	0.09	-0.11	0.41	0.04	0.75
<i>Neobrachiella spinicephala</i>	0.15	0.28	0.11	0.43	0.53	<0.01
<i>Trifur tortuosus</i>	—	—	0.41	<0.01	0.39	<0.01

BAP, Buenos Aires Province; NG, Nuevo Gulf; SMG, San Matías Gulf.

Individual fish were distributed mainly along the first axis, a pattern readily observed by representing the group means [Fig. 2(a)]. Dimensionality tests for group separation showed that the zones were significantly separated in both dimensions ( $\chi^2$ , d.f. = 20,  $P < 0.001$ ), although fish from both Patagonian gulfs were closer to each other [Fig. 2(b)]. Each host was classified correctly to the three zones with an accuracy of 87.01% (Table IV), while the per cent of correctly classified samples relative to chance was 35.06%.

The importance of each parasite species with respect to discrimination between groups (Fig. 3), evaluated as the contribution of each variable to the total sum of Mahalanobis distances, showed that *Grillotia* sp., the most important species in determining the position of samples, was related to fish from BAP. A similar pattern was observed for *C. australe* and *S. polymorphus*, the third and fifth species in order of importance, respectively. *Trifur tortuosus* and Opecoelidae gen sp., the second and fourth species in order of importance, respectively, were important in determining the position of Patagonian samples, especially those from Nuevo Gulf.

None of the species was clearly related to fish from San Matías Gulf. The remaining species, whose removal resulted in a decrease <5% of the total sum of Mahalanobis distances, were excluded from Fig. 3.

## DISCUSSION

*Pinguipes brasiliianus* harboured a varied and abundant parasite fauna in the region studied, with 18 out of the 21 metazoan species being new host records. *Neobrachiella spinicephala*, *Cucullanus carioca* and *P. argentinensis* have been described previously as parasites of *P. brasiliianus* (Ringuet, 1945; Vicente & Fernandes, 1973; Etchegoin *et al.*, 2006; Timi *et al.*, 2007).

A smaller number of species accounted for the majority of parasites found, a common feature of parasite communities, in which few species are well-represented and others are rare (Poulin, 1996). The identity of these dominant species varied between zones and some of them showed significant correlations with fish size.

TABLE III. Comparisons of prevalence and abundance of selected parasite species of *Pinguipes brasiliensis* among three zones of the Argentine Sea

	Prevalence				Abundance					
	$\chi^2$	P	BAP-SMG	BAP-NG	SMG-NG	Anova F	P	BAP-SMG	BAP-NG	SMG-NG
<i>Microcotyle pseudopercis</i>	0.45	NS	—	—	—	0.08	NS	—	—	—
<i>Neolebouria georginascimentoi</i>	53.19	<0.01	BAP < SMG*	BAP < NG*	SMG < NG*	29.39	<0.01	BAP < SMG*	BAP < NG*	SMG < NG**
<i>Opelcoelidae</i> gen. sp.	30.32	<0.01	BAP < SMG*	BAP < NG*	SMG < NG*	16.83	<0.01	NS	BAP < NG*	SMG < NG*
<i>Grillotia</i> sp.	136.78	<0.01	BAP > SMG*	BAP > NG*	SMG > NG*	379.86	<0.01	BAP > SMG*	BAP > NG*	NS
<i>Scolex polymorphus</i>	64.41	<0.01	BAP > SMG*	BAP > NG*	NS	53.27	<0.01	BAP > SMG*	BAP > NG*	NS
<i>Terranova</i> sp.	10.95	0.01 < P < 0.05	A	BAP < NG*	SMG < NG*	5.51	<0.01	A	BAP < NG**	SMG < NG**
<i>Pseudoterranova</i> sp.	10.95	0.01 < P < 0.05	A	BAP < NG*	SMG < NG*	5.45	<0.01	A	BAP < NG**	SMG < NG**
<i>Paracapillaria argentinensis</i>	0.83	NS	—	—	—	0.11	NS	—	—	—
<i>Corynosoma australe</i>	23.66	<0.01	BAP > SMG*	BAP > NG*	SMG > NG*	85.91	<0.01	BAP > SMG*	BAP > NG*	NS
<i>Neobrachiella spinicephala</i>	3.40	NS	—	—	—	3.65	0.03	NS	NS	SMG < NG**
<i>Trifur tortuosus</i>	70.42	<0.01	BAP < SMG*	BAP < NG*	SMG < NG*	56.84	<0.01	BAP < SMG*	BAP < NG*	SMG < NG*

A, absent in both zones; BAP, Buenos Aires Province; NG, Nuevo Gulf; NS, non-significant ( $P > 0.05$ ); SMG, San Matías Gulf.\* $P < 0.01$ .\*\* $0.01 < P < 0.05$ .

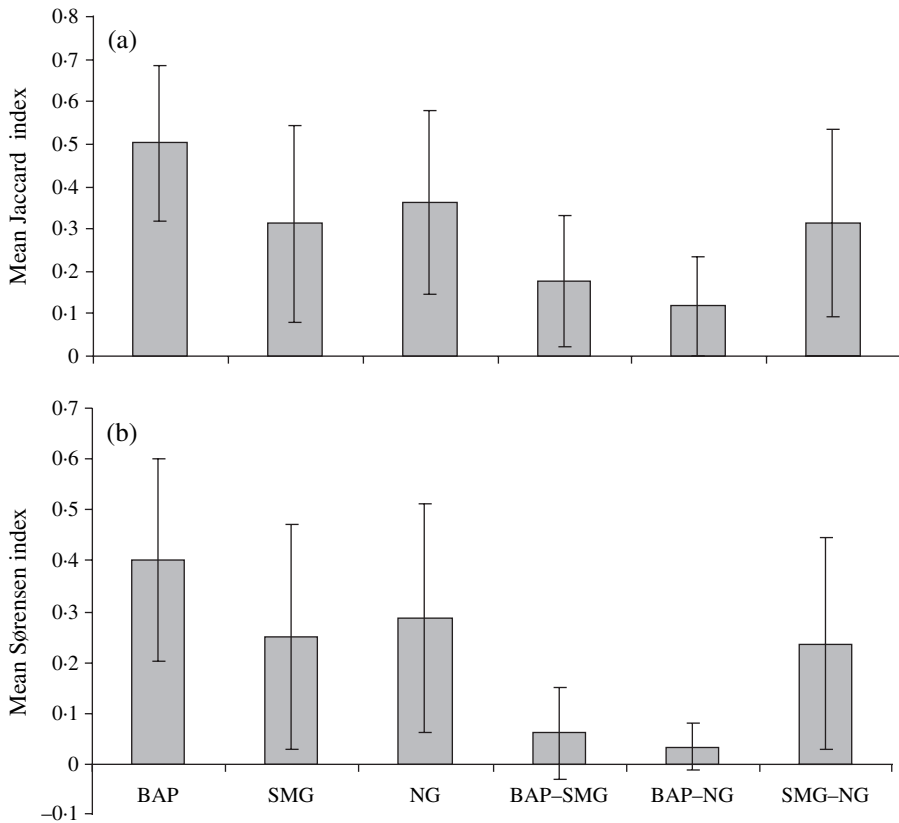


FIG. 1. Mean  $\pm$  s.d. similarity indices of parasite infracommunities of *Pinguipes brasiliensis* in three zones of the Argentine Sea (BAP, Buenos Aires Province; SMG, San Matías Gulf; NG, Nuevo Gulf). (a) Qualitative similarity within each zone and between zones and (b) quantitative similarity within each zone and between zones.

Intensity of infection by parasites in fish populations commonly increases with the age or size of fish hosts (Dogiel *et al.*, 1958). This increase sometimes ceases beyond a certain host age or size, but the positive relationship generally holds when several size classes are pooled (Poulin, 2000). Thus the effect of fish size on parasite populations can account for a large proportion of variability in parasite community attributes such as species richness (Guégan *et al.*, 1992; Poulin & Valtonen, 2001; Timi & Poulin, 2003), and therefore can interfere in comparative studies if it is not controlled.

In the present study, fish from NG were significantly larger than those in the other two zones; however, the effect of these differences on comparisons among zones of parasite population and community structure can be disregarded first because there was not a consistent pattern of parasite abundance–host size relationship for any species in all zones; second, some species (*e.g.* *P. argentinensis* and *N. spinicephala*), in spite of showing significant increases with fish size in NG, displayed similar abundances in other zones where fish were smaller; third, some parasite larvae (*e.g.* *Grillotia* sp. and *S. polymorphus*), with a cumulative effect observed in BAP, were

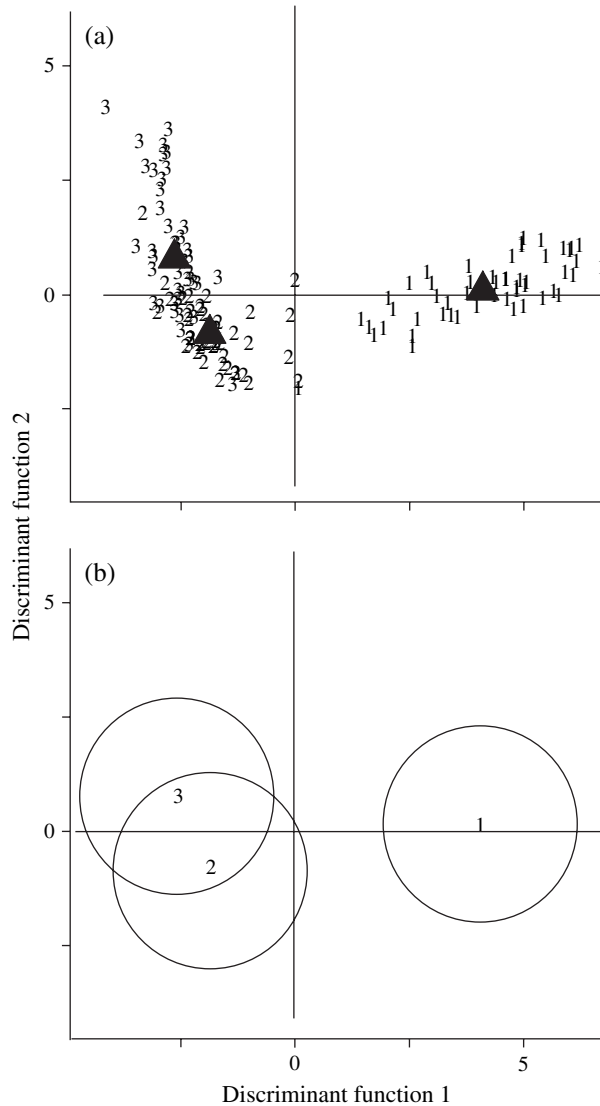


FIG. 2. Sample scores of the first two discriminant functions for specimens of *Pinguipes brasilianus* in three zones of the Argentine Sea (1, Buenos Aires Province; 2, San Matias Gulf; 3, Nuevo Gulf). (a) Numbers represent each fish and  $\blacktriangle$  represent group averages and (b) numbers represent group averages.  $\circ$  around group means represent the 90% tolerance regions (e.g. 90% of the observations in a group are expected to lie in this region).

more abundant in this zone than in NG where fish were larger; fourth, three species (*N. georgenascimentoi*, Opecoelidae gen. sp. and *T. tortuosus*), were significantly more abundant in SMG than in BAP, despite no differences in host size being observed between the two zones. Therefore a cause other than host size must account for the observed differences between zones.

In aquatic systems, food web structure has been hypothesized to have some influence on transmission rates of some parasite species (Marcogliese, 2002), so



TABLE IV. Discriminant analysis classification showing the numbers and percentages of fish classified in each zone (rows correspond to group memberships)

	BAP	SMG	NG	Percentage of correctly classified fish per zone
BAP	53	1	0	98.15
SMG	0	43	7	86.00
NG	0	12	38	76.00

BAP, Buenos Aires Province; NG, Nuevo Gulf; SMG, San Matías Gulf.

certain host features, such as feeding habits, can influence the number of parasite species exploiting a host species (Luque *et al.*, 2004). Although the feeding habits of Brazilian sandperch were similar among zones, variations in diet composition could explain the differences found in both faunal composition and endoparasite burden. Accordingly, differential prey availability and its impact on diet composition have been highlighted as causes of spatial changes in parasite burden at a regional scale for other fish species in the same region (Timi, 2003; Sardella & Timi, 2004). On the other hand, the geographical distribution of ectoparasite species, with direct life cycles, is mainly determined by environmental conditions. These conditions also have an influence on the distribution of endoparasites, either directly, or *via* their effects on other host species (MacKenzie & Abaunza, 1998). Indeed, local environmental factors can regulate the survival and transmission success of infective stages causing inter-population variations of parasite burdens (Pietroock & Marcogliese, 2003).

At parasite population level, significant differences in both prevalence and abundance between zones for most parasite species demonstrate the potential for discriminating three discrete Brazilian sandperch stocks by their parasites. The higher similarity observed between infracommunities in Patagonian samples than the comparisons with northern fish corroborate the finding at parasite population level. This is not surprising since in analyses of similarity of parasite communities and their relationship with habitat characteristics, the distance

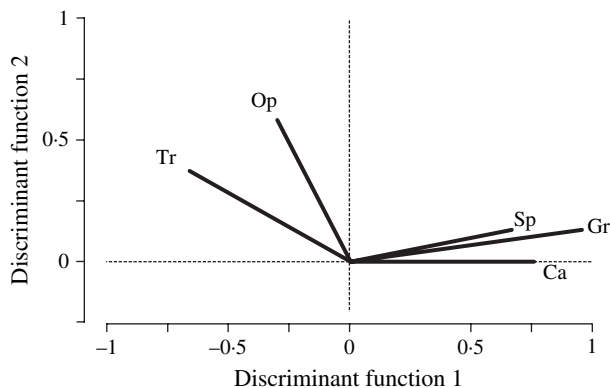


FIG. 3. Canonical correlations between the first two discriminant functions and the parasites of *Pinguipes brasiliensis* selected as biological tags (Ca, *Corynosoma australe*; Gr, *Grillotia* sp.; Op, Opecoelidae gen. sp.; Sp, *Scolex polymorphus* and Tr, *Trifur tortuosus*).

between sampling localities usually emerges as the best predictor of similarity (Poulin & Morand, 1999) and a decay of the similarity in the composition of parasite communities is a common phenomenon (Poulin, 2003).

The structure of parasite communities in those hosts inhabiting the boundaries of their geographical distribution is affected mainly by ecological factors (Kennedy & Bush, 1994). Brazilian sandperch living in the Patagonian gulfs are at the southern limit of their distribution (Froese & Pauly, 2007) which is probably the cause of the heterogeneity of their parasite infracommunities, specially in SMG where parasite burdens are comparatively lower, and these low prevalences of parasitism for most species produced lower values of Jaccard indexes.

Evidence supporting the existence of three different stocks was also obtained by multivariate analysis. Results of discriminant analysis clearly showed that fish from the three areas represented discrete units, although fish from SMG and NG clumped closer to each other. The parasite species which contributed most to the separation of the samples, agreed with those identified as potential biological markers in the analyses at population level. Northern samples were characterized by higher values of prevalence and abundance of *Grillotia* sp., *C. australe* and *S. polymorphus*. *Trifur tortuosus* and Opecoelidae gen sp., however, were important in determining the position of Patagonian samples particularly those from the Nuevo Gulf, whereas none of the species was clearly related to fish from San Matías Gulf.

Parasite species characteristic of Brazilian sandperch from Buenos Aires Province (*Grillotia* sp., *C. australe* and *S. polymorphus*), which displayed very low specificity among fish species in this area, have been used previously as biological tags for other host species in these regions (Timi, 2003; Sardella & Timi, 2004; Timi *et al.*, 2005), confirming their value as tags for stock delineation.

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