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## Similarity in parasite community structure may be used to trace latitudinal migrations of *Odontesthes smitti* along Argentinean coasts

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The aims of this study were to determine the existence of migratory movements and to identify ecological stocks of the silverside *Odontesthes smitti* along its distribution in the Southern Atlantic Ocean, using metazoan parasites as biological tags. Samples were obtained from San José Gulf (SJ) (42° 25' S; 64° 07' W) and Nuevo Gulf (NG) (42° 47' S; 65° 02' W) in north Patagonia during winter and summer and in waters off Mar del Plata (MDP) (38° 03' S; 57° 32' W), Bonaerense region, during winter. Fifteen parasite species were collected. Multivariate statistical procedures on parasite community data showed strong effect of host size on the structure of parasite assemblages. Taking into account the variations among samples due to host size, the differential structure of parasite communities between SJ and NG suggests that fish inhabiting these localities could belong to different ecological stocks. Fish from MDP and SJ caught in summer showed similar composition in their parasite assemblages, which is congruent with a migratory cycle that implies that fish caught in MDP during winter inhabit SJ during summer. Further evidence of the Patagonian origin of MDP *O. smitti* is the presence of the digenean *Proctotrema bartolii* in fish from both regions. *Proctotrema bartolii* is acquired by *O. smitti* only in the Magellanic province, where its intermediate host, *Darina solenoides*, is distributed. The analyses suggest that *O. smitti* inhabiting north Patagonian gulfs could belong to different ecological stocks and that *O. smitti* caught in MDP could have come from SJ.

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**Key words:** Atherinopsidae; biological tags; ecological stocks; metazoan parasites; south-western Atlantic Ocean.

### INTRODUCTION

The silverside *Odontesthes smitti* (Lahille 1929) (Atherinopsidae) is distributed in the south-west Atlantic Ocean from southern Uruguay to Tierra del Fuego, including the Malvinas Islands (Cousseau & Perrotta, 2004) and in southern Chile in the

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Pacific Ocean (Sielfeld & Vargas, 1999; Dyer, 2006). This species is one of the main components of artisanal fisheries in north Patagonian gulfs, Argentina, where it constitutes an important regional resource (Elías *et al.*, 1991). *Odontesthes smitti* represents an important target of artisanal fisheries, in terms of volume and availability, all year round in north Patagonian gulfs, such as Nuevo Gulf (Elías *et al.*, 1991). Nevertheless, it is present only during the colder months (July to August) at lower latitudes (Cousseau & Perrotta, 2004), where it is targeted mainly by recreational anglers.

The presence of *O. smitti* for such a short period in the northern Argentine sea (Bonaerense region) means that it migrates seasonally to that region from southern areas, but their geographical origin has not been assessed. A migratory behaviour from north Patagonia to Bonaerense waters implies not only long migratory periods but also their transit through waters with dissimilar oceanographic conditions belonging to two different faunistic provinces of the South American coasts: the Argentine Province (Bonaerense region) and the ecotone between the Argentine and Magellanic Provinces (Bogazzi *et al.*, 2005; Cousseau & Figueroa, 2010).

In the Argentine Sea, discriminating fish populations between Patagonian and Bonaerense waters by using parasites has been successful, and parasite species typical of Bonaerense waters have been identified (Timi, 2003, 2007; Sardella & Timi, 2004; Braicovich & Timi, 2008; Timi *et al.*, 2008; Timi & Lanfranchi, 2009a); even the same hosts compared between north Patagonian gulfs have shown different composition of their parasite faunas, and consequently are identified as different populations (Timi *et al.*, 2008).

The parasite fauna of *O. smitti* from north Patagonian gulfs has been reported by Carballo & Navone (2007), Carballo (2008) and Carballo *et al.* (2011a), but there are no previous studies on this species in the Bonaerense region, except for a record of the copepod *Bomolochus globiceps* (Vervoort & Ramírez, 1968; Timi & Etchegoin, 1998). There is no information on whether schools of *O. smitti* arriving in Bonaerense waters during winter come from different southern gulfs, but if this is the case, no differential abundance of parasite species typical of lower latitudes should be observed among *O. smitti* caught in Patagonian gulfs. This hypothesis was tested by comparing the parasite burdens of *O. smitti* caught during cold and warm seasons in San José and Nuevo Gulfs (Patagonia) with samples from the Bonaerense region, caught during winter. Secondary aims were to identify the existence of different ecological stocks in north Patagonian gulfs and to characterize the parasite fauna of *O. smitti* in the Bonaerense region.

## MATERIALS AND METHODS

A total of 177 adult only specimens of *O. smitti* were examined for parasites to minimize the possible influence of host size on parasite burden. Fish were caught by artisanal fishermen using coastal nets in three zones along the coast of the Argentine Sea, two of them in north Patagonian gulfs, San José (SJ) and Nuevo Gulfs (NG) and in waters off Mar del Plata, Bonaerense region (MDP) (Fig. 1). Because its migratory behaviour depends on water temperature, rather than being strictly seasonal, samples were collected during summer and winter periods. Details of fish samples are given in Table I.

Fish were either kept in plastic bags fresh or deep frozen at  $-18^{\circ}\text{C}$  until examination. After thawing, *O. smitti* were measured for total length  $L_T$  (cm), and parasites recovered

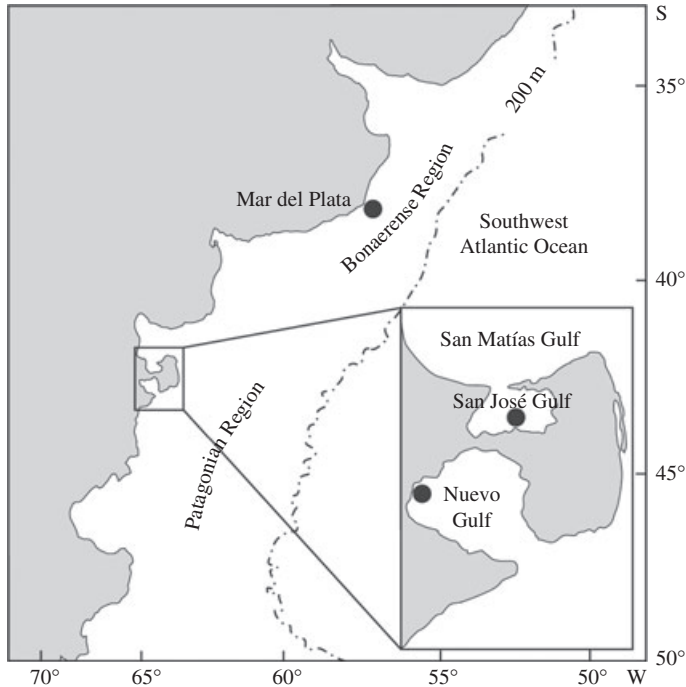


FIG. 1. Map showing the study areas and the sampling sites.

from the body surface, fins, gills, branchial and body cavities and viscera (stomach, intestine, liver, gonads and mesenteries) using stereoscopic microscopy.

The  $L_T$  was compared between samples by means of a one-way ANOVA (Zar, 1984). Prevalence, mean abundance and mean intensity were calculated for each parasite species in each sample following Bush *et al.* (1997).

Multivariate statistical procedures on parasite community data were implemented in PRIMER v. 6 (Clarke & Warwick, 2001; Clarke & Gorley, 2006) and PERMANOVA+ for PRIMER (Anderson *et al.*, 2008) packages. PERMANOVA is a non-parametric multivariate procedure that performs distance-based analysis of variance to test hypotheses with permutation procedures. It avoids the assumption of normal distributions of the response

TABLE I. Composition of samples of *Odontesthes smitti* in three areas of the south-western Atlantic Ocean

Area	Position	Season	Date of capture	$n$	$L_T$ (mean $\pm$ s.d.) (cm)
San José Gulf	42° 25' S; 64° 07' W	Winter	July 2004	32	22.62 $\pm$ 1.23
		Summer	January 2005	31	28.29 $\pm$ 2.26
Nuevo Gulf	42° 47' S; 65° 02' W	Winter	August 2004	23	26.02 $\pm$ 2.10
		Summer	February 2005	32	21.73 $\pm$ 1.55
Mar del Plata	38° 03' S; 57° 32' W	Winter	July 2008	59	25.69 $\pm$ 1.64

$L_T$ , total length;  $n$ , sample size.

variables, which are not met when analysing some community data (Anderson *et al.*, 2008), including those with large numbers of species absences for infrequent taxa. The Bray-Curtis index, which takes into account differences in abundance of each shared parasite species (Magurran, 1988), was used as similarity measure in all analyses. The original data were fourth-root transformed using the transformation module of the PRIMER program to reduce the importance of very abundant species, so that the less dominant and even rare species play some role in determining the similarity of samples (Clarke & Gorley, 2006).

First, the structure of parasite infracommunities between gulfs and seasons ( $2 \times 2$  factorial design, both as fixed factors) were compared, testing for main effects and factor interactions, after 9999 permutations. Where differences were detected by PERMANOVA, pair-wise comparisons were used to determine which groups differed within factors. In this analysis, samples from MDP were excluded because of the higher geographical distance separating them from both gulfs and because only fish caught in winter (as this fish species is absent from this zone during summer) were available for comparison. The differences in community composition were tested by means of a two-way crossed permutational multivariate analysis of the variance (PERMANOVA). Following Anderson *et al.* (2008), a permutation of residuals under a reduced model was used as the method of permutation. A sequential sum of squares (type I SS) was applied because samples were unbalanced (different numbers of fish examined by sample) and because host size was introduced as a covariable (ANCOVA model). The  $L_T$  was included as a covariable because differences in host size were detected among samples. Therefore, the procedure was repeated after swapping the order of factors. Since PERMANOVA is sensitive to differences in multivariate dispersion between groups (*sensu* homogeneity of variances, which can inflate type I error even when centroids have identical locations), the same models were tested for differences in dispersion using the routine PERMDISP (Anderson *et al.*, 2008). Dispersions were measured as distances to the centroids, and each term in the analysis was tested using 9999 permutations, with significant terms investigated using *a posteriori* pair-wise comparisons with the PERMANOVA *t* statistic (Anderson *et al.*, 2008). As significant differences were observed between all pairs of samples, samples from MDP were then included in a one-way PERMANOVA to detect differences between samples.

Differences in infracommunities among samples were examined in more detail using canonical analysis of principal co-ordinates (CAP) (Anderson & Willis, 2003; Anderson *et al.*, 2008). This is a canonical discriminant analysis on a subset of principal co-ordinate (PCO) axes from a metric (classical) multidimensional scaling. A major advantage of this method is that, through the use of PCO, the constrained analysis can be based on any distance measure that is appropriate for the type of dataset and hypotheses being addressed (Bray-Curtis in this case). The CAP ordination displays multivariate data in reference to *a priori* hypotheses. Within the routine, principal co-ordinates (PCO) were calculated from the resemblance matrix, and potential over-parameterization was prevented by choosing the number of PCO axes (*m*) that maximised leave-one-out allocation success to groups (Anderson & Robinson, 2003). To test for significant differences in infracommunities among the samples, a permutation 'trace' test (sum of squared canonical eigenvalues) was applied, and *P* was obtained after 9999 permutations. For CAP analyses, hosts from MDP were divided randomly into two subsamples; a subsample of 20 hosts was used as a 'blind' sample in the 'adding new samples' subroutine of the CAP analyses. The remaining hosts were considered as representatives of MDP to perform the CAP model, and the new samples were placed onto the canonical axes of the obtained CAP model, with each being classified into one of the existing groups.

An indication of the underlying species differences in community structure was obtained by the strength of their correlation with the canonical discriminant axes co-ordinates, with diagnostic compounds visualized using vector overlays based on Spearman correlations. Only species correlations of  $|r| > 0.3$  were considered significant.

Prevalence of parasites is commonly used as a quantitative descriptor of parasites as biological tags, so to visualize patterns of component community level, non-metric multidimensional scaling (MDS) was conducted using the Bray-Curtis similarity index on data of parasite prevalence in each sample. The fit of MDS ordinations was quantified by a value of stress. Hierarchical agglomerative clustering was performed for both the samples and the parasite species using group-average linking, and resemblance levels were overlaid on the MDS plot (Clarke & Gorley, 2006).

## RESULTS

### GENERAL RESULTS

Mean host  $L_T$  were significantly different among samples ( $F_{4,172} = 69.6$ ,  $P < 0.01$ ), with only fish from SJ winter and NG summer, and from NG winter and MDP, showing no differences in  $L_T$  (both  $P > 0.05$ ).

Only three *O. smitti* in the overall sample were free of parasites, all of them from the NG summer sample. All other hosts were parasitized by at least one of 15 parasite species (Table II), including two ectoparasite species, seven larval endoparasites and six adult endoparasites. The maximum infracommunity species richness (seven parasite species) was found in only one fish. A total of 38 641 metazoan parasites were found in the overall sample, which results in a mean total abundance of 218.3 per fish. *Prosorhynchoides* sp. was the dominant species in all samples, accounting for 81.2% of all parasites found. The highest mean total abundance was observed in fish from SJ and the lowest in samples from NG (Table II).

### MULTIVARIATE ANALYSES

Uninfected fish ( $n = 3$ ) were excluded from the multivariate analyses. PERMANOVA results showed a strong effect of host size on the response variables and therefore on community structure (Table III). The lack of interaction of host  $L_T$  with both factors shows that the nature of the relationship between the covariate and the multivariate responses did not differ among levels of the factors. Nevertheless, taking into account the variations among samples due to host size, significant variability was detected among the assemblages at each level of both factors. Parasite infracommunity composition was significantly different between gulfs and seasons with a significant interaction of both factors (Table III), which shows that differences in the composition of assemblages between seasons were not uniform across gulfs. Pair-wise comparisons between seasons, carried out separately in each gulf, showed that there were significant differences in both localities (SJ:  $t = 1.98$ ,  $P$  (perm)  $< 0.01$ ; NG:  $t = 2.33$ ,  $P$  (perm)  $< 0.001$ ). The same results were obtained for pair-wise tests between gulfs, within each season (winter:  $t = 2.96$ ,  $P$  (perm)  $< 0.001$ ; summer:  $t = 2.47$ ,  $P$  (perm)  $< 0.01$ ). Significant differences were also observed in all pair-wise comparisons after changing the order of factors.

PERMANOVA results on the five samples (including fish from MDP) showed a strong effect of host size on community structure (Table IV). The lack of interaction between host  $L_T$  and locality, however, showed that the nature of the relationship between the covariate and the multivariate responses did not differ among levels of this factor. Nevertheless, taking into account the variations among samples due to host size, significant variability was detected among the levels of this factor. Pair-wise tests between localities showed that there were significant differences between most pairs of localities, except for those samples, with highest average similarity, from SJ caught in summer and MDP (Table V), which also showed the highest values of average similarity. Comparisons involving *O. smitti* caught during summer in NG always displayed lower values of average similarity, whereas those caught in winter in the same gulf showed similarity levels equivalent to those found in the comparisons between both samples from SJ and MDP.

TABLE II. Taxonomic composition, microhabitat, prevalence and mean abundance of *Odontesthes smitti* in three areas of the Argentine Sea during winter and summer. The development stage is only indicated for non-adult species

	Site	Prevalence				Mean $\pm$ s.d. abundance							
		SJ		NG		SJ		NG		MDP			
		winter	summer	winter	summer	winter	summer	winter	summer	winter	summer		
<b>Digenea</b>													
<i>Proserfynchooides</i> sp. (ME)	Fins, eyes, muscle, opercula, heart	100	100	96	81	100	138.5 $\pm$ 97.9	312.1 $\pm$ 100.5	128.2 $\pm$ 107.3	130.2 $\pm$ 179.0	233.8 $\pm$ 131.4		
<i>Proctotremia bartolii</i>	Stomach/intestine	9	3	4	0	3	152 $\pm$ 582.0	0.5 $\pm$ 3.0	18.5 $\pm$ 88.6	0.0 $\pm$ 0.0	3.3 $\pm$ 24.7		
<i>Lecithaster</i> sp.	Intestine	66	0	4	3	3	6.7 $\pm$ 12.9	0.0 $\pm$ 0.0	0.04 $\pm$ 0.2	0.03 $\pm$ 0.2	0.03 $\pm$ 0.2		
<i>Aponurus laguncula</i>	Stomach/intestine	6	10	0	0	7	0.1 $\pm$ 0.6	0.1 $\pm$ 0.4	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.1 $\pm$ 0.3		
<i>Diphtherostomum</i> sp.	Intestine	6	0	0	3	3	0.1 $\pm$ 0.2	0.0 $\pm$ 0.0	0.0 $\pm$ 1.0	0.03 $\pm$ 0.2	0.5 $\pm$ 3.6		
<b>Cestoda</b>													
<i>Scolex polymorphus</i> (PL)	Stomach	3	0	0	3	10	0.3 $\pm$ 1.8	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.03 $\pm$ 0.2	0.9 $\pm$ 3.5		
<i>Bothrioccephalidea</i> gen. sp. (PL)	Intestine	0	0	0	0	3	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.1 $\pm$ 0.4		
<b>Nematoda</b>													
<i>Huffmanella moravecii</i>	Gills/opercula/fins	31	26	70	12	22	0.8 $\pm$ 1.7	0.6 $\pm$ 1.4	2.3 $\pm$ 3.8	0.2 $\pm$ 0.4	0.5 $\pm$ 1.2		
<i>Cucullianus marplatensis</i>	Stomach/intestine	75	84	100	59	85	2.8 $\pm$ 3.4	11.4 $\pm$ 18.3	11.5 $\pm$ 6.3	1.7 $\pm$ 2.1	4.9 $\pm$ 5.2		
<i>Cosmocephalus obvelatus</i> (LIII)	Liver	0	0	0	6	7	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.1 $\pm$ 0.2	0.1 $\pm$ 0.4		
<i>Ascarophis marina</i> (LIII)	Body cavity	0	0	0	0	2	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.03 $\pm$ 0.3		
<i>Pseudoterranova</i> sp. (LIII)	Muscle of body wall	3	0	0	0	0	0.3 $\pm$ 0.2	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0		
<b>Acanthocephala</b>													
<i>Corynosoma australe</i> (JU)	Body cavity/liver	31	48	17	3	54	0.7 $\pm$ 1.6	0.8 $\pm$ 1.2	0.2 $\pm$ 0.5	0.03 $\pm$ 0.2	1.1 $\pm$ 1.5		
<b>Copepoda</b>													
<i>Bomolochus globiceps</i>	Opercula/branchial arches	53	45	30	28	46	0.9 $\pm$ 1.4	0.8 $\pm$ 1.2	0.4 $\pm$ 0.7	0.4 $\pm$ 0.9	0.7 $\pm$ 0.9		
<i>Penniculus</i> sp.	Fins/body surface	3	6	30	0	5	0.03 $\pm$ 0.2	0.1 $\pm$ 0.2	0.3 $\pm$ 0.6	0.0 $\pm$ 0.0	0.05 $\pm$ 0.2		
<b>Total</b>		100	100	100	81	100	303.0 $\pm$ 587.3	326.5 $\pm$ 108.7	163.5 $\pm$ 185.0	16.7 $\pm$ 20.7	246.1 $\pm$ 131.4		

JU, Juvenile; LIII, third stage larva; MDP, Mar del Plata; ME, metacercaria; NG, metacercaria; PL, plerocercoid; SJ, San José Gulf.



TABLE III. Two-factors crossed PERMANOVA results of fourth-root transformed infracommunity abundance data of parasites of *Odontesthes smitti*, based on Bray-Curtis dissimilarity measures, for gulfs (fixed, two levels) and season (fixed, two levels), with host total length ( $L_T$ ) as covariable. The  $P$ -values were obtained using 9999 permutations

Source	d.f.	SS	MS	$F$	$P$ (perm)
$L_T$	1	13 058	13 058	17.93	<0.001
Gulf	1	16 291	16 291	22.37	<0.001
Season	1	7618.3	7618.3	10.46	<0.001
$L_T \times$ gulf	1	677.2	677.2	0.93	>0.05
$L_T \times$ season	1	1614	1614	2.21	>0.05
Gulf $\times$ season	1	3602.6	3602.6	4.95	<0.001
$L_T \times$ gulf $\times$ hboxseason	1	406.8	406.8	0.56	>0.05
Residual	106	77 215	728.4		
Total	113	1.2048e <sup>5</sup>			

Dispersion of parasite infracommunities varied among samples in terms of their deviations from centroids (Table VI), with significant variability ( $F_{4,169} = 8.6966$ ;  $P$  (perm) < 0.001). *A posteriori* tests underlined significant differences for five of the 10 pair-wise comparisons (Table V).

The CAP analysis showed significant differences among samples ( $tr = 0.95$ ;  $P < 0.001$ ) (Fig. 2). The selected orthonormal PCO axes ( $m = 4$ ) described 88% of the variation in the data 'cloud', although the percentage of correct allocations was low (50.65%). The first two canonical axes resulting from CAP analysis clearly separated samples, and strong association between the multivariate data 'cloud' and the hypothesis of group differences was indicated by the reasonably large size of their canonical correlations ( $\delta_1 = 0.83$  and  $\delta_2 = 0.49$ ). Indeed, samples from NG were distinguishable from those of SJ and MDP and were situated at the left of the CAP biplot, while those from summer occupied the upper corner, separated from the winter samples. No clear separation was observed among the remaining samples, but those from MDP were situated at the upper right corner of the CAP biplot. When vectors corresponding to Spearman correlations of individual species were superimposed with the CAP axes (restricted to those species having  $r > 0.3$ ), *Prosohynchoides* sp. and *Corynosoma australe* were related to northern samples,

TABLE IV. One-way PERMANOVA results of fourth-root transformed infracommunity abundance data of parasites of *Odontesthes smitti*, based on Bray-Curtis dissimilarity measures, for localities (fixed, five levels), with host total length ( $L_T$ ) as covariable. The  $P$ -values were obtained using 9999 permutations

Source	d.f.	SS	MS	$F$	$P$ (perm)
$L_T$	1	14 906	14 906	22.33	<0.001
Locality	4	34 496	8264	12.92	<0.001
$L_T \times$ locality	4	2471	61 774	0.93	>0.05
Residual	164	1.0946e <sup>5</sup>	66 747		
Total	173	1.6134e <sup>5</sup>			

TABLE V. Average similarity (Bray-Curtis index) between parasite infracommunities of five samples of *Odontesthes smitti* and result of pair-wise tests comparing their multivariate community structure (PERMANOVA) and dispersion (PERMDISP)

Comparisons	Average Similarity	PERMANOVA		PERMDISP	
		<i>t</i>	<i>P</i> (perm)	<i>t</i>	<i>P</i> (perm)
SJw–SJs	62.43	1.98	0.0047	4.18	0.0004
SJw–NGw	57.28	2.96	0.0001	1.70	>0.05
SJw–NGs	45.77	3.88	0.0001	1.50	>0.05
SJw–MDP	62.08	2.49	0.0002	2.83	0.0119
SJs–NGw	64.24	4.28	0.0001	1.83	>0.05
SJs–NGs	46.86	2.40	0.0026	4.41	0.0001
SJs–MDP	73.02	1.33	>0.05	1.98	>0.05
NGw–NGs	50.91	2.28	0.0025	2.52	0.0161
NGw–MDP	62.61	4.30	0.0001	0.38	>0.05
NGs–MDP	48.05	3.63	0.0001	4.08	0.0004

MDP, Mar del Plata; NG, Nuevo Gulf; s, summer; SJ, San José Gulf; w, winter.

whereas the abundances of *H. moravecii* and *C. marplatensis* were mainly associated to the separation of *O. smitti* from NG along the second axis.

Cross validation results showed that the lowest per cent of correctly allocated fish occurred for the MDP subsample, with near 44% of hosts allocated to the SJ winter sample and only 33% to their own sample. Conversely, a high proportion of fish from SJ summer were allocated to MDP. Both samples from NG showed the highest values of correctly allocated hosts, especially those from summer. Finally,

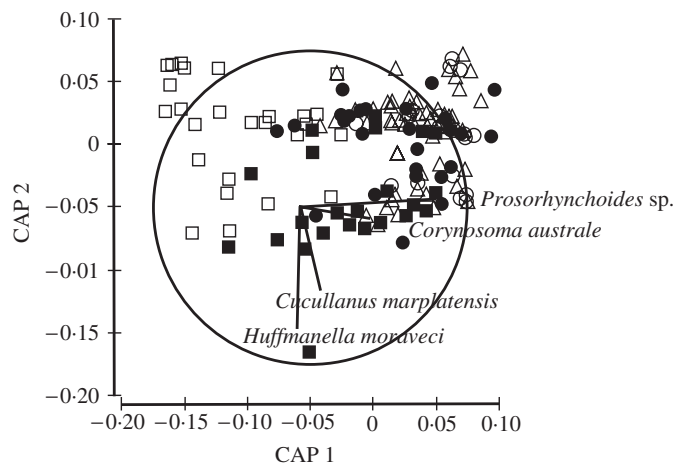


FIG. 2. Canonical analysis of principal co-ordinates (CAP) biplot of parasite abundance in five samples of *Odontesthes smitti* from three areas and two seasons (■, Nuevo Gulf winter; □, Nuevo Gulf summer; ●, San José Gulf winter; ○, San José Gulf summer; △, Mar del Plata) in the Argentine Sea. The analysis was based on Bray-Curtis dissimilarities calculated from untransformed data, and vector overlay are Spearman correlations of parasite species with the CAP axes (restricted to those having  $r > 0.3$ ).



TABLE VI. Average similarity (Bray-Curtis index) between parasite infracommunities and average dispersion in terms of deviations from centroids within five samples of *Odontesthes smitti*

Sample	Average similarity	Average dispersion	S.E. dispersion
SJ winter	62.62	26.192	1.8304
SJ summer	75.98	16.979	1.2016
NG winter	68.97	21.316	2.2221
NG summer	53.75	31.574	3.172
MDP	70.76	20.47	1.1081

MDP, Mar del Plata; NG, Nuevo Gulf; SJ, San José Gulf.

*O. smitti* from the 'blind' MDP sample were mainly allocated to samples from SJ (75% of them), especially to the summer sample (45%), with only 15% of fish being correctly allocated to MDP, which is below the 20% allocation success for five groups expected by chance alone (Table VII).

Multidimensional scaling of parasite prevalences revealed an apparent pattern of separation between samples from both gulfs, and the stress level (0.0) indicated a community structure substantially different from random (Fig. 3). Fish from MDP were clearly associated to those from SJ caught in summer, and both samples clustered together with those from SJ winter. *Odontesthes smitti* from NG caught in summer were the most different in terms of parasite component community structure, calculated in terms of prevalence.

## DISCUSSION

Most parasite species harboured by *O. smitti* were present in the three samples, with the exception of Bothriocephalidea gen. sp. and *Ascarophis marina*, which were only present in the Bonaerense region. Nonetheless, their low values of prevalence and abundance, as well as those of *Pseudoterranova* sp., suggest that these species are accidental parasites of *O. smitti*. The exclusion of these species leads to a value

TABLE VII. Results of the cross validation of analysis of principal co-ordinates (CAP) analyses (leave-one-out allocation of individual *Odontesthes smitti* to one of five samples for the choice of  $m = 4$ )

Origin group	Classified to					% Correct
	SJ winter	SJ summer	NG winter	NG summer	MDP	
SJ winter	12	8	4	2	6	37.500
SJ summer	2	16	2	0	11	51.613
NG winter	4	2	13	3	1	56.522
NG summer	2	0	3	24	0	82.759
MDP	4	17	5	0	13	33.333
MDP 'blind'	6	9	2	0	3	15.000

MDP, Mar del Plata; NG, Nuevo Gulf; SJ, San José Gulf.

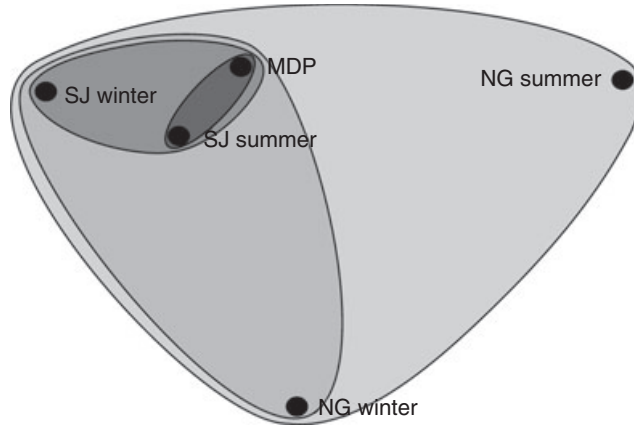


FIG. 3. Non-metric two-dimensional (MDS) ordination plot using Bray-Curtis similarity based on untransformed prevalence data in five samples of *Odontesthes smitti* from two seasons and three areas (MDP, Mar del Plata; NG, Nuevo Gulf; SJ, San José Gulf) of the Argentine Sea. Results of a hierarchical agglomerative clustering performed for samples are overlaid on the MDS plot with similarity levels represented by a grey scale. □, 60; ▤, 70; ▥, 80; ▦, 90.

of total species richness ( $S = 12$ ) similar to those recorded in other small-bodied fishes that also occupy a low level in the regional trophic web (Lanfranchi *et al.*, 2009; Timi & Lanfranchi, 2009b). In fact, small size and low trophic level are important host features determining low species richness in the parasite assemblages of marine fishes (Guégan *et al.*, 1992; Poulin, 2000; Luque *et al.*, 2004; Luque & Poulin, 2008).

A recurrent pattern seen in the parasite assemblages of marine fishes from the Bonaerense region, where several of these assemblages have been studied, is the higher species richness and numerical dominance of larval parasites with respect to adult endoparasites (Timi, 2007; Lanfranchi *et al.*, 2009; Timi & Lanfranchi, 2009b; Timi *et al.*, 2010). On the other hand, little is known about the structure of parasite communities in fishes from north Patagonian gulfs (González *et al.*, 2006; Carballo, 2008; Timi *et al.*, 2010). The parasite communities of *O. smitti* and *Odontesthes nigricans* (Richardson 1848) from north Patagonian gulfs showed lower species richness than those of fishes from the Bonaerense region, and only two of the larval endoparasites (*Proisorhynchoides* sp. and *C. australe*) had a high prevalence and abundance (Carballo *et al.*, 2011a). In the same region, adult endoparasites are the richest guild in the assemblages harboured by the sandperch *Pinguipes brasiliensis* Cuvier 1829 (Timi *et al.*, 2010). Although more studies would be necessary to detect a possible repeated pattern in the structure of parasite assemblages from north Patagonian gulfs, the richness of adult endoparasites, even in samples of *O. smitti* from MDP, is different from that of fishes from the Bonaerense region. Thus, the structure of the assemblages harboured by this *O. smitti* could be indicative of their Patagonian origin, either in the life span of individual fish or on a longer time scale.

The observed strong effect of host size on the structure of parasite assemblages is not surprising because time combined with ontogenetic shifts in habitat or diet can generate differences between hosts of different ages. After taking into account the

effect of host  $L_T$ , significant differences were observed between the gulfs and the seasons.

The differences in parasite community structure observed between gulfs are consistent with the hypothesis that fish inhabiting these localities belong to different ecological stocks. Previous studies using parasites as biological tags to discriminate fish populations between NG and San Matías Gulf (to which SJ is connected) have shown the same results, *i.e.* for *P. brasiliensis* (Timi *et al.*, 2008). Indeed, those fish species inhabiting San Matías Gulf studied so far, from a parasitological perspective, harbour parasite assemblages different enough to be considered as discrete ecological stocks; this is the case for the hake *Merluccius hubbsi* Marini 1933 (Sardella & Timi, 2004), the flathead *Percophis brasiliensis* Quoy & Gaimard 1825 (Braicovich & Timi, 2008) and *P. brasiliensis* (Timi *et al.*, 2008). The observed seasonal variations can not only arise from the seasonal dynamics of individual parasite species but also from the migratory behaviour of *O. smitti*, or a combination of both variables. The inclusion of samples from MDP, after considering those from the gulf as independent samples, again showed a strong effect of host size and sample, with no interaction between the variables. The effect of differences in the variability of their infracommunities could account for the differences observed between some pairs of samples, especially between seasonal samples within each gulf, which displayed higher *P*-values in the PERMANOVA analysis. In spite of these differences, which could be artifacts due partially or wholly to differences in the dispersion of their infracommunities, the differences in dispersion could indicate that real biological processes underpin these dispersion patterns. These could involve differential environmental and biological processes between gulfs, as well as migratory behaviour of fish, not only between gulfs but also from and to southern populations. In fact, the similarity between *O. smitti* caught during winter in NG with those from SJ obtained in MDS could be the outcome of migratory movements of fish between these gulfs. The higher prevalence of *C. australe* and *Proisorhynchoides* sp. in winter compared to summer could indicate that the main direction of the displacement is from SJ to NG in winter. In any case, only fish from MDP and SJ caught in summer showed similar composition in their parasite assemblages, which is congruent with a migratory cycle that implies that fish caught in MDP during winter inhabit SJ during summer. The temporal persistence of the structure of parasite assemblages of *O. smitti* is also indirectly supported by the fact that the most similar samples (MDP and SJ) were caught 4 years apart.

The CAP analysis provided further evidence of the differences between samples, especially between gulfs. The low per cent of correct allocations was mainly due to misclassifications between MDP and SJ summer, although a large proportion of fish from SJ winter were also wrongly assigned to SJ summer, being responsible for the poor discrimination among fish from SJ. Similarity between MDP and SJ summer samples was also supported by the allocation of *O. smitti* from the 'blind' sample.

The higher abundances of *Proisorhynchoides* sp. and *C. australe* in samples from SJ and MDP, and also of *Proisorhynchoides* sp. in NG winter, were reflected in the superimposition of their vectors on the CAP axis. According to Lester & MacKenzie (2009), only permanent parasites (those expected to be recognizable for most of the life of the fish) are useful for stock discrimination. In the present study, although the whole parasite community was included in the comparisons, *Proisorhynchoides*

sp. and *C. australe* were the main taxa responsible for the differences in community structure. Given that they were found as larvae parasitizing tissues of *O. smitti*, they can be considered as long-lived and, therefore, good candidates for discriminating between fish populations (MacKenzie, 2002; Lester & MacKenzie, 2009) and tracking their migrations. Whereas the distribution and definitive hosts of *Prosorhynchoides* sp. are still unknown, *C. australe*, a parasite of pinnipeds (Sardella *et al.*, 2005), is mainly distributed in warmer (northern) waters in this region (Timi, 2007) and has been identified as one of the best indicators for discriminating Bonaerense from Patagonian fish populations (Timi, 2003, 2007; Sardella & Timi, 2004; Braicovich & Timi, 2008; Timi *et al.*, 2008; Timi & Lanfranchi, 2009a). The higher prevalence and abundance of this acanthocephalan in fish from MDP could indicate that it is acquired mainly in this region, during winter migrations. Variations in the abundances of *C. marplatensis* and *H. moravecii*, specific parasites of *O. smitti* found as adults in the hosts, could be due to seasonal changes in their population dynamics. This also seemed to be true for the species of *Lecithaster* and *Penniculus*, which showed high prevalence in only one of the samples. Such short-lived parasites are likely to be lost as fish migrate, so predictions about host movement from their presence may be unreliable (Lester & MacKenzie, 2009).

Results of MDS applied to parasite prevalences revealed the same general trend as those analyses based on abundances, a close resemblance between MDP and SJ summer and of both samples in relation to SJ winter. It is also noteworthy that the sample from NG winter was more similar to those from SJ and MDP compared to NG summer.

Further evidence of the Patagonian origin of MDP *O. smitti* was the presence of *Proctotrema bartolii* in fish caught in all sampling zones. *Proctotrema bartolii* is transmitted by the clam *Darina solenoides* (Mactridae), its first and second intermediate host (Carballo *et al.*, 2011b). This clam is distributed exclusively in sandy beaches from Patagonia. The northernmost limit of its distribution is the San Matías Gulf (Carcelles, 1950), thus determining the northern limit of the endemic area of *P. bartolii*. The presence of the short-lived *P. bartolii* in *O. smitti* from MDP supports the hypothesis of migration of *O. smitti* from north Patagonian gulfs to lower latitudes, especially from SJ, where high prevalence and abundance of *P. bartolii* were recorded (Carballo *et al.*, 2011b).

The results are consistent with the hypothesis that *O. smitti* inhabiting north Patagonian gulfs belong to different ecological stocks and that *O. smitti* caught in MDP come from SJ. These interpretations, however, should be taken cautiously, because all fish from MDP were caught in a single day, whereas other schools from southern populations could arrive at other times to this Bonaerense region (especially taking into account the unpredictable date of arrival and the variable time spent by *O. smitti* at MDP that has been observed for many years). Further research is necessary to establish the existence of migratory movements of *O. smitti* between gulfs during winter as well as from and to other populations inhabiting southern Patagonian regions whose boundaries and parasite faunas remain unknown.

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