



Stocks and migrations of the demersal fish *Umbrina canosai* (Sciaenidae) endemic from the subtropical and temperate Southwestern Atlantic revealed by its parasites



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ABSTRACT

The Argentine croaker *Umbrina canosai* (Sciaenidae) is a demersal fish distributed along the coasts of central Brazil, Uruguay and northern Argentina. In recent years, an increasing impact of commercial fishing has been reported, representing a high risk of collapse for this resource and enhancing the need of knowledge on its population structure. In the southwestern Atlantic fish parasites have already been demonstrated to be useful as biological tags for such purposes in other resources and are, here, analyzed to delimit the stocks of *U. canosai* and confirm its migratory route between Brazil and Argentina. A total of 193 Argentine croakers, distributed in six samples from five different localities, were examined for metazoan parasites. *Umbrina canosai* harboured 28 parasite taxa among which long-lived larvae of *Grillotia carvajalregorum*, *Corynosoma australe* and *Hysterothylacium* sp., and the adult monogenean, *Nicolasia canosorum*, were the most prevalent. Host size was related to both diversity and structure of parasite infracommunities in the whole sample, but no effect of fish sex was detected. The multivariate analyses, at both infracommunity and component community levels, showed clear geographic patterns with fish from Rio de Janeiro differentiated from their southern counterparts, confirming the presence of two discrete stocks. The results also evidenced the homogeneity of samples caught in southern Brazil and Northern Argentina, regardless of the long distances separating them, confirming the existence of a single stock with seasonal migrations between southern Brazil and northern Argentina. The information on the stock structure and migratory pathways of *U. canosai* represents a first step to the implementation of measures for a sustainable management of this important fishery resource.

1. Introduction

The Argentine croaker *Umbrina canosai* Berg, 1895 (Sciaenidae) is a demersal fish endemic in shelf waters from the subtropical and temperate Southwestern Atlantic between São Tomé cape in Brasil (22 °S) (Figueiredo and Menezes, 1980) and the north of San Matías gulf in Argentina (41° 30' S) (Cousseau and Perrotta, 2013). Warm tropical and cold temperate waters seem to limit its distribution; in northern Argentina and Uruguay it is usually found at depths between 8 m and 130 m, however, in waters where Brazilian current has greater influence, this species can be found in noticeably deeper waters, reaching 210 m deep (Nion et al., 2013). Along Rio de Janeiro it is associated

with the upwelling of colder waters on the shelf (Zaneti Prado, 1979).

Umbrina canosai is commercially exploited in Brazil, Uruguay and Argentina, being mainly fished in southern Brazil where it represents one of the main targets of gillnet and bottom trawl fisheries in coastal waters (Haimovici et al., 2006). In recent years, mid-water trawl fishing assisted by sonars is being implemented increasingly in southern Brazil (Haimovici and Cardoso, 2016). The use of this fishing gear has resulted in high yields since it captures reproductive schools of large specimens which are moving northwards along the outer shelf in autumn and winter (Haimovici and Cardoso, 2016).

Along southern Brazil *U. canosai* already was considered overfished in the early 2000's (Haimovici et al., 2006). Nowadays, increasing

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fishing pressure by mid water trawlers could lead to the economic collapse of its fishery (Haimovici and Cardoso, 2016), therefore better management of this fishing resource is necessary. An important step for sustainable management is to identify management units (Cadrin et al., 2014; Timi and MacKenzie, 2015). There is a considerable uncertainty on the connectivity of *U. canosai* along its distributional range. In fact, previous studies have suggested the existence of two stocks of *U. canosai*, one of them inhabiting the coasts of São Paulo and Rio de Janeiro states and a second one more abundant and migratory, between south of Brazil and northern Argentina (Gonzalez Alberdi and Nani, 1967; Haimovici et al., 2006). However, this stock composition has never been confirmed, although the latter has been more intensely studied (Zaneti Prado, 1979; Haimovici and Reis, 1984; Haimovici, 1988; Haimovici and Cousin, 1989; Haimovici et al., 1989, 2006; Haimovici and Cardoso, 2016). Several studies have shown that they are late winter and spring multiple spawners (Haimovici and Cousin, 1989); with larger females being the first to start spawning and to move southward (to Argentina) for feeding, followed by males and smaller females (Haimovici et al., 1989). In southern Brazil, adults are common throughout the year, however they are more abundant during winter and spring months when spawning takes place (Haimovici and Cousin, 1989). All these evidences came from observational data on catch composition, but no studies confirming the origin of Argentine migrants during warmer seasons have been so far carried out.

The use of parasites as biological tags is a methodology with increasing acceptance worldwide (Timi and MacKenzie, 2015). In the Southwest Atlantic Ocean it has been used for stock discrimination in several species of commercial fish (Timi, 2007; Cantatore and Timi, 2015), with particularly successful results when applied to resources from central-southern Brazil and northern Argentina (Timi et al., 2005, 2010a; Braicovich et al., 2012, 2016; Pereira et al., 2014; Alarcos et al., 2016; Soares et al., 2018) or for tracking migrations along the coasts (Carballo et al., 2012; Alarcos and Timi, 2013).

The aim of this study is, therefore, with the use of parasites of the Argentine croaker as indicators to 1) contribute with the delimitation of the stocks of *U. canosai* distributed between Rio de Janeiro state (Brazil) and Buenos Aires Province (Argentina), and 2) confirm the origin of migratory specimens arriving in Argentina during warmer seasons.

2. Materials and methods

2.1. Fish and parasites sampling

A total of 193 Argentine croakers, distributed in six samples from five different localities in the southwestern Atlantic (two of them caught at Rio Grande do Sul (RS) in two consecutive months: RS1 and RS2), were examined for metazoan parasites (Fig. 1, Table 1). Fish were obtained from commercial catches and deep frozen in individual plastic bags until examination. After thawing, each fish was measured (TL, cm) and sexed. Parasites were recovered from the body surface, gills, branchial and body cavities, and viscera (stomach, intestine, liver, gonads, and mesenteries) after examination under a stereoscopic microscope. Each parasite was identified and counted and the prevalence and mean abundance were calculated, for each species in each sample, following Bush et al. (1997). The Brillouin's index of diversity (HB) (Magurran, 1988) was calculated for each individual fish harbouring two or more parasite species.

The total length of fish was compared across samples by a one-way permutational multivariate analysis of the variance (PERMANOVA, Anderson et al., 2008) on the Euclidean distances (1×6 factorial design, 'sample' as fixed factor), testing for main effects after 9999 permutations and subsequent post-hoc pair-wise comparisons. Following Anderson et al. (2008), an unrestricted permutation of raw data was used as method of permutation (Anderson et al., 2008). A PERMANOVA based on Euclidean distances, including host size as a covariable, was also used to compare the diversity (Brillouin index) across

infracommunities (including all parasites).

2.2. Similarity analysis

Similarity in parasite assemblages was computed at infracommunity and component community levels *sensu* Bush et al. (1997). Bray-Curtis similarity indices (Magurran, 1988) were calculated for parasite abundance between all possible pairs of hosts (infracommunities), whereas at component community level, this index was calculated on values of both prevalence and mean abundance between all possible pairs of samples. Due to the large differences in parasite loads across parasite species, data were square-root transformed previous to all analyses in order to down-weight the importance of very prevalent/abundant species so that the less dominant species played some role in determining similarity among samples (Clarke and Gorley, 2015).

Since population parameters of short-lived parasites (ectoparasites and adult gastrointestinal parasites) can be heterogeneous and temporally variable at small spatial scales (Cantatore and Timi, 2015; Soares et al., 2018), being therefore unsuitable as indicators for stock discrimination, comparisons among samples were carried out on a subset of species, represented by long-lived larval parasites (*Grillotia carvajalregorum*, *Callitetrarhynchus gracilis*, *Corynosoma australe*, *Corynosoma cetaceum*, *Hysterothylacium* sp., *Anisakis simplex* s.l., *Contracaecum* sp. and *Pseudoterranova cattani*), as recommended for studies on fish stock discrimination (Lester and MacKenzie, 2009; Braicovich et al., 2016). Species found at low prevalence in all samples (< 10%) were also excluded from comparisons.

Given the differential migratory behavior displayed by males and females, possible gender differences on parasite community structure were tested using a PERMANOVA on the Bray-Curtis similarity matrix, introducing host size as a covariable (ANCOVA model; 1×2 factorial design, 'sex' as fixed factor) and testing for main effects after 9999 permutations and subsequent post-hoc pair-wise comparisons. Following Anderson et al. (2008) a permutation of residuals under a reduced model was used as method of permutation. A sequential sum of squares (Type I SS) was applied because host length was introduced as a covariable.

In order to assess whether or not the geographic origin of the samples can be differentiated based on the abundance values of their parasites, nonmetric multidimensional scaling (nMDS) of the similarity matrix was performed between all infracommunities and their centroid differences were visualized by means of bootstrap averaging based on repeated resampling (with replacement, 50 iterations) from the original dataset (Clarke and Gorley, 2015). Average values were then visualized in a nMDS using as many dimensions as needed to closely match the original distance matrix (correlation coefficient of $\rho = 0.99$) and the fit of the nMDS ordinations was quantified by a value of stress. For this and all subsequent multivariate analyses, a single fish harbouring no long-lived parasites was excluded (a 24 cm TL male, from RJ).

Differences between infracommunities among samples were examined in more detail using canonical analysis of principal coordinates (CAP) (Anderson and Willis, 2003; Anderson et al., 2008). The potential for over-parameterization was prevented by choosing the number of PCO axes (m) that maximized a leave-one-out allocation success to groups (Anderson and Robinson, 2003). To test for significant differences between infracommunities among the samples, a permutation "trace" test (sum of squared canonical eigenvalues) was applied; the P was obtained after 9999 permutations.

Since both nMDS and CAP analyses do not consider host size, the significance of the differences among samples was further tested by using a PERMANOVA, including host size as a covariable (1×6 factorial design, 'sample' as fixed factor).

Because 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



Fig. 1. Study area showing sampling localities. NE: Necochea; RJ: Rio de Janeiro; RS: Rio Grande do Sul; SC: San Clemente del Tuyú; VG: Villa Gesell.

PERMDISP (Anderson et al., 2008). Dispersions were measured as distance to the centroid, and each term in the analysis was tested using 9999 permutations.

At the component community level, non-metric multidimensional scaling analyses (nMDS) (Clarke and Gorley, 2015) were performed on the Bray-Curtis similarity index on both prevalence and mean abundance data to visualize possible geographic patterns in the composition of parasite assemblages across the samples. A hierarchical agglomerative clustering was applied to the component communities using group-average linking, and resemblance levels were overlaid on the nMDS plot (Clarke and Gorley, 2015).

All similarity and distance measures, as well as all multivariate analyses were implemented in PERMANOVA + for PRIMER package (Anderson et al., 2008).

Table 2

PERMANOVA results of comparisons of total length, diversity and structure of parasite communities of *Umbrina canosai* across six samples from the south-western Atlantic. *P*-values obtained after 9999 permutations.

Response variable	Source	d.f.	SS	MS	Pseudo <i>F</i>	<i>P</i> (<i>perm</i>)
Univariate						
Fish total length (Euclidean distance)	Sample	5	1732	346.4	29.751	< 0.01
	Residual	187	2177.3	11.6		
	Total	192	3909.4			
Brillouin index (Euclidean distance)	Host Size	1	2.7	2.7	62.418	< 0.01
	Sample	5	4.6	0.9		
	Residual	186	8.2	0.04		
Total	192	15.5				
Multivariate						
Infracommunity structure (Bray-Curtis similarity)	Host size	1	41066	41066	69.765	< 0.01
	Sample	5	1.12 e ⁰⁵	22596		
	Residual	180	1.09 e ⁰⁵	588.6		
Total	191	2.63 e ⁰⁵				

Table 1

Composition of six samples of *Umbrina canosai* in five localities of the southwestern Atlantic, ordered by increasing latitude.

Locality	Locality code	Latitude and longitude of capture	Date of capture	n	Total length ± SD (range) (cm)
Rio de Janeiro (Brazil)	RJ	22°50'S 41°58'W ¹	10/2016	36	34.5 ± 5.2 (24.0 – 44.5)
Rio Grande do Sul (Brazil)	RS1	32°28'S 51°56'W ¹	08/2018	26	35.2 ± 2.5 (29.4 – 40.5)
Rio Grande do Sul (Brazil)	RS2	32°28'S 51°56'W ¹	09/2018	25	39.3 ± 6.6 (34.4 – 53.5)
San Clemente del Tuyú (Argentina)	SC	36°50'S 56°39'W	08/2016	27	28.9 ± 6.6 (25.0 – 32.0)
Villa Gesell (Argentina)	VG	37°20'-37°40'S 56°15'-56°45'W	12/2015	40	37.2 ± 2.2 (32.0-42.0)
Necochea (Argentina)	NE	38°33'-39°13'S 57°41'-59°05'W	04/2017	39	36.7 ± 3.5 (28.0-44.0)

¹ Landing port.

Table 3
Prevalence of parasites of *Umbrina canosai* in six samples from the southwestern Atlantic coasts, their site of infection and stage of development.

Parasite	Site	Stage	Locality					
			RJ	RS1	RS2	SC	VG	NE
Monogenea								
<i>Nicolasia canosorum</i>	Gills	Adult	100	96.15	100	55.56	100	97.44
Diclidophoridae gen. sp.	Gills	Adult	41.67	34.62	48.00	0.00	45.00	0.00
<i>Encotyllabe spari</i>	Mouth	Adult	0.00	0.00	4.00	3.70	0.00	2.56
Digenea								
<i>Lecithochirium microstomum</i>	Stomach	Adult	5.56	3.85	8.00	0.00	2.50	0.00
<i>Aponurus laguncula</i>	Stomach	Adult	16.67	26.92	28.00	0.00	0.00	0.00
<i>Pachycreadium gastrocotylum</i>	Stomach	Adult	0.00	0.00	0.00	0.00	2.50	0.00
<i>Otodistomum</i> sp.	Stomach wall	Metacercaria	0.00	0.00	0.00	0.00	0.00	2.56
Didymozoidae gen sp.	Mesenteries	Metacercaria	2.78	0.00	0.00	0.00	0.00	0.00
Cestoda								
<i>Grillotia carvajalregorum</i>	Mesenteries/gut wall	Plerocercus	47.22	100	100	96.30	97.50	100
<i>Callitetrarhynchus gracilis</i>	Mesenteries	Plerocercus	33.33	0.00	0.00	0.00	0.00	0.00
<i>Scolex polymorphus</i>	Intestine	Plerocercoid	0.00	0.00	16.00	0.00	5.00	0.00
Acanthocephala								
<i>Corynosoma australe</i>	Mesenteries	Juvenile	25.00	100	100	100	100	100
<i>Corynosoma cetaceum</i>	Mesenteries	Juvenile	0.00	46.15	64.00	22.22	30.00	35.90
<i>Bolbosoma turbinella</i>	Mesenteries	Juvenile	5.56	0.00	4.00	0.00	0.00	5.13
Unidentified	Intestine	Adult	0.00	3.85	0.00	0.00	0.00	0.00
Nematoda								
<i>Dichelyne sciaenicola</i>	Intestine	Adult	25.00	46.15	52.00	33.33	77.50	35.90
<i>Ascarophis marina</i>	Stomach	Adult	0.00	0.00	0.00	7.41	0.00	0.00
Cystidicolidae gen. sp.	Stomach wall	Larva III	2.78	0.00	0.00	0.00	0.00	0.00
<i>Hysterothylacium</i> sp.	Mesenteries	Larva III	97.22	100	100	40.74	100	100
<i>Anisakis simplex</i> s.l.	Mesenteries	Larva III	11.11	0.00	4.00	0.00	7.50	2.56
<i>Contracaecum</i> sp.	Mesenteries/gut wall	Larva III	0.00	0.00	0.00	0.00	0.00	17.95
<i>Pseudoterranova cattani</i>	Mesenteries	Larva III	0.00	0.00	8.00	0.00	0.00	15.38
<i>Terranova</i> sp.	Mesenteries	Larva III	0.00	0.00	4.00	0.00	0.00	0.00
Copepoda								
<i>Lernanthropus leidy</i>	Gills	Adult	0.00	3.85	0.00	0.00	0.00	0.00
<i>Colobomatus</i> sp.	Sensory cephalic channels	Adult	2.78	0.00	0.00	0.00	0.00	0.00
<i>Caligus</i> sp.	Skin	Adult	0.00	3.85	0.00	0.00	0.00	0.00
Isopoda								
Gnathiidae gen.sp.	Gills	Praniza	0.00	00.00	4.00	0.00	0.00	0.00
Cymothoidae gen sp.	Gills	Manca	0.00	3.85	00.00	0.00	0.00	0.00

RJ: Rio de Janeiro; RS: Rio Grande do Sul; SC: San Clemente del Tuyú; VG: Villa Gesell; NE: Necochea.

3. Results

3.1. General results

Mean host length was significantly different between samples (Table 2), with fish from SC being significantly smaller than those of the other samples (all $P_{perm} < 0.01$; Table 2).

Umbrina canosai harboured 28 parasite taxa, represented by 91,990 individuals in the whole sample. However, only twelve of those taxa showed a prevalence > 10% in at least one of the samples (Table 3), whereas other ten taxa were represented by single specimen by a single host (Table 4). Among parasites, *Nicolasia canosorum*, *Grillotia carvajalregorum*, *Corynosoma australe* and *Hysterothylacium* sp., the most prevalent taxa, accounted for almost 98.8% of all specimens found. The diversity measured by the Brillouin index varied significantly among samples (Table 2; Fig. 2), with Argentine croakers from RJ and SC harbouring similarly diverse infracommunities ($P_{perm} > 0.01$), both significantly less diverse than the rest of the samples (all $P_{perm} < 0.01$), which in turn, did not differ from each other ($P_{perm} > 0.01$).

3.2. Similarity analyses

The structure of infracommunities of long-lived parasites was similar between male and female fish in all samples (Table 5). No differences in the multivariate dispersions of parasite infracommunities in terms of their deviations from centroids were observed (all $P_{perm} > 0.01$). Consequently both genders were pooled for subsequent analyses. Host length was significantly related to both diversity and structure of parasite infracommunities in the whole sample (Table 2).

The bootstrap-average-based nMDS ordination (Fig. 3) of long-lived parasites showed an apparent pattern of separation between samples, with a low level of stress (0.04). Indeed, the parasite assemblages from RJ were clearly separated from the rest, especially along the first axis; fish from SC were also situated apart from other samples, especially from a rather homogeneous group of samples composed by RS1, RS2, VG and NE along the second axis.

The CAP analysis showed significant differences among samples ($tr = 1.45$; $P < 0.001$) (Fig. 4). The selected orthonormal PCO axes ($m = 5$) described 98.5% of the variation in the data 'cloud', although the percentage of correct allocations was low (47.92%). The first two canonical axes resulting from CAP analysis clearly separated some samples, and a strong association between the multivariate data 'cloud' and the hypothesis of group differences was indicated by their high canonical correlations ($d1 = 0.93$ and $d2 = 0.869$). Argentine croakers from RJ were distinguishable from those of southern localities and were situated at the left of the CAP biplot, while those from SC occupied the upper right corner, separated from the rest of samples, RS1, RS2, VG and NE, which constituted a homogeneous group. When vectors corresponding to Spearman correlations of individual species were superimposed with the CAP axes (restricted to those species having $r > 0.3$), *C. australe*, *G. carvajalregorum* and *C. cetaceum* were mainly associated with southern samples, however their lower abundances separated fish from SC along the CAP2. On the other hand *Hysterothylacium* sp. and *Callitetrarhynchus gracilis* were strongly associated with RJ. The superimposition of the fish size (TL) as an additional explanatory variable showed that this factor was relevant in the differences between SC and the rest of southern samples. Cross validation results (Table 6) showed that both RJ and SC had the highest percentages of correctly allocated

Table 4
Mean abundance (range) of parasites of *Umbrina canosai* in six samples from the southwestern Atlantic coasts, their site of infection and stage of development.

Parasite	Locality					
	RJ	RS1	RS2	SC	VG	NE
Monogenea						
<i>Nicolasia canosorum</i>	58.14 (2-199)	39.15 (0-178)	66.12 (3-257)	1.26 (0-4)	75.83 (10-198)	27.15 (0-99)
Diclidophoridae gen. sp.	1.03 (0-8)	0.38 (0-2)	0.88 (0-4)	–	0.70 (0-6)	–
<i>Encotylabe spari</i>	–	–	0.04 (0-1)	0.04 (0-1)	–	0.03 (0-1)
Digenea						
<i>Lecithochirium microstomum</i>	0.06 (0-1)	0.27 (0-7)	0.16 (0-3)	–	0.05 (0-2)	–
<i>Aponurus laguncula</i>	0.28 (0-4)	0.46 (0-3)	1.40 (0-13)	–	–	–
<i>Pachycreadium gastrocotylum</i>	–	–	–	–	0.60 (0-24)	–
<i>Otodistomum</i> sp.	–	–	–	–	–	0.03 (0-1)
Didymozoidae gen sp.	0.03 (0-1)	–	–	–	–	–
Cestoda						
<i>Grillotia carvajalregorum</i>	14.19 (0-183)	187.85 (14-480)	464.04 (55-5224)	18.67 (0-178)	183.00 (0-900)	156.38 (2-459)
<i>Callitetrarhynchus gracilis</i>	2.08 (0-11)	–	–	–	–	–
<i>Scolex polymorphus</i>	–	–	5.28 (0-117)	–	1.10 (0-42)	–
Acanthocephala						
<i>Corynosoma australe</i>	14.61 (0-304)	275.69 (18-855)	291.20 (19-800)	70.93 (20-140)	367.68 (28-928)	290.00 (27-701)
<i>Corynosoma cetaceum</i>	–	1.65 (0-14)	2.76 (0-25)	0.37 (0-4)	1.65 (0-11)	0.79 (0-5)
<i>Bolbosoma turbinella</i>	0.11 (0-3)	–	0.04 (0-1)	–	–	0.10 (0-3)
Unidentified	–	0.04 (0-1)	–	–	–	–
Nematoda						
<i>Dichelyne sciaenidicola</i>	0.56 (0-6)	1.15 (0-13)	1.88 (0-18)	0.44 (0-4)	4.03 (0-34)	0.85 (0-5)
<i>Ascarophis marina</i>	–	–	–	0.07 (0-1)	–	–
Cysticolidae gen. sp.	0.03 (0-1)	–	–	–	–	–
<i>Hysterothylacium</i> sp.	106.22 (0-624)	41.35 (1-287)	46.12 (10-205)	2.22 (0-29)	26.75 (3-125)	27.36 (1-72)
<i>Anisakis simplex</i> s.l.	0.14 (0-2)	–	0.08 (0-2)	–	0.08 (0-1)	0.03 (0-1)
<i>Contracaecum</i> sp.	–	–	–	–	–	0.28 (0-3)
<i>Pseudoterranova cattani</i>	–	–	0.16 (0-2)	–	–	0.26 (0-3)
<i>Terranova</i> sp.	–	–	0.04 (0-1)	–	–	–
Copepoda						
<i>Lernanthropus leidy</i>	–	0.04 (0-1)	–	–	–	–
<i>Colobomatus</i> sp.	0.03 (0-1)	–	–	–	–	–
<i>Caligus</i> sp.	–	0.04 (0-1)	–	–	–	–
Isopoda						
Gnathiidae gen.sp.	–	–	0.04 (0-1)	–	–	–
Cymothoidae gen sp.	–	0.04 (0-1)	–	–	–	–

RJ: Rio de Janeiro; RS: Rio Grande do Sul; SC: San Clemente del Tuyú; VG: Villa Gesell; NE: Necochea.

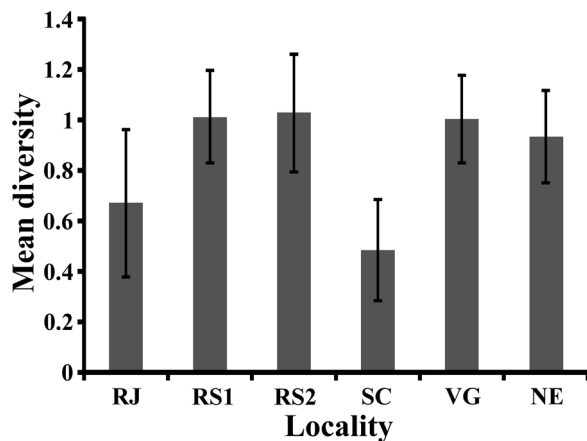


Fig. 2. Mean infracommunity diversity (Brillouin index) ± standard deviation of parasites of *Umbrina canosai* in six samples in the southwestern Atlantic. NE: Necochea; RJ: Rio de Janeiro; RS: Rio Grande do Sul; SC: San Clemente del Tuyú; VG: Villa Gesell.

fish, whereas the rest of samples showed a low proportion of correctly allocated fish, many of which were misclassified among them. Indeed, when the correct allocations of fish to the homogeneous group were considered, the percentages increased notably.

The results of PERMANOVA analyses on infracommunities, showed a significant effect of fish length on the multivariate parasite abundance (Table 2). Furthermore, taking into account the variations due to fish size, significant variability of parasite assemblages was detected among

Table 5
PERMANOVA results comparing the structure of long-lived parasite communities between sexes of *Umbrina canosai* in six samples from the southwestern Atlantic. P-values obtained after 9999 permutations.

Sample (Bray-Curtis dissimilarity)	Source	d.f.	SS	MS	Pseudo F	P (perm)
Rio de Janeiro	Host size	1	19919	19919	16.142	< 0.01
	Host sex	1	977.9	977.9	0.792	> 0.01
	Residual	31	38253	1234		
	Total	33	59150			
Rio Grande do Sul 1	Host size	1	1679.6	1679.6	4.783	> 0.01
	Host sex	1	13.6	13.645	0.039	> 0.01
	Residual	23	8075.8	351.1		
	Total	25	9769.1			
Rio Grande do Sul 2	Host size	1	413.4	413.4	1.487	> 0.01
	Host sex	1	97.4	97.4	0.350	> 0.01
	Residual	21	5839.5	278.1		
	Total	23	6350.3			
San Clemente del Tuyú	Host size	1	734.9	734.9	2.246	> 0.01
	Host sex	1	706.5	706.5	2.159	> 0.01
	Residual	23	7524.6	327.1		
	Total	25	8966			
Villa Gesell	Host size	1	2772.1	2772.1	7.829	< 0.01
	Host sex	1	483.4	483.4	1.365	> 0.01
	Residual	35	12393	354.1		
	Total	37	15649			
Necochea	Host size	1	4696.8	4696.8	14.192	< 0.01
	Host sex	1	96.177	96.2	0.291	> 0.01
	Residual	35	11583	330.9		
	Total	37	16376			

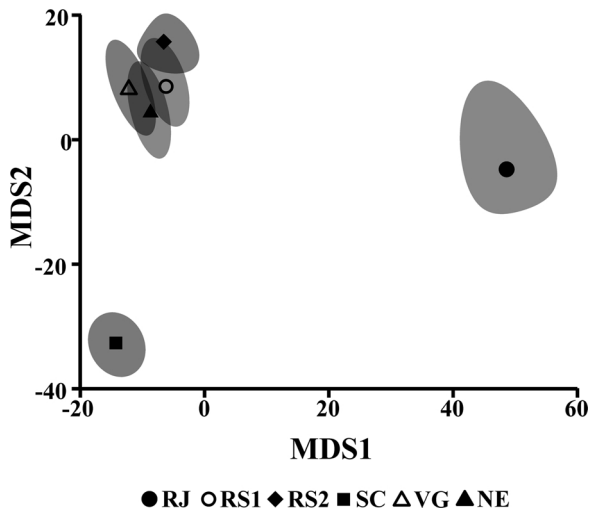


Fig. 3. Non-metric multi-dimensional scaling plot (nMDS) of bootstrap averages (50 repetitions) of parasite infracommunities in six samples of *Umbrina canosai* in the southwestern Atlantic based on Bray-Curtis dissimilarity of square root-transformed data. Individual repetitions are based on random draw and replacement of samples from the original dataset. Black symbols represent the overall centroids across all repetitions. Grey areas represent 95% confidence regions. NE: Necochea; RJ: Rio de Janeiro; RS: Rio Grande do Sul; SC: San Clemente del Tuyú; VG: Villa Gesell (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

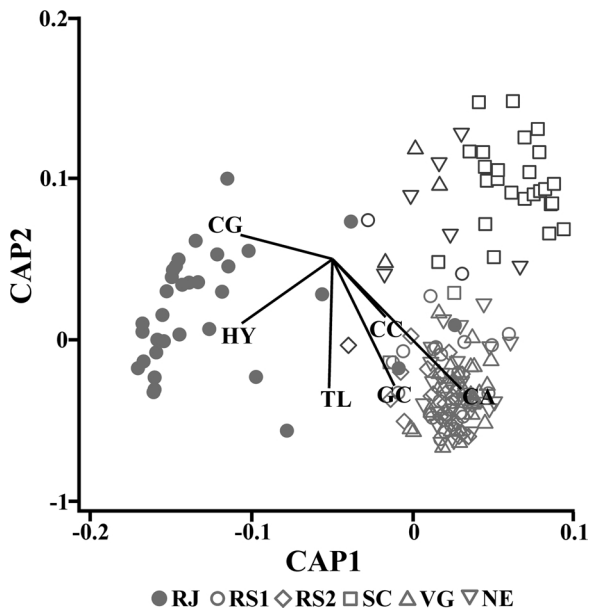


Fig. 4. Canonical analysis of principal co-ordinates (CAP) bi-plot based on Bray-Curtis dissimilarities on square-root abundance data of long-lived parasites of *Umbrina canosai* in six samples in the southwestern Atlantic. Vectors represent Pearson correlations of abundance of individual parasite species, as well as of fish total length, with the CAP axes. NE: Necochea; RJ: Rio de Janeiro; RS: Rio Grande do Sul; SC: San Clemente del Tuyú; VG: Villa Gesell. CA: *Corynosoma australe*; CC: *Corynosoma cetaceum*; CG: *Callitetrarhynchus gracilis*; GC: *Grillotia carvajalregorum*; HY: *Hysterothylacium* sp.; TL: Host total length.

the samples. Pairwise tests agreed in general with both bootstrap-average-based nMDS ordinations and CAP analysis. Indeed, both RJ and SC differed significantly (both $P_{perm} < 0.01$) from each other and from all the other samples, which, in turn, showed no differences among them (all $P_{perm} > 0.05$) for abundance of long-lived parasites. No differences in the multivariate dispersions of parasite infracommunities in

Table 6

Results of the cross validation of principal co-ordinates analysis (CAP) (leave-one-out allocation of individual fish to one of six samples). Rows correspond to group memberships, including the percentage of correctly classified fish to their individual sample or group of homogeneous samples (RS1 + RS2 + VG + NE).

Original sample	RJ	RS1	RS2	SC	VG	NE	Total	% (sample)	% (group)
RJ	31	1	1	1	0	1	35	88.6	88.6
RS1	0	2	13	1	1	9	26	7.7	96.2
RS2	0	4	14	0	5	2	25	56.0	100
SC	0	1	0	24	0	2	27	88.9	88.9
VG	0	1	16	2	13	8	40	32.5	95.0
NE	0	4	16	5	6	8	39	20.5	87.2

RJ: Rio de Janeiro; RS: Rio Grande do Sul; SC: San Clemente del Tuyú; VG: Villa Gesell; NE: Necochea.

terms of their deviations from centroids, were observed from most of comparisons (all $P_{perm} > 0.01$), except for all those including RJ (all $P_{perm} < 0.01$).

Considering fish from RS1, RS2, VG and NE as a single group, their significant differences with both RJ and SC evidenced by PERMANOVA, are clearly consequence of the differential abundance of the indicator species identified in the CAP analysis (Fig. 5).

The MDS and cluster analyses carried out at the level of component communities showed the same pattern than infracommunities. For both, prevalence (Fig. 6a) and mean abundance (Fig. 6b), RJ and SC were located apart from the group composed by RS1, RS2, VG, and NE, all with the same set of parasite species as indicators. However, the cluster analyses detected a higher similarity of fish from SC to the other southern samples than to RJ.

4. Discussion

Umbrina canosai harbours a diverse parasite fauna, comparable in terms of richness to that of other sciaenids with similar or higher trophic levels in the region (Timi et al., 2005; Luque et al., 2010). At present, studies on parasites of this host have mostly dealt with taxonomic aspects (Luque and Tavares, 2007; Santos et al., 2008; Luque et al., 2011; Cohen et al., 2013; Alves et al., 2017). To the best of our knowledge, this is the first quantitative study on the parasite fauna of Argentine croaker, which extends the list of parasites cited for *U. canosai* adding 19 taxa to previously recorded species. Those taxa with higher values of prevalence and abundance were mainly long-lived larvae of endoparasites, with the exception of *N. canosorum*, an adult monogenean found in gills. The same long-lived endoparasites taxa have been shown to be useful biological tags in previous studies on stock discrimination for several fish species in the study region (Cantatore and Timi, 2015). Therefore, their dominance, high burdens and proved value as stock indicators provided a set of tools to evaluate the stock structure along most of the distribution range of *U. canosai*, as well as to trace their migratory patterns in the southern boundaries of this species.

The observed infracommunity diversity was variable across samples, with those from RJ and SC being significantly less diverse than the rest, which composed a homogenous group. Such lower diversity has, however, different causes. Whereas the differences of fish from RJ are due to their geographic origin (see below), an ontogenetic effect on the formation of parasite assemblages explains the lower diversity observed in SC. Indeed, an effect of host size/age is to be expected for long-lived parasites, which tend to accumulate over time (Braigovich et al., 2016) with bigger hosts harbouring more abundant and diverse parasite communities (Luque et al., 2004; Timi et al., 2010b). Thus, in the case of SC sample, its low diversity is likely to be due to the significantly smaller size of fish specimens. In addition to the size and time of exposure as drivers of the differences observed for fish from SC, these are

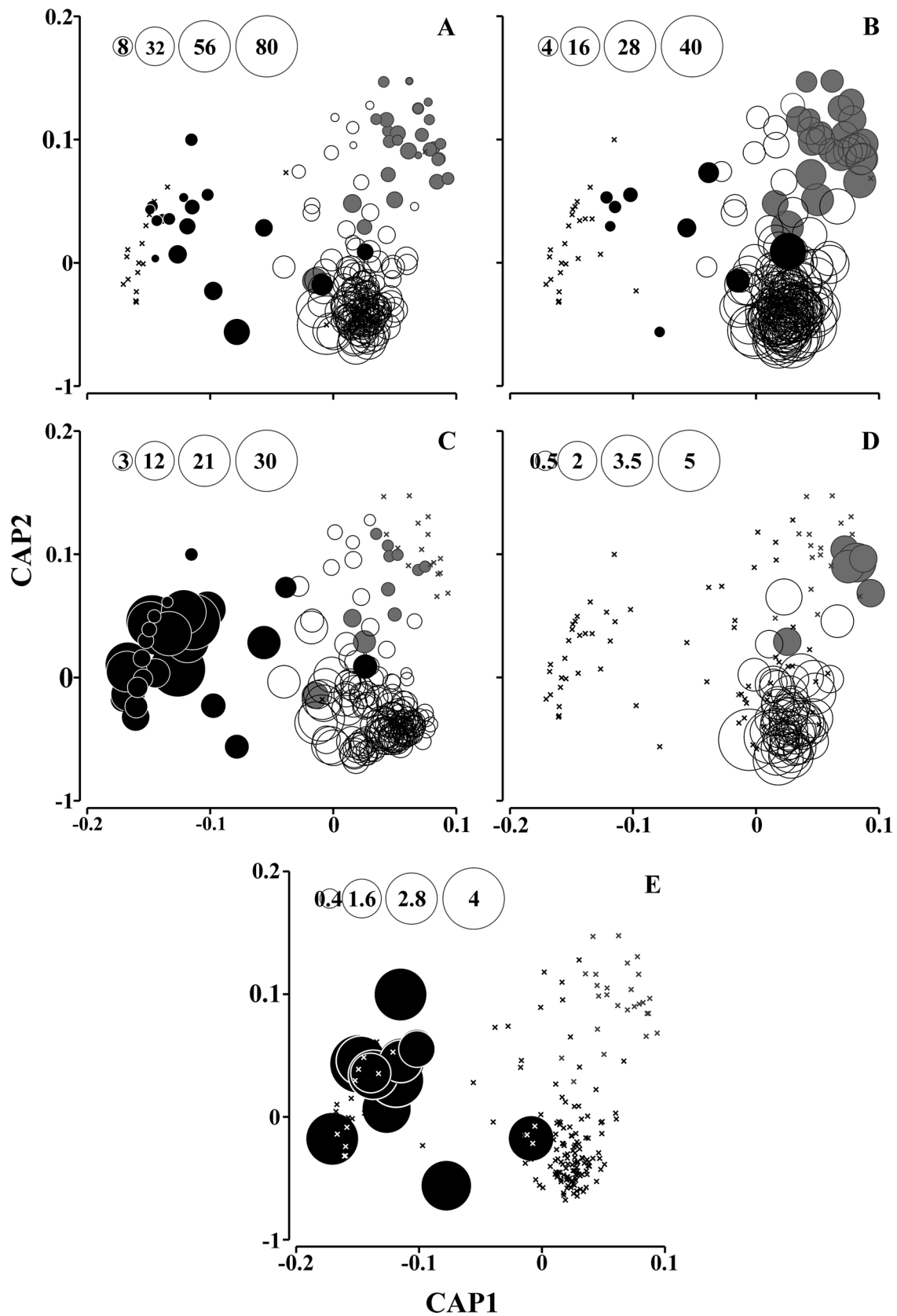


Fig. 5. Canonical analysis of principal co-ordinates (CAP) bi-plot based on Bray-Curtis dissimilarities on square-root abundance data of long-lived parasites of *Umbrina canosai* in six samples in the southwestern Atlantic. Circle diameters scaled according to the square root of the abundance for the five main indicator species for stock discrimination in each individual fish (references in upper circles); crosses indicate unparasitized fish. Black circles: Rio de Janeiro; grey circles: San Clemente del Tuyú; white circles: combined samples from Rio Grande do Sul, Villa Gesell and Necochea. A: *Grillotia carvajalregorum*; B: *Corynosoma australe*; C: *Hysterothylacium* sp.; D: *Corynosoma cetaceum*; E: *Callitetrarhynchus gracilis* (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

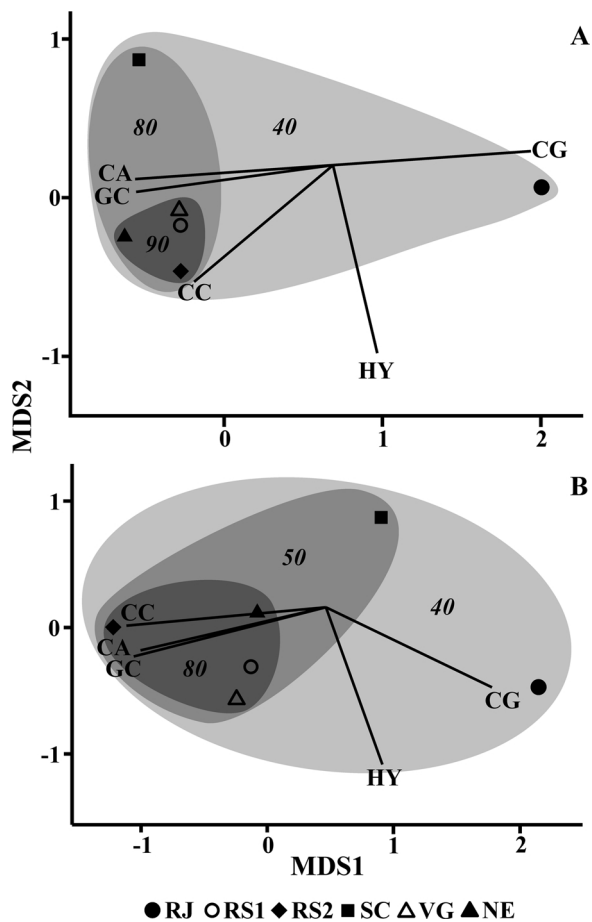


Fig. 6. Non-metric multi-dimensional scaling plot (nMDS) and cluster analyses of six component communities (Bray-Curtis dissimilarity) of long-lived parasites of *Umbrina canosai* in the southwestern Atlantic. A: prevalence; B: mean abundance. Results of a hierarchical agglomerative clustering are overlaid on the nMDS plot with similarity levels represented by a grey scale, with its value given as a number inside each grey area. Vectors represent Pearson correlations of prevalence (A; $R > 0.85$) and mean abundance (B; $R > 0.80$) of individual parasite species with the nMDS axes. NE: Necochea; RJ: Rio de Janeiro; RS: Rio Grande do Sul; SC: San Clemente del Tuyú; VG: Villa Gesell. CA: *Corynosoma australe*; CC: *Corynosoma cetaceum*; CG: *Callitetrarhynchus gracilis*; GC: *Grillotia carvajalregorum*; HY: *Hysterothylacium* sp. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

enhanced by trophic differences displayed by younger *U. canosai* compared with adults. In fact, pronounced changes in trophic habits have been observed for this species, with crustaceans (particularly amphipods and mysids) dominating the diet of smaller specimens, while adults have a broader diet, mainly composed of polychaetes, ophiuroids, molluscs, decapods and fishes (Haimovici et al., 1989). These trophic changes throughout its life cycle may consequently lead to different exposure to parasites in terms of both quantity and diversity, and therefore harboring unequal parasite loads.

Differences in parasite infections between males and females are often reported, mostly due to sexual dimorphism in many traits, including morphology, physiology, life history, behavior and feeding habits (Rohde, 1993; Duneau and Ebert, 2012). Males and females of *U. canosai* display a differential migratory timing. Whereas larger females are earlier migrants, males remain in spawning areas for longer, until the end of spring (Haimovici and Cousin, 1989), the structure of infracommunities was similar between sexes. This result is consistent with previous studies on feeding habits of *U. canosai*, which have found that both males and females have almost equal diets (Haimovici et al.,

1989), leading to equivalent parasite assemblages. Therefore, both sexes can be combined for comparative purposes in further studies.

Regarding the remaining samples, all comparative analyses, at both infracommunity and component community levels, showed evident geographic patterns. Fish from RJ were consistently different from their southern counterparts, being characterized by higher loads of *C. gracilis* and *Hysterothylacium* sp. Thus the present findings confirm the presence of a discrete stock in the northern region of the study area, as it was proposed by Vazzoler (1976). This is anticipated result, since all previous studies involving samples from these regions, most of them based on the same or a similar set of parasite indicators, have arrived at similar conclusions (Timi et al., 2005, 2010a; Braicovich et al., 2012, 2016; Pereira et al., 2014; Alarcos et al., 2016; Soares et al., 2018) mirroring the zoogeographic classifications of South American Atlantic waters and the differences in oceanographic and biological features of each region (Lanfranchi et al., 2016; Braicovich et al., 2017). Given the migratory behavior of *U. canosai*, the evident differences in the structure of parasite assemblages between its northern and southern stocks will allow the detection of their boundaries in further studies at smaller spatial scales in intermediate regions.

Finally, the same results evidenced the homogeneity of samples caught in southern Brazil and Northern Argentina, regardless of the long distances separating them. Indeed, the cross validation of discriminant analyses showed a high degree of misclassifications among them, with none of their specimens being allocated to RJ. Consequently, it is confirmed that a single stock, with seasonal migrations to Argentine coasts, is distributed in southern Brazil, Uruguay and Argentina such as has been proposed by Gonzalez Alberdi and Nani (1967) and Haimovici et al. (2006).

In the case of fish from SC, they were caught in an intermediate region, during late winter. Taking into account that its differences from other samples are due to their smaller size, their geographic location, as well as their similarity with other southern samples at component community level, that sample should be considered as composed of early migrants from the southern stock.

The confirmation of the stock structure and migratory patterns of *U. canosai* is a first step towards the implementation of measures to ensure a sustainable management of this important resource, under commercial exploitation by three different countries, which moreover is evidencing signs of overexploitation and a verified risk of collapse.

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References

- Alarcos, A.J., Timi, J.T., 2013. Stocks and seasonal migrations of the flounder *Xytreureus rasile* as indicated by its parasites. *J. Fish Biol.* 83, 531–541. <https://doi.org/10.1111/jfb.12190>.
- Alarcos, A.J., Pereira, A.N., Taborda, N.L., Luque, J.L., Timi, J.T., 2016. Parasitological evidence of stocks of *Paralichthys isosceles* (Pleuronectiformes: Paralichthyidae) at small and large geographical scales in South American Atlantic coasts. *Fish. Res.* 173, 221–228. <https://doi.org/10.1016/j.fishres.2015.07.018>.
- Alves, P.V., De Chambrier, A., Scholz, T., Luque, J.L., 2017. Annotated checklist of fish cestodes from South America. *Zookeys* 650, 1–205. <https://doi.org/10.3897/zookeys.650.10982>.
- Anderson, M.J., Robinson, J., 2003. Generalized discriminant analysis based on distances. *Aust. N. Z. J. Stat.* 45, 301–318. <https://doi.org/10.1111/1467-842X.00285>.
- Anderson, M.J., Willis, T.J., 2003. Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. *Ecology* 84, 511–525. [https://doi.org/10.1890/0012-9658\(2003\)084\[0511:CAOPCA\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2003)084[0511:CAOPCA]2.0.CO;2).
- Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA + For PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth.
- Braicovich, P.E., Luque, J.L., Timi, J.T., 2012. Geographical patterns of parasite

- infracommunities in the rough scad, *Trachurus lathami* Nichols, in the Southwestern Atlantic Ocean. *J. Parasitol.* 98, 768–777. <https://doi.org/10.1645/GE-2950.1>.
- Braicovich, P.E., Ieno, E.N., Sáez, M., Despos, J., Timi, J.T., 2016. Assessing the role of host traits as drivers of the abundance of long-lived parasites in fish-stock assessment studies. *J. Fish Biol.* 89, 2419–2433. <https://doi.org/10.1111/jfb.13127>.
- Braicovich, P.E., Pantoja, C., Pereira, A.N., Luque, J.L., Timi, J.T., 2017. Parasites of the Brazilian flathead *Percophis brasiliensis* reflect West Atlantic biogeographic regions. *Parasitology* 144, 169–178. <https://doi.org/10.1017/S0031182016001050>.
- Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: margolis et al. revisited. *J. Parasitol.* 83, 575–583. <https://doi.org/10.2307/3284227>.
- Cadrin, S.X., Kerr, L.A., Mariani, S., 2014. *Stock Identification Methods: Applications in Fishery Science*. Academic Press, Waltham.
- Cantatore, D.M.P., Timi, J.T., 2015. Marine parasites as biological tags in South American Atlantic waters, current status and perspectives. *Parasitology* 142, 5–24. <https://doi.org/10.1017/S0031182013002138>.
- Carballo, M.C., Cremonese, F., Navone, G.T., Timi, J.T., 2012. Similarity in parasite community structure may be used to trace latitudinal migrations of *Odontesthes smitti* along Argentinean coasts. *J. Fish Biol.* 80, 15–28. <https://doi.org/10.1111/j.1095-8649.2011.03125.x>.
- Clarke, K.R., Gorley, R.N., 2015. *PRIMER v7: User manual/tutorial*. PRIMER-E Ltd., Plymouth.
- Cohen, S.C., Justo, M.C.N., Kohn, A., 2013. South American Monogeneoidea parasites of fishes, amphibians and reptiles. Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Sao Paulo.
- Cousseau, M.B., Perrotta, R.G., 2013. *Peces marinos de Argentina: biología, distribución, pesca*, fourth ed. Instituto Nacional de Investigación y Desarrollo Pesquero, Mar del Plata.
- Duneau, D., Ebert, D., 2012. Host sexual dimorphism and parasite adaptation. *PLoS Biol.* 10 (2), e1001271. <https://doi.org/10.1371/journal.pbio.1001271>.
- Figueiredo, J.L., Menezes, N.A., 1980. *Manual de peixes marinhos do sudeste do Brasil. III. Teleostei*. Museu de Zoologia, Universidade de São Paulo, São Paulo.
- Gonzalez Alberdi, P., Nani, A., 1967. Contribución al conocimiento de la biología del pargo blanco *Umbrina canosai* Berg de la región de Mar del plata (Argentina). *CARPAS Docum. Tec. Num* 10, 1–36.
- Haimovici, M., 1988. Análisis de cohortes del stock de pargo blanco (*Umbrina canosai*) explotado en el sur de Brasil, Uruguay y Argentina. *Publ. Com. Téc. Mix. Fr. Mar.* 4, 33–40.
- Haimovici, M., Cardoso, L.G., 2016. Colapso do estoque de *Umbrina canosai* do sul do Brasil devido à introdução do arrasto-de-meia-água. *Bol. Ins. Pesca* 42, 258–267. <https://doi.org/10.5007/1678-2305.2016v42n1p258>.
- Haimovici, M., Cousin, J.C.B., 1989. Reproductive biology of the castanha *Umbrina canosai* (Pisces, Sciaenidae) in Southern Brazil. *Rev. Bras. Biol.* 49, 523–537.
- Haimovici, M., Reis, E.G., 1984. Determinação de idade e crescimento da castanha *Umbrina canosai* (Pisces, Sciaenidae) do sul do Brasil. *Atlntica* 7, 25–46.
- Haimovici, M., Teixeira, R.L., Arruda, M., de, C., 1989. Alimentação da castanha *Umbrina canosai* no litoral sul de Brasil. *Rev. Bras. Biol.* 49, 511–522.
- Haimovici, M., Absalonsen, L., Velasco, G., Miranda, L.V., 2006. Diagnóstico do estoque e orientações para o ordenamento da pesca de *Umbrina canosai* (Berg, 1895). In: Rossi-Wongtschowski, C.L.D.B., Ávila-da-Silva, A.O., Cergole, M.C. (Eds.), *Análise das Principais Pescarias Comerciais da Região Sudeste-Sul do Brasil: Dinâmica Populacional das Espécies em Exploração – II*. Universidade de São Paulo, São Paulo, pp. 77–85.
- Lanfranchi, A.L., Braicovich, P.E., Cantatore, D.M.P., Alarcos, A.J., Luque, J.L., Timi, J.T., 2016. Ecotonal marine regions – ecotonal parasite communities: helminth assemblages in the convergence of masses of water in the southwestern Atlantic. *Int. J. Parasitol.* 46, 809–818. <https://doi.org/10.1016/j.ijpara.2016.07.004>.
- Lester, R.J.G., MacKenzie, K., 2009. The use and abuse of parasites as stock markers for fish. *Fish. Res.* 97, 1–2. <https://doi.org/10.1016/j.fishres.2008.12.016>.
- Luque, J.L., Tavares, L.E.R., 2007. Checklist of Copepoda associated with fishes from Brazil. *Zootaxa* 1579, 1–39. <https://doi.org/10.11646/zootaxa.1579.1.1>.
- Luque, J.L., Mouillot, D., Poulin, R., 2004. Parasite biodiversity and its determinants in coastal marine teleost fishes of Brazil. *Parasitology* 128, 671–682. <https://doi.org/10.1017/S0031182004005050>.
- Luque, J.L., Cordeiro, A.S., Oliva, M.E., 2010. Metazoan parasites as biological tags for stock discrimination of whitemouth croaker *Micropogonias furnieri*. *J. Fish Biol.* 76, 591–600. <https://doi.org/10.1111/j.1095-8649.2009.02515.x>.
- Luque, J.L., Aguiar, J.C., Vieira, F.M., Gibson, D.I., Santos, C.P., 2011. Checklist of Nematoda associated with the fishes of Brazil. *Zootaxa* 3082, 1–88.
- Magurran, A.E., 1988. *Ecological Diversity and Its Measurement*. Princeton University Press, Princeton, New Jersey. <https://doi.org/10.1007/978-94-015-7358-0>.
- Nion, H., Marín, Y.H., Meneses, P., Puig, P., 2013. Distribución batimétrica de la familia Sciaenidae (Perciformes) en el Atlántico Sudoccidental y consideraciones sobre las pesquerías de los peces de esta familia. *Fr. Mar.* 23, 105–132.
- Pereira, A.N., Pantoja, C., Luque, J.L., Timi, J.T., 2014. Parasites of *Urophycis brasiliensis* (Gadiformes: Phycidae) as indicators of marine ecoregions in coastal areas of the South American Atlantic. *Parasitol. Res.* 113, 4281–4292. <https://doi.org/10.1007/s00436-014-4106-3>.
- Rohde, K., 1993. *Ecology of Marine Parasites: An Introduction to Marine Parasitology*, second ed. Cab International.
- Santos, C.P., Gibson, D.I., Tavares, L.E.R., Luque, J.L., 2008. Checklist of the Acanthocephala associated with the fishes of Brazil. *Zootaxa* 1938, 1–22. <https://doi.org/10.5281/zenodo.184999>.
- Soares, I.A., Lanfranchi, A.L., Luque, J.L., Haimovici, M., Timi, J.T., 2018. Are different parasite guilds of *Pagrus pagrus* equally suitable sources of information on host zoogeography? *Parasitol. Res.* 117, 1865–1875. <https://doi.org/10.1007/s00436-018-5878-7>.
- Timi, J.T., 2007. Parasites as biological tags for stock discrimination in marine fish from South American Atlantic waters. *J. Helminthol.* 81, 107–111. <https://doi.org/10.1017/S0022149X07726561>.
- Timi, J.T., MacKenzie, K., 2015. Parasites in fisheries and mariculture. *Parasitology* 142, 1–4. <https://doi.org/10.1017/S0031182014001188>.
- Timi, J.T., Luque, J.L., Sardella, N.H., 2005. Parasites of *Cynoscion guatucupa* along South American Atlantic coasts: evidence for stock discrimination. *J. Fish Biol.* 67, 1603–1618. <https://doi.org/10.1111/j.1095-8649.2005.00867.x>.
- Timi, J.T., Lanfranchi, A.L., Luque, J.L., 2010a. Similarity in parasite communities of the teleost fish *Pinguipes brasilianus* in the southwestern Atlantic: Infracommunities as a tool to detect geographical patterns. *Int. J. Parasitol.* 40, 243–254. <https://doi.org/10.1016/j.ijpara.2009.07.006>.
- Timi, J.T., Luque, J.L., Poulin, R., 2010b. Host ontogeny and the temporal decay of similarity in parasite communities of marine fish. *Int. J. Parasitol.* 40, 963–968. <https://doi.org/10.1016/j.ijpara.2010.02.005>.
- Zanetti Prado, E.M., 1979. Bionomia e ciclo de vida de *Umbrina canosai*, Berg (1895). *Bolm. Inst. Oceanogr.* 28, 119–165.
- Vazzoler, A.E.A. de M., 1976. Alguns aspectos do ciclo de vida de *Umbrina canosai* na região da plataforma continental brasileira entre Cabo Frio (lat 23°S) e Torres (lat 29°21'S). *Resumos da 28 Reunião Anual da Soc. Brasileira para o Progresso da Ciência*.