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Segregation of SE Pacific and SW Atlantic southern blue whiting stocks: integrating evidence from complementary otolith microchemistry and parasite assemblage approaches

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Abstract Southern blue whiting *Micromesistius australis* support one of the largest industrial fisheries in South America. Two main spawning/nursery grounds are known: one in the SW Atlantic Ocean (SWA), southwest from the Falkland (Malvinas) Islands; and other in the SE Pacific Ocean (SEP), south from the Taitao Peninsula. Juveniles originating from both grounds are believed to mix during migration and/or in feeding areas in the Scotia Sea. Previous efforts to distinguish stocks in this area have yielded contradictory results between genetics and otolith microchemical analyses. In the present work we revisited the null hypothesis of a single stock occurring in the broader SWA-SEP region by comparing and integrating results from different approaches: trace metals (Ca, Sr, Ba, Mg, Mn) and stable isotopes ($\delta^{13}C$, $\delta^{18}O$) in otolith cores, and parasite assemblage compositions in adults from SWA and SEP spawning grounds. We found significant differences in Sr:Ca, $\delta^{13}C$ and $\delta^{18}O$ mean ratios between spawning grounds. The best trace element discriminant model classified 83% of the samples. Each stable isotope discriminated >90% of the samples, while combining them into a bivariate discriminant model led to 100% classification success. Higher $\delta^{18}O$ levels in the SWA samples agreed with lower mean temperature and higher ambient $\delta^{18}O$ levels in that area. Parasite assemblage compositions also showed significant differences between grounds regarding the prevalence of C*hondracanthus*, *Contracaecum, Hepatoxylon* and *Grillotia* and the abundance of *Diclidophora, Anisakis*, *Contracaecum*, *Hysterothylacium* and *Hepatoxylon.* Parasite-based discriminant models supported 90–100% correct assignment of samples to capture location. Although preliminary due to limited sampling coverage, our results support the existence of at least two ecologically distinct sub-populations of southern blue whiting in South America. The joint use of otolith microchemistry and parasitological techniques showed to be a promising way to test hypotheses concerning ecological stocks in marine fishes.

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Introduction

The worldwide representation of genus *Micromesistius* is limited to just two species: blue whiting *M. poutassou* (Risso 1826) that inhabits the North Atlantic Ocean and the Mediterranean Sea, and Southern blue whiting *M. australis* (Norman, 1937), found in the Southern hemisphere. Although circa one order of magnitude smaller than the M*icromesistius poutassou* fishery (which ranks fifth in the world), the *M. australis* fishery sustains important factory vessel fleets in New Zealand, Chilean, Argentinean, British and international waters. The South American fishery started in the Southwest Atlantic area, in the late 1970s, reaching an historical peak of 258 000 metric ton in 1983. From that point, the fishery declined to annual catches between 41 000 and 84 000 ton during the last decade (FAO 2005). This fishery is currently considered a moderately over-exploited fishery, in which recent fishing mortality rates exceed sustainable levels by circa 35% (Wöhler et al. 2007). In the contiguous Southeast Pacific area, *M. australis* exploitation started, as bycatch, in the mid 1970s, with reported captures around 2500 ton. Only after the mid 1990s did the species become targeted by surimi factory vessels, producing a rapid increase in annual catches that have exceeded 20 000 ton during the last 16 years. In spite of its importance, the population and stock structure of *M. australis* around South America remains poorly known.

M. australis shares very similar morphological, biological and ecological features with *M. poutassou*. Both are relatively long lived, highly mobile mesopelagic species that inhabit a wide depth range and engage yearly reproductive migrations to shallower waters in autumn, with massive spawning events in late winter-early spring. *M. australis* is associated predominantly with sub-Antarctic waters, at temperatures between 3 and 10°C (Figueroa et al. 1998; Cassia 2000; Agnew 2002). This species is ubiquitous along the outer shelf and slope (130–800 m) of South America (South from 37°S), Antarctic Peninsula, Falkland (Malvinas) Islands, Scotia Ridge islands and New Zealand Plateau. Given its abundance and distribution, *M. australis* is considered one of the most important prey species for several top predators in the Sub-Antarctic ecosystem, including southern hake (*Merluccius australis*), king clip (*Genypterus blacodes*) and black-browed albatross, *Diomedea melanophrys impavida* (Payá 1992; Cherel et al. 1999; Nyegaard et al. 2004).

Three main distribution areas occur for *M. australis*: the southwest Pacific area around the New Zealand Plateau (SWP), the southeast Pacific area (SEP) along the Chilean coast, and the southwest Atlantic area (SWA) that extends from the Argentinean coast to the South Georgia Islands. Strong evidence indicates the SWP and the SWA stocks correspond to genetically distinct populations (Ryan et al. 2002). This work, based on mini- and microsatellite loci analyses, supported former work by Inada and Nakamura (1975), who classified SWP and SWA groups into two separate sub-species: *M. australis pallidus* in the SWP and *M. australis australis* in the SWA.

Due to its geographic and genetic proximity to the SWA area, the SEP stock has been considered as *M. a. australis* by most authors (Cohen et al. 1990; Arkhipkin et al. 2009). However, the population and stock structure of this subspecies has not been fully clarified. While *M. a. australis* presents a nearly continuous distribution around the Patagonian tip of South America (Fig. 1), genetic studies have failed to find significant differences between SEP and SWA groups (Shaw 2003, 2005; Galleguillos et al. 2009), suggesting all *M. a. australis* might belong to the same genetic population, which agrees with early morphometric work from the 1960s and 1970s (reviewed by Arkhipkin et al. 2009). More recent studies indicate, however, important levels of ecological segregation between the SEP and the SWA stocks. These studies have shown significant differences in exploitation trajectories, age at first maturity, size structure and growth patterns (Roa-Ureta 2009), as well as in trace metals composition in otolith cores and edges (Arkhipkin et al. 2009).

Two separate spawning grounds and diverging spawning migration circuits of *M. a. australis* are well known and regularly tracked by commercial fleets on both sides of Patagonia. On the SEP side, *M. a. australis* adults reach the southernmost tip of the Chilean shelf (~55°S) in May–June, most probably from Atlantic waters. From there, they move

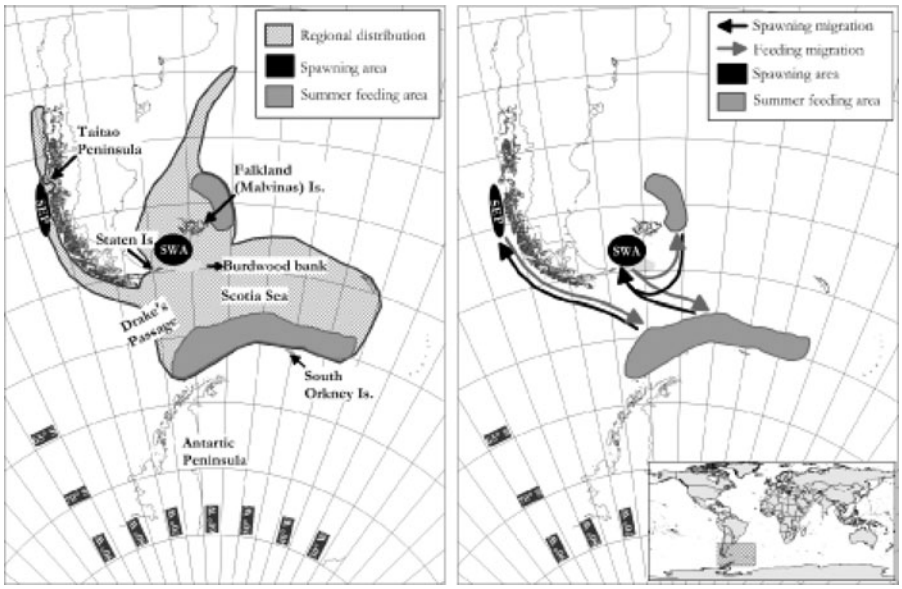

Fig. 1 Known regional distribution, spawning grounds and summer feeding areas of *Micromesistius australis australis* in the Patagonian area (*left panel*). Simplified migratory routes hypothesized for this species in Patagonia (*right panel*). Modified from Gordon (1991) based upon interpretations from Bailey (1982), Perrota (1982), Sánchez et al. (1986), Barrera-Oro and Tomo (1988), Wiecaszek (1988), Cassia (2000), Agnew (2002), Agnew et al. (2003), Saavedra et al. (2007) and Arkhipkin et al. (2009)

northwest to reach the SEP spawning ground, located between 47° and 51°S along the continental shelf (Fig. 1) where they spawn in late winter, between mid July and mid September (Céspedes et al. 1998; Lillo et al. 2002; Saavedra et al. 2007). Soon after, adults initiate a reverse (southward) migration, leaving Chilean waters in November (Céspedes et al. 1998) to cross Drake's Passage (Agnew et al. 2003) in search of summer feeding grounds (Fig. 1), probably located in the Southern Scotia Sea (Bailey 1982).

In the SWA area, massive westward migrations occur during the early winter, crossing the Burdwood Bank towards the Staten Island (Agnew 2002), where they tend to concentrate in August as gonads mature (Macchi and Pájaro 1999). From there, they seem to move northeast to reach the Falkland (Malvinas) spawning grounds (Fig. 1), located in the SSW section of the Falkland Plateau, between 51 and 53°S (Agnew 2002). After spawning during September and October (Agnew 2002), adults disperse into feeding grounds located along the Patagonian shelf NE of the Falkland (Malvinas) Islands (Perrota 1982; Agnew 2002) or in Antarctic waters of the Scotia Sea (Bailey 1982; Barrera-Oro and Tomo 1988; Wöhler et al. 2001; Agnew et al. 2003). Here adults from both the SWA and the SEP spawning grounds might overlap (Céspedes et al. 1998). Early life stages are believed to develop in the proximity of the spawning grounds (Arkhipkin et al. 2009), although existing evidence is restricted to observations of larval stages (Ehrlich et al. 1999; Agnew 2002; Balbontin et al. 2004).

Seasonal and lifetime migration patterns depicted above for the SEP and SWA areas still require formal and systematic clarification. Thus, major uncertainties remain on the actual degree of stock segregation and philopatry, including natal homing and spawning site fidelity (for term definitions see Secor 2010, this issue) between the SEP and the SWA stocks, including the periods and regions where these two stocks overlap. Answering these questions is not only of scientific interest, but also highly relevant to the assessment and management of domestic and international fisheries. As acknowledged by both Chilean and Argentinean scientists, *M. a. australis* stock assessment models are highly sensitive to assumptions about fishing mortality rates outside jurisdictional waters and reproductive subsidies from neighboring populations (Payá et al. 2002; Wöhler et al. 2007).

In the present work, we conducted a preliminary study aimed to obtain, compare and integrate evi-

dence about the stock structure and life history segregation of *M. a. australis* in Patagonia by using three complementary techniques: trace metals and stable isotopes in otolith cores (chemical tags), and parasite assemblages in adult tissues (biological tags). Otoliths have been shown to incorporate trace chemicals from early life, which can reflect habitat and physiological changes due to fish movements (Elsdon et al. 2008). In the present work, we focused on identifying multiple tracers (chemical fingerprint) incorporated during the first year of life (otolith core), which might be suitable for assessing nursery areas segregation and natal homing (Rooker et al. 2008), and to obtain retrospective information about fish habitat and metabolism (Sherwood and Rose 2003; Dufour et al. 2008). Here, we measured and compared otolith core concentrations of oxygen and carbon stable isotopes ($\delta^{18}O$ and $\delta^{13}C$), expected to reflect known regional differences in seawater composition and temperature between spawning areas (Thorrold et al. 1997; Rooker et al. 2008). Further, we sought to compare the discriminating power between stable isotope and trace metals. Although not planned and with a different focus, we analyzed some of the same otolith tracers utilized by Arkhipkin et al. (2009): barium (Ba), magnesium (Mg), manganese (Mn), and strontium (Sr).

While otolith chemistry reflects chemical properties from the environment at specific time intervals, parasites integrate biological signatures throughout the life-span of each host individual, which reflect the relative abundance of parasite infective stages in its prey and habitats across time (Lester 1990). Moreover, from the species composition and life-span of prevalent parasites it is possible to obtain valuable insights about the interannual consistency and life-history periods when such trophic segregation affected the host population. This information has been used to identify stocks that are ecologically separated and use distinct feeding areas (MacKenzie 2002; MacKenzie et al. 2008), and might be a valuable complement to otolith microchemistry techniques when no significant chemical differences exist in the environment, as could be the case in neighboring feeding areas along the Antarctic shelf.

By combining otolith chemistry and parasitological techniques we aimed to reach a better understanding of *M. a. australis* stock structure and life-history in Patagonia. To the best of our knowledge, the combination of these two techniques has not been attempted previously. In this preliminary study we compared the relative discriminating ability of these techniques and tested the feasibility of integrating different sources of information into a single statistical model for estimating stock mixture ratios.

Materials and methods

Otolith micro-chemistry

Two groups of 15 otoliths were randomly selected from available collections obtained from spawning adults (5–21 years old) caught at SEP and SWA spawning grounds in 2006 and 2007. Otoliths were embedded in a plastic resin (Secor et al. 1992) and then sectioned using an ISOMET (Beuhler ®) low speed wafering saw. Transverse sections through the otolith core were cut to 2 mm thickness. The embedded and sectioned otolith was glued to a 2 mm thick plastic wafer such that the glue occurred only under plastic regions. This assembly was then mounted to a petrographic slide using thermoplastic glue. The otolith assembly was mounted onto the sample plate of a New Wave Research MicroMill and a $1.2 \times 0.7 \times 2$ mm section was extracted from a region circumscribed by the first annulus. An image template was used to ensure a consistent region of the core was selected. The area included in this section represented several months within the first year of life of each specimen. The extracted core sections were physically cracked into several smaller fragments. Approximately half of this material (2.3 mg±0.105 SD) was used for stable isotope and the other half for trace elements analysis.

Otolith fragments were decontaminated according to techniques described in Rooker et al. (2001). Fragments were immersed for 5 min in 1% nitric acid to remove surface contamination, and then rinsed with double distilled water ($DDIH_2O$) for 5 min to remove the acid. Finally, they were dried under a Class 100 laminar flow hood, weighed to the nearest 0.01 mg, and stored in clean plastic vials. All instruments were cleaned with 10% HCL, $DDIH_2O$, and dried with ultra clean nitrogen air between samples. For stable isotope analysis, otolith fragments were powdered and submitted to the University of Arizona Isotope Geochemistry Laboratory for analysis following procedures detailed

by Kerr et al. (2007). Stable isotopes were measured with an automated carbonate preparation device (KIEL-III) attached to a gas-ratio spectrometer (Finnigan MAT 252). Otolith samples were reacted with dehydrated phosphoric acid under vacuum at 70°C. The CO_2 was then analyzed for $\delta^{18}O$ and $\delta^{13}C$ and reported as per mil relative to a standard (Vienna Pee Dee Belemnite [VPDB], international standards NBS-19 and NBS-18). Precision estimates reported by the Isotope Geochemistry Laboratory indicated relative standard deviations of 16.2% and 2.0% for $\delta^{13}C$ and $\delta^{18}O$, respectively. For trace metals analysis, digested samples were introduced into a Hewlett-Packard 4500 quadrupole ICP-MS. Levels of magnesium (Mg), manganese (Mn) and barium (Ba) were quantified using the method of standard additions; levels of calcium (Ca) and strontium (Sr) were determined using otolith Certified Reference Material (Yoshinaga et al. 2000) produced at the National Institute of Environmental Studies (NIES) of Japan. Standard samples were analyzed at intervals during machine runs to evaluate accuracy, obtaining relative standard deviation estimates of 2.1, 10.6, 8.7, 1.8 and 18.9% for Ca, Mg, Mn, Sr and Ba, respectively. Trace metal results were expressed as ratios to Ca concentrations ($\mu mol \cdot mol\ Ca^{-1}$).

Infracommunities

Ninety *M. a. australis* specimens were randomly selected from 2006–2007 commercial fishery trawls conducted at the SEP and the SWA spawning grounds, with sample sizes of 49 and 41 individuals, respectively. Since collected specimens were allocated and sent to different labs, specimens used for parasite analysis differed from those used for otolith microchemistry. Specimens were measured (fork length), weighed, sexed and dissected following standard practices (George-Nascimento and Arancibia 1994; George-Nascimento 1996), which focus on viscera, coelomic cavity, white muscle and gills examination. Ages were estimated from growth models available for the same stocks and period (Roa-Ureta 2009), indicating specimens spanned 5 to 10 years olds. Parasites were classified to the genus level whenever possible, otherwise to the lowest possible taxonomic category, recording total abundance for each taxon and individual host. Total parasite abundance (sum of all abundances across parasite taxa within hosts), taxonomic richness (total number of nominal taxa within hosts), diversity (Brillouin's index) and dominance (Berger-Parker's index) were calculated for parasite assemblages found in each fish host (= infracommunity, see Bush et al. 1997).

Seawater relevant data for SEP and SWA spawning grounds

Temperature and salinity values were extracted from oceanographic data gathered at the World Ocean Atlas 2005 (Antonov et al. 2006; Locarnini et al. 2006). Relative concentrations of $\delta^{18}O$ were obtained from published global gridded data (LeGrande and Schmidt 2006). All salinity, temperature and $\delta^{18}O$ records available in the proximity of each spawning ground were averaged across the 0–300 m depth stratum.

Statistical analysis

Trace metals and stable isotopes results were analyzed using a combination of univariate (ANOVA) and multivariate (MANOVA) analysis of variance. ANOVA was conducted within a linear mixed models framework (Searle 1987; Littel et al. 1996), and used to test pairwise differences in trace metals and stable isotopes between spawning grounds, after accounting for random effects from fish age (or cohort year) and fixed effects from fish length and otolith sample mass. Significant effects ($p<0.05$) were found for fish length upon Ba:Ca ratio (positive linear effects), and for otolith sample mass upon Mn:Ca and Mg:Ca ratios (negative linear effects). Therefore, a linear regression approach (Jónsdóttir et al. 2006) was used to adjust for length and mass effects in subsequent univariate and multivariate analyses.

Linear discriminant analysis, LDA (Fisher 1936), was used to construct and compare classification success from alternative discriminating functions based upon either trace metals, stable isotopes or parasite infracommunities. Age (cohort) was incorporated and tested as a covariate in all LDA models. This multivariate approach was also used to reduce dimensions in trace metals and parasite multivariate sub-models that were integrated into a common mixture distribution model (see below). Backward stepwise procedures were used to subset variables and produce a more parsimonious classification model that included trace metal, stable isotopes, and parasites (exclusion threshold, F-test, $p=0.05$). Canonical correlations were used to assess the association

between the groups formed by the response variables and each discriminant function, both in qualitative and quantitative terms (likelihood ratio test). Error rates were estimated through a cross-validation procedure where error-count estimates are produced after each observation is iteratively excluded from the training set (Lachenbruch and Mickey 1968).

Parasite prevalence (= percent of fish infected in a sample), and abundance were compared between SEP and SWA by means of Chi-square (or exact probability Fisher's) and Wilcoxon-Mann-Whitney (WMW) tests, respectively. Infracommunity descriptors and age were compared between spawning grounds using one-way ANOVAs. Linear discriminant analysis (LDA) was initially dismissed since observed abundances did not fulfill normality assumptions, even after several transformations were applied. However, an exploratory analysis indicated very similar results between LDA and a non-parametric discriminant analysis with a kernel set to the first nearest neighbor (NPDA). Thus, we present results from both a full data set NPDA model, and a LDA model based upon raw abundance responses of those taxa that satisfied the exclusion threshold used in backward stepwise selection procedures.

Information obtained from all three different sources (trace elements, stable isotopes and composition of parasite assemblages) was integrated into a two-stocks finite mixture distribution model (Everitt and Hand 1981), where means and variances by response variable and stock, and mix proportions by spawning ground were fit simultaneously through a maximum likelihood procedure. While not all responses were obtained from the same individuals, we assumed univariate normal distributions for the six response variables included in the model: $\delta^{13}C$, $\delta^{18}O$, and the first and second canonical roots obtained from 1) trace metals and 2) parasite LDAs. Binomial distributions were assumed for the stock proportion estimates. In practice, we maximized the joint likelihood of the data by estimating the parameters vector Ψ, given the mixture model,

$$l(\psi|y_{i,j,m}) = \sum_{m=1}^{2}\sum_{k=1}^{2}\sum_{j=1}^{6}\sum_{i=1}^{n_{j,m}}\left[p_{k,m}\cdot\exp\left(\frac{\log(2\pi)}{2}+\frac{\left(y_{i,j,m}-\overline{y}_{j,k}\right)^2}{2V\left[y_{j,k}\right]}+log\left(V\left[y_{j,k}\right]\right)\right)\right]$$

where,

p_k	$\frac{1}{1+\exp(-\beta_k)}$; natural-scaled proportion of stock k at spawning ground m
β_k	logit-scaled proportion of stock k at spawning ground m
$\overline{y}_{j,k}$	mean value of response variable j in stock k
$V[y_{j,k}]$	variance for response variable j in stock k
$y_{i,j,m}$	value of the response variable j observed in specimen i, collected at spawning ground m.

Following previous model specifications, $\widehat{p}_{k,m}$ was back-calculated from the logit-scaled estimate $\widehat{\beta}_{k,m}$. Under this two-stock model, it is enough to estimate $\widehat{\beta}\ \widehat{p}_{k,m}$ for one stock, as the other can be simply computed by difference $\widehat{p}_{k,m}$. The variance of $\widehat{p}_{k,m}$ was approximated from $\widehat{V}\left[\widehat{\beta}_{k,m}\right]$ as,

$$\widehat{V}\left[\widehat{p}_{k,m}\right] = \frac{1}{\left(1-e^{-\widehat{\beta}_{k,m}}\right)^4}\widehat{V}\left[\widehat{\beta}_{k,m}\right]$$

The parameters vector Ψ contained 26 elements, which can be grouped into two categories. The first category corresponded to stock-specific mean and variance estimates for each of the six response variables. The second one corresponded to the logit-scaled proportions estimated for each stock at each spawning ground (see details below). Mean and variance estimates represented the underlying signature of response variables attributed to each stock, instead of sample means and variances computed from their observed values, as it would be the case under a classical discriminant analysis. Thus, the mixture model maximized the joint likelihood of individual responses (specimens within spawning grounds) as a function of a) estimated means and variances by response variable and stock, and b) estimated stock proportions by spawning ground. Since this was a two-stock model, the dominant stock proportion $\widehat{p}_{k,m}$ ranged from 0.5 (even mix, no segregation) to 1.0 (no mix, complete segregation). As a result, $\widehat{p}_{k,m}$ can be interpreted as a maximum likelihood estimate of stock segregation rate, while its

logit-scaled equivalent $\widehat{\beta}_{k,m}$ can be used for testing the null hypothesis of no segregation (i.e., $\widehat{\beta}_{k,m}=0$; $\widehat{p}_{k,m}=0.5$). As $\widehat{p}_{k,m}$ results directly from modeling the data, this segregation rate is not strictly equivalent to a classification success rate. In fact, there is no discriminant model involved in this analysis.

No evidence of lack of normality was found by Shapiro-Wilk´s univariate test for any of the trace metals, stable isotopes or parasite variables considered in this work, except for parasite abundances. Multivariate normality was graphically assessed by Q-Q plots and by applying Shapiro-Wilk´s test on the first and last principal component derived from the original variables. Null hypotheses for all analyses in the present work were rejected under a significance level (α) of 0.05.

Results

Otolith trace elements

We found significantly higher Sr:Ca ratios in adult otolith cores from the SWA than in those from the SEP spawning ground, but no significant differences were detected between SWA and SEP areas for Ba:Ca, Mg:Ca and Mn:Ca ratios (Table 1, Fig. 2). MANOVA failed to show significant differences between spawning grounds for the full model when all trace metals were considered (Table 1). MANOVA results indicated, however, significant differences between spawning grounds for the reduced model (backward procedure), when response variables were limited to Sr:Ca and Mg:Ca (Table 1, Fig. 3). No evidence of significant age effects was found, neither for the full, nor for the reduced model (Table 1). Linear discrimination based upon these two ratios reached a classification success of 83% for the samples (Table 2). Squared canonical correlation indicated circa 25% of the variance in discriminant scores was explained by this discriminant model.

Otolith stable isotopes

Mean $\delta^{13}C$ was significantly higher in SEP otolith cores, while mean $\delta^{18}O$ was higher in samples from the SWA spawning ground (Table 3, Fig. 2). MANOVA detected significant differences ($p<0.001$) in both stable isotope ($\delta^{13}C$ and $\delta^{18}O$) between the two spawning grounds, without evidence of significant age (cohort) effects (Table 3). Both variables, $\delta^{13}C$ and $\delta^{18}O$, were selected by the LDA stepwise procedure into a model that explained 89% of the variability in discriminant scores, and assigned correctly 100% of the samples to their respective spawning ground (Table 2, Fig. 3).

Parasites assemblages

None of the infracommunity descriptors showed significant differences between SEP and SWA (Table 4). Most prevalent parasites were *Anisakis, Kudoa* and *Hysterothylacium.* More abundant ones were *Kudoa*, *Anisakis* and *Diclidophora* (Table 5). Although 10 out of the 17 observed parasite taxa were

Table 1 Univariate and multivariate analysis of trace metal ratios (μmol·mol Ca^{-1}) in otolith cores from adult *Micromesistius australis australis* captured at the Southeast Pacific (SEP) and Southwest Atlantic (SWA) spawning grounds.

SE=standard error. MANOVA full model includes all four trace metals. Reduced model (backward stepwise procedure) only includes Mg:Ca and Sr:Ca

Analysis	Response variable	Mean (SE)		F	*p*-value
		SEP	SWA		
ANOVA	Ba:Ca	10.5(0.77)	11(1.29)	0.04	>0.1
	Mg:Ca	320(12)	300(16)	1.067	>0.1
	Mn:Ca	20(0.49)	18(1.5)	0.49	>0.1
	Sr:Ca	2070(76)	2340(85)	5.31	<0.05
MANOVA full model	Spawning ground			2.12	>0.1
	Fish age			1.54	>0.05
MANOVA reduced model	Spawning ground			3.42	<0.05
	Fish age			1.24	>0.1

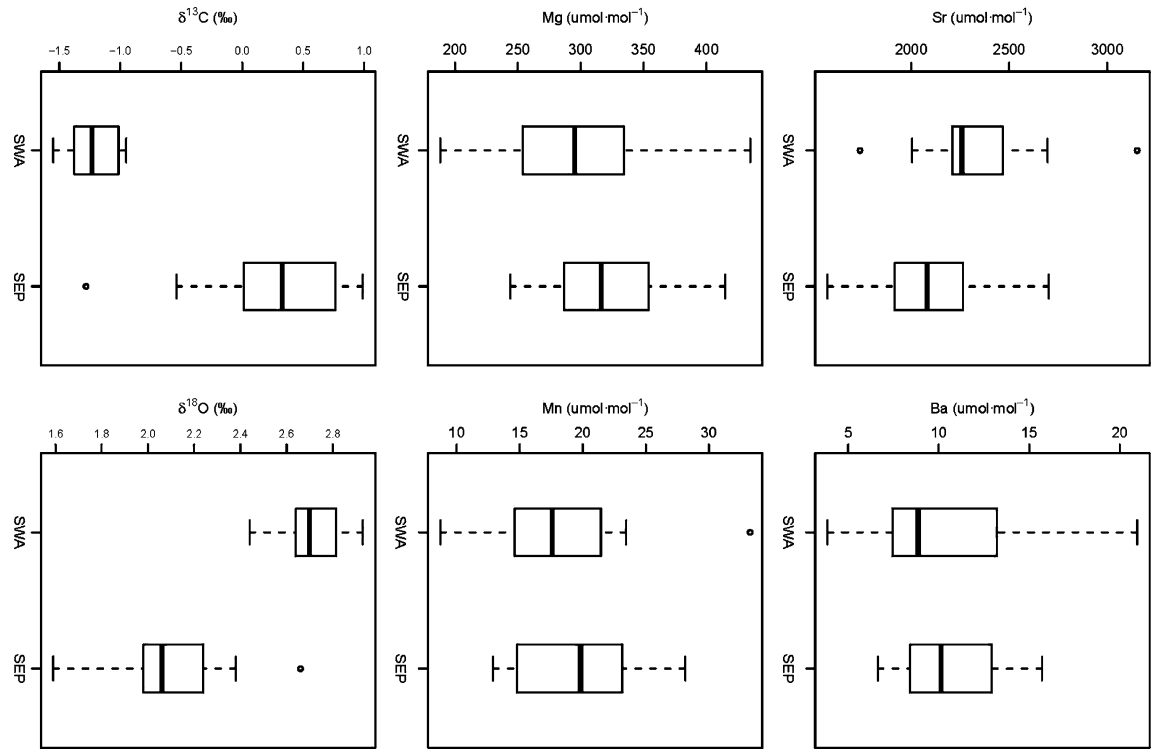

Fig. 2 Trace metal (Sr, Ba, Mg, Mn) and stable isotope ($\delta^{13}C$ and $\delta^{18}O$) ratios in core sections of adult *Micromesistius australis australis* otoliths from Southeast Pacific (SEP) and Southwest Atlantic (SWA) spawning areas. Mg and Mn values corrected for mass effects. Ba values are corrected for fish length effects. Metal ratios are relative to Ca concentration (μmol·mol Ca^{-1}). Isotope ratios are relative to international standards NBS-19 and NBS-18 (parts per thousand)

common to both spawning grounds (Table 5), Chi-square tests indicated significant differences in parasites prevalence between SEP and SWA areas. Larger differences in prevalence corresponded to *Chondracanthus*, *Contracaecum, Hepatoxylon* and *Grillotia* (Table 5). There were also significant differences (WMW test) in the abundance of some parasite taxa between spawning grounds, particularly *Diclidophora, Anisakis*, *Contracaecum*, *Hysterothylacium* and *Hepatoxylon* (Table 5).

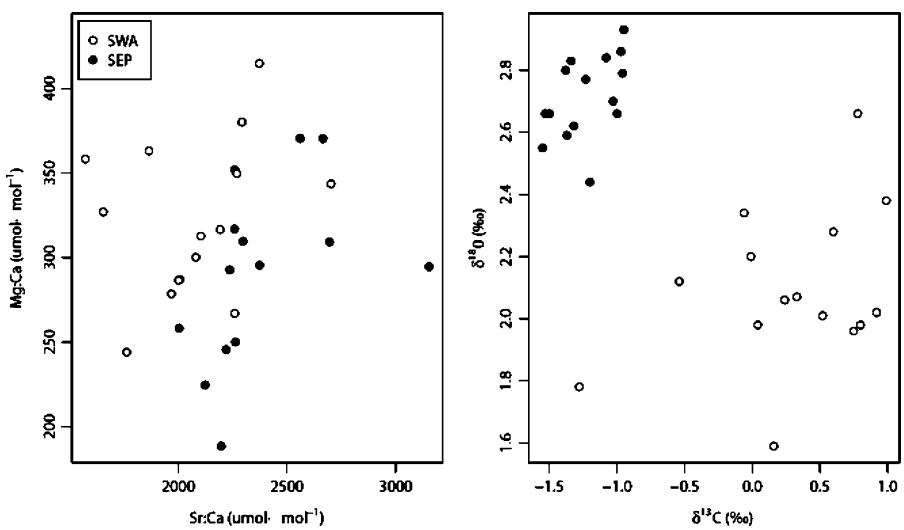

Fig. 3 Bivariate plots for metals (Sr, Mg) and stable isotopes ($\delta^{13}C$ and $\delta^{18}O$) in otolith cores from adult *Micromesistius australis australis* from Southeast Pacific (SEP) and Southwest Atlantic (SWA) spawning areas. Metal ratios are relative to Ca concentration (μmol·mol Ca^{-1}). Isotope ratios are relative to international standards NBS-19 and NBS-18 (parts per thousand)

Table 2 Assignment matrix from four discriminant models conducted on *Micromesistius australis australis* captured at Southeast Pacific (SEP) and Southwest Atlantic (SWA) spawning grounds. Otolith core microchemistry models include i) trace metals linear LDA (reduced model, Sr:Ca and Mg:Ca) and ii) stable isotopes LDA ($\delta^{13}C$ and $\delta^{18}O$). Parasite models included iii) reduced LDA model (five taxa), and iv) full NPDA model (17 taxa). LDA: linear discriminant analysis. NPDA: Non-parametric discriminant analysis. All error rates estimated by cross-validation. *n.a.* non-applicable

Predictive variables	From	Into		Percent correct	Canonical correlation	*p*-value
		SWA	SEP			
i) Trace metals LDA (reduced model)	SWA	12	3	80.0	0.45	<0.05
	SEP	2	13	86.7		
	Total	14	16	83.3		
ii) Stable isotopes LDA	SWA	15	0	100	0.94	<0.0001
	SEP	0	15	100		
	Total	15	15	100		
iii) Parasites LDA (reduced model)	SWA	36	5	89.8	0.79	<0.0001
	SEP	5	44	87.8		
	Total	41	49	87.9		
iv) Parasites NPDA (full model)	SWA	41	0	100	n.a.	<0.0001
	SEP	0	49	100		
	Total	41	49	100		

MANOVA indicated overall differences between spawning grounds in parasite abundance, both for the full (Wilks's lambda=0.366, $F_{(17, 72)}=7.34$, $p<0.0001$) and reduced (Wilks' lambda=0.378, $F_{(5, 84)}=27.67$, $p<0.0001$) models. Complete differences in composition of parasite infracommunities between spawning grounds were revealed by NPDA, with 100% correct assignment to locality of capture (Table 2). An overall 87.9% correct assignment was obtained by LDA (Table 2) based upon five selected (backward stepwise procedure) taxa: *Hepatoxylon*, *Anisakis*, Anisakidae (undetermined genus), *Ascarophis* and cysts of unknown ethiology. The first canonical root from this model (Fig. 4) contributed >95% of the explained variance and was unrelated to age ($p>0.1$). The taxa most positively and negatively associated to this first root were *Hepatoxylon* and *Anisakis,* respectively.

Integrated analysis

The fully integrated mixture model, where all three discriminating sources (trace metals, stable isotopes and parasite infracommunities) where included, estimated segregation ratios of 0.97±0.024 (SE) for the SEP area, and of $1.0\pm4.5\times10^{-5}$ (SE) for the SWA spawning ground (Table 6). These estimated ratios were significantly different from 0.5 (i.e., from the null hypothesis of no segregation) at both spawning grounds. Mixture models built upon individual sources (trace metals, stable isotopes or parasites) yielded segregation ratio estimates that ranged from 0.9 to 1.0, depending on the spawning ground and the predictive variables used (Table 6). Highest levels of uncertainty in segregation ratio estimates occurred for the trace metals mixture model (Table 6).

Table 3 Univariate and multivariate analysis of stable isotope ratios in otolith cores from adult *Micromesistius australis australis* captured at the outheast Pacific (SEP) and Southwest Atlantic (SWA). Isotope ratios are relative to international standards NBS-19 and NBS-18

Analysis	Response variable	Mean (SE)		F	*p*-value
		SEP	SWA		
ANOVA	$\delta^{13}C$	0.28(0.156)	−1.23(0.056)	81.72	<0.001
	$\delta^{18}O$	2.09(0.066)	2.71(0.034)	68.63	<0.001
MANOVA	Spawning ground			116.89	<0.001
	Fish age			1.51	>0.1

Table 4 Mean (standard deviation) of fish age, total abundance, taxonomic richness, Brillouin's diversity index and Berger-Parker's dominance index in parasite infracommunities of southern blue whiting (*Micromesistius australis australis*) at Southeast Pacific (SEP) and Southwest Atlantic (SWA) spawning grounds

	SEP n=49	SWA n=41	F	p>F
Total abundance	44.59 (30.03)	33.07 (17.26)	0.43	>0.1
Richness	5.84 (1.56)	5.29 (1.56)	2.89	>0.05
Diversity	1.57 (0.39)	1.45 (0.39)	1.94	>0.1
Dominance	0.24 (0.13)	0.29 (0.15)	2.17	>0.1

Seawater data

The analysis of published oceanographic data indicated important differences between spawning grounds regarding salinity, temperature and $\delta^{18}O$ concentration in seawater. The 0–300 m stratum at the SEP spawning ground was characterized by a lower and more variable salinity (32.2±1.44 SD), a higher mean temperature (8.4°C±0.71 SD) and a lower mean concentration of $\delta^{18}O$ (0.074 ‰±0.104 SD). Mean values for the SWA spawning ground yielded a salinity of 34.1±0.099 SD, a temperature of 4.5°C±0.49 SD and a $\delta^{18}O$ ratio of 0.211 ‰±0.148 SD.

Discussion

All three sources of evidence suggested high levels of stock segregation between the SEP and the SWA subpopulations of *M. a. australis*, at opposing sides of South America. There where noticeable differences in the relative strength of the three discriminant technique applied. Thus, in spite of a relatively small

Table 5 Prevalence and abundance of 17 parasite taxa found in 90 *Micromesistius australis australis* specimens sampled at Southeast Pacific (SEP) and Southwest Atlantic (SWA) spawning grounds. n=number of hosts examined. Asterisks indicate significant univariate differences in prevalence or abundance (p<0.05) between spawning grounds

Parasite Taxa	SEP n=49		SWA n=41	
	Prevalence %	Abundance (SD)	Prevalence %	Abundance (SD)
Ecotoparasites				
Diclidophora	61.2	1.94(2.33)*	82.9	3.71(4.54)
Chondracanthus	10.2*	0.28(1.02)	0.0	–
Adult Endoparasites				
Ascarophis	14.3	0.33(0.92)	4.9	0.05(0.22)
Cucullanus	0.0	–	2.4	0.02(0.16)
Derogenes	10.2	0.10(0.31)	22.0	0.24(0.49)
Hemiuridae	20.4	0.63(1.89)	31.7	0.46(0.78)
Kudoa	81.6	21.94(22.69)	87.8	11.10(11.23)
Larval Endoparasites				
Anisakis	100	9.12(6.32)*	97.6	13.66(7.26)
Hysterothylacium	81.6	3.57(3.24)*	80.5	1.88(1.58)
Contracaecum	69.4*	1.39(1.51)*	24.4	0.41(0.86)
Anisakidae indet.	36.7	0.84(1.36)	61.0	1.05(0.97)
Hepatoxylon	73.5*	3.69(3.65)*	24.4	0.39(0.80)
Diphyllobothrium	6.1	0.08(0.34)	0.0	–
Pseudophyllidea	4.1	0.06(0.32)	0.0	–
Grillotia	0.0*	–	9.8	0.10(0.30)
Corynosoma	8.2	0.10(0.37)	0.0	–
Others				
Cysts of unknown etiology	8.2	0.51(2.32)	0.0	–
Total	100	44.59(30.03)	100	33.07(17.26)

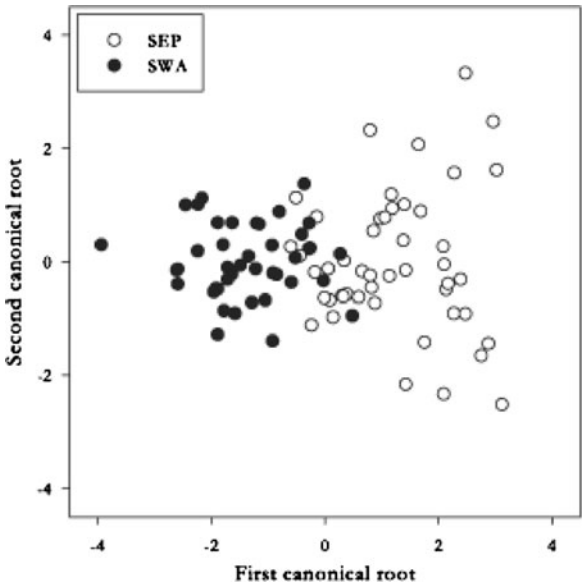

Fig. 4 Bivariate plot of the first two canonical roots corresponding to linear discriminant analysis (LDA) based upon 17 parasite taxa observed in 90 *Micromesistius australis australis* adults from Southeast Pacific (SEP) and Southwest Atlantic (SWA) spawning grounds

sample size (n=30), stable isotopes analysis provided uncertainty (MANOVA p-value, Table 3), error rates (LDA, Table 2) and segregation ratio estimates (Table 6) similar to those obtained from parasite assemblage analysis, which had a much larger sample size (n=90). A lower discriminant performance was observed, on the other hand, for trace metals, which presented smaller mean differences between spawning grounds, implying a need for larger sample sizes to obtain statistical power equivalent to that obtained from stable isotopes analysis. Overall, stable isotopes results exceeded what was expected for a preliminary study. For instance, in spite of a large age range in the samples, the variance contribution from inter-annual variability was relatively small and did not mask variability between spawning grounds, as probably occurred to some degree with trace metal results.

The existence of two segregated stocks of *M. a. australis* off South America is consistent with previous otolith trace metal work conducted in this area by Arkhipkin et al. (2009). These authors report a proportion of misclassified fish, after a clustering procedure, that averaged 20%. Although statistical methods are not fully comparable and our sample size is much smaller, this quantity is similar to the 18% misclassification ratio yielded by our LDA model based upon trace metals. It must be highlighted that misclassification and mix ratios should not be interpreted as equivalent and comparable quantities. We can illustrate this difference by comparing results from our LDA and mixture models. Taking, for example, LDA and mixture models based upon trace metals (Table 6), we obtain an average misclassification ratio of 18%, much larger than the average 5% obtained for the mix ratio. When all three sources of stock discrimination data were used segregation estimates increased substantially to nearly 100% for both regions.

Stock segregation levels indicated by chemical and parasitological tags are relevant to an ecological time-scale. Thus, results reported here are not necessarily inconsistent with genetics evidence that failed to show discrimination between the SWA and the SEP stocks (Perrota 1982; Shaw 2003, 2005; Galleguillos et al. 2009). Genetic segregation results from processes occurring at evolutionary time-scales and requires nearly full isolation to yield significant levels of discrimination between groups.

Table 6 Stock segregation ratios estimated for *Micromesistius australis australis* in the Southeast Pacific (SEP) and Southwest Atlantic (SWA) spawning grounds. Estimates obtained from alternative mixture models based upon i) stable isotopes (δ^{13}C and δ^{18}O), ii) the first two canonical roots from trace metals linear discrimination model (LDA), iii) the first two canonical roots from parasites LDA; and iv) all previous sources of information combined.

	SEP		SWA	
	Natal homing ratio (SE)	p-value (t-test, $\widehat{5}_{k,m}$=0.5)	Natal homing ratio (SE)	p-value (t-test, $\widehat{5}_{k,m}$=0.5)
Trace metals	$1.00(6.5\times10^{-4})$	<0.0001	0.9(0.15)	<0.05
Stable isotopes	0.91(0.059)	<0.0001	$1.00(8.0\times10^{-5})$	<0.0001
Parasites	$1.00(9.7\times10^{-5})$	<0.0001	$1.00(1.0\times10^{-4})$	<0.0001
Integrated analysis	0.96(0.026)	<0.0001	$1.00(4.5\times10^{-5})$	<0.0001

The large and consistent difference in stable isotopes composition we found in the otolith cores indicated a very large majority of adults captured at a given spawning ground spent their first year of life at a chemically distinct area. Considering that spawning areas are located in relative proximity to spawning grounds (Agnew 2002; Arkhipkin et al. 2009), it is very likely that most adults in the sample actually returned to their natal ground. In fish otoliths, $\delta^{18}O$ have been found to be incorporated in direct proportion to $\delta^{18}O$ ratios in the ambient seawater, but in inverse relation to seawater temperature (Kalish 1991a, b; Thorrold et al. 1997; Høie et al. 2003). Using mean $\delta^{18}O$ and temperature values computed from published oceanographic data (LeGrande and Schmidt 2006; Locarnini et al. 2006) we found that the higher $\delta^{18}O$ we measured in SWA otolith cores were consistent with both higher mean $\delta^{18}O$ (0.106‰) and lower mean temperature (4.5°C) in seawater at this spawning ground (0–300 m depth). This match in $\delta^{18}O$ between otolith cores and seawater suggest that a large fraction of *M. a. australis* adults may return to the grounds where they spent their fist year of life, suggesting a high degree of life-history containment. While this containment might result from both natal homing and spawning site fidelity behaviors, future microchemistry analysis of juvenile otoliths would be required to provide direct support to a natal homing interpretation of our findings.

Differences in $\delta^{13}C$ between otolith cores from different ecosystems are harder to interpret since $\delta^{13}C$ in otoliths is not in full equilibrium with seawater $\delta^{13}C$ (Kalish 1991b). Thus, although otolith $\delta^{13}C$ is primarily affected by $\delta^{13}C$ in seawater dissolved inorganic carbon (DIC), it is also affected by $\delta^{13}C$ ratios in ingested prey (Radtke et al. 1996; Solomon et al. 2006) and by progressive fractionation (depletion of $\delta^{13}C$) through metabolic pathways (Kalish 1991a; Gauldie et al. 1994; Høie et al. 2003; Sherwood and Rose 2003; Dufour et al. 2008). Seawater DIC tends to get enriched in $\delta^{13}C$ at areas where freshwater influence is larger (Fry 2002; Kerr et al. 2007). Hence, the higher $\delta^{13}C$ observed in SEP otoliths might be a consequence of the relative proximity of this spawning ground to the coast, and the much higher freshwater inflow that characterizes this coastal area (Pickard 1971; Silva and Palma 2000), reflected in the lower mean salinity estimated from published oceanographic data (Antonov et al. 2006). Following the same rationale higher $\delta^{13}C$ would be also expected in SEP prey species, agreeing with our findings in otoliths from this location. Metabolic depletion of $\delta^{13}C$ would be expected, however, to be higher at this more temperate spawning ground, which could dampen to some unknown degree enrichment of $\delta^{13}C$ due to trophic effects.

The similarity in species richness and diversity between parasite infracommunities from adult *M. a. australis* sampled at SEP and SWA, and the consistency observed in LDA results across ages suggest we obtained a robust and representative characterization of these assemblages. These findings support the past use of this tool to assess stock segregation in this species (Williams et al. 1992; MacKenzie 2002; Mackenzie et al. 2008). Hence, the significant differences found in the prevalence, abundance and composition of parasite infracommunities of *M. a. australis* from both spawning grounds indicate a consistent trophic segregation between stocks throughout most or all their life cycles. This assertion arises from the fact that all taxa (except *Diclidophora*) are transmitted to *M. a. australis* through predation, and that some of them correspond to long standing cumulative infections in teleost fishes (*Anisakis* and *Hepatoxylon* larvae). Most taxa we found become adults in *M. a. australis* (*Ascarophis*) or in its predators, including teleost fishes (*Hysterothylacium* sp.), elasmobranchs (*Grillotia*, *Hepatoxylon*) and mammals (*Anisakis*, *Contracaecum*, *Diphyllobothrium*, Pseudophyllidea, *Corynosoma*). Moreover, all five taxa selected as part of the parasite LDA analysis are cumulative along host ontogeny, except for *Ascarophis*.

The segregation between feeding grounds indicated by the parasite infracommunity analysis is consistent with results from elemental composition in otolith edges (Arkhipkin et al. 2009), which suggested the summer feeding grounds located NE from the Falkland Islands were used by SWA but probably not by SEP spawners. What remains to be evaluated, however, is the actual level of mixing of these two stocks at the Scotia Sea feeding grounds. Arising evidence about stock segregation of *M. a. australis* both in nursery areas and adult feeding grounds also leads to new questions about the degree of overlap between stock specific migratory routes, particularly around the Tierra del Fuego and Staten Islands shelfs,

where an important fishery occurs every year. Answering these new questions will be a high priority for the sustainable management of these stocks from both national and international perspectives.

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