

# Myxozoans as biological tags for stock identification of the Argentine hake, *Merluccius hubbsi* (Gadiformes: Merlucciidae)

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## SUMMARY

Myxozoans have been successfully used as tags for fish stock identification around the world. However, few studies using myxozoan tags have been carried out in the Southern Atlantic, a region with complex oceanography that constitutes a potentially suitable scenario for testing the utility of myxozoans as indicators. Its usefulness was tested using six samples of *Merluccius hubbsi* in two different regions of the Argentine Sea. Generalized linear models were performed to assess the effects of fish size and sex, and year and region of capture and selected using the Information Theoretic approach. Three myxozoan species were recorded: *Kudoa rosenbuschi*, *Myxoproteus meridionalis* and *Fabespora* sp. Results of modelling species individually showed differential capabilities for detecting geographical population structure at different spatial scales, with *K. rosenbuschi* and *Fabespora* sp. allowing the discrimination of northern and southern stocks, but *Fabespora* sp. also as a promissory indicator of intrapopulation sub-structure due to different migratory routes during non-reproductive periods. This work confirms that myxozoans offer a set of suitable markers at different spatial scales, which can be selected individually or in any combination, depending on the geographical extent of the study, constituting tools adaptable to the objectives of further research on fish population structure.

Key words: Biological markers, *Kudoa rosenbuschi*, *Myxoproteus meridionalis*, *Fabespora* sp., common hake, stock discrimination, South West Atlantic.

## INTRODUCTION

The use of parasite species as biological tags (BTs) is one of several approaches applied to identify fish stocks and to trace movements of fish populations (MacKenzie and Abaunza, 2005). Fish stock identification is essential for fishery sciences since it is a prerequisite for the assessment of exploited resources and their population dynamics, in order to provide information necessary for their proper management (Cadrin *et al.* 2005). Over recent decades, the use of parasites as BTs has been increasingly applied in such sense, being at present a well-established methodology (Timi and MacKenzie, 2015).

Parasite species that are ideal as BTs are characterized by showing significantly different levels of infection among localities, persisting for long periods in the host, being easily detected and identified, and not causing serious damage to the host

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(MacKenzie and Abaunza, 2013; Catalano *et al.* 2014). Some myxozoans meet the requirements, being host/site specific and easily identified by their distinctive spore shapes, with some species known to persist for years in their hosts (Lom and Dyková, 1992). Indeed, many myxozoan species have been successfully used as tags for stock identification of valuable fish species around the world (Moles and Short 1989; Khan and Tuck, 1995; Urawa and Nagasawa, 1995; Larsen *et al.* 1997; Campbell, 2005; MacKenzie *et al.* 2005). Moreover, this group of parasites proved to be a very effective BT on the coast and the open seas of the Pacific Ocean in North America (Marcogliese and Jacobson, 2015). The use of parasites as BTs has been successfully applied to delineate fish stocks in the South West Atlantic (Timi, 2007; Cantatore and Timi, 2015), only few studies using myxozoan species as BTs have been carried out in this region, with most of them corresponding to stock assessments of deep water fishes inhabiting the southern boundaries of the continental shelf (Agnew *et al.* 2003; George-Nascimento *et al.* 2011; MacKenzie *et al.* 2013). Nonetheless, due to the oceanographic complexity of Southwest Atlantic at lower latitudes, it is expected that the

discriminatory capability of myxozoans is at least equivalent to those demonstrated for several helminth species in that region (Cantatore and Timi, 2015).

In this region the Argentine hake, *Merluccius hubbsi* Marini, 1933 is one of the principal sources of income in the Argentine and Uruguay fisheries (Bezzi *et al.* 2004). This temperate-cold species is widely distributed in the Southwest Atlantic Ocean, inhabiting Brazilian, Uruguayan and Argentine waters, from 22° to 55°S at depths ranging between 50 and 500 m (Cousseau and Perrotta, 2004).

Based upon evidence from ichthyological studies, two different stocks of *M. hubbsi*, limited by the 41°S parallel, have been identified in Argentine waters and considered for management purposes: the northern stock called Bonaerense and the southern one, known as Patagonian (Bezzi *et al.* 1995). Each stock has its main spawning and nursery area and season; the northern area situated in the Argentine–Uruguayan Common Fishing Zone at the Rio de la Plata estuarine front during the austral autumn–winter (May–July) and the southern area in coastal waters of Chubut Province during the austral spring–summer (with a main peak in December and January) (Macchi *et al.* 2004, 2007). It has also been established that hakes of both stocks perform trophic and reproductive seasonal migrations between the coast (near the 50 m isobath) and deeper waters (more than 100 m depth) (Pájaro *et al.* 2005; Macchi *et al.* 2007). The existence of an independent third group, restricted to the San Matías Gulf basin (41°–42°S, 63°–65°W), has also been proposed based on fishery biology and population studies (Ehrlich, 1998; Sardella and Timi, 2004; Machado-Schiaffino *et al.* 2011).

Two previous studies have used parasite tags to discriminate stocks of *M. hubbsi* in the region; the first by MacKenzie and Longshaw (1995) studied the potential value of parasites as BTs for Argentine hake in southern deep waters of Patagonia. However, as stated by the authors, their samples consisted of small numbers of fish of mixed sizes and ages, which precluded them from reaching reliable conclusions regarding stock identification and migrations of this host species. Nevertheless a number of parasite species were pointed out as the most promising BTs; among them, the authors identified the myxozoans *Alatospora merluccii* Kalavati, Longshaw and MacKenzie, 1995, *Kudoa rosenbuschi* (Gelormini, 1944), *Myxidium baueri* Kovaleva and Gaevskaya, 1982 and *Myxoproteus meridionalis* Evdokimova, 1977, the four myxozoan species so far known to infect the hake. The second by Sardella and Timi (2004) identified three stocks of hake in the Argentine Sea using parasites as BTs, one composed

of hakes from the Argentine–Uruguayan Common Fishing Zone, a second stock in San Matías Gulf and a third comprising samples from both San Jorge Gulf and the Patagonian Shelf. However, of the two myxozoan species found in this study, only *K. rosenbuschi* was useful as a discriminating species. The other species found, *M. meridionalis*, was not included in analyses because not all hakes were examined for it (Sardella and Timi, 2004). Based on these findings, myxozoans could be a good ancillary tool for delineating hake stocks, even at smaller spatial scales than in previous studies.

The main objectives of the study were to identify myxozoan species that parasitize *M. hubbsi* in the northern and central Argentine Sea and to assess the feasibility of using them as BTs to (i) discriminate among different hake stocks, and (ii) to trace movements of hake populations in the region.

## MATERIALS AND METHODS

### *Fish samples and parasites inventories*

In total, 213 specimens of Argentine hake were caught in two regions: in Patagonian waters (Chubut Province, 42°–46°S/63°–66°W) during a research cruise in January 2009 and by commercial trawlers in January 2014, and off the coast of Buenos Aires Province (36°–39°S/53°–57°W) by commercial trawlers in July 2009 and May 2014 (Table 1). The two most recent samples, caught 5 years later, were included in order to evaluate temporal stability of parasite prevalence. As *M. hubbsi* displays different migratory patterns at both sampling regions concentrating in two separate and well-known reproductive areas (Bezzi *et al.* 1995), the samples were collected during the reproductive season to ensure their belonging to each individual stock. Moreover, data on the exact geographical coordinates of capture available only for hakes caught during the research cruise (January 2009), allows comparative analyses among samples Patagonian 1, 2 and 3 to test for differences in parasite faunas obtained in the regions of origin previous to reproductive migrations.

Fish were kept frozen at –18° C until examination. Each specimen was measured (total length), sexed and thoroughly examined for myxozoan infections. Any tissue lesion or cyst observed under a stereoscopic microscope, as well as smears of brain, liver, kidney and spleen, and contents of gall and urinary bladders were examined with the aid of a light microscope equipped with differential interference contrast. Myxozoans were collected and preserved in 10% formaldehyde solution for subsequent morphological identification following the recommendations made by Lom and Arthur (1989) and Lom and Dyková (1992).

Table 1. Composition of samples of *Merluccius hubbsi* Marini, 1933 (Gadiformes: Merlucciidae) in two regions of the South West Atlantic

Sample	Position	Date of collection	Number of examined fish			Mean total length $\pm$ s.d. (range) in cm
			Males	Females	Total	
Bonaerense 1	36°–39°S/53°–57°W	July 2009	18	24	42	44.5 $\pm$ 3.5(39.8–55.0)
Bonaerense 2	36°–39°S/53°–57°W	May 2014	0	20	20	48.0 $\pm$ 3.6(42.5–56.0)
Patagonian1	44°4'2"S/63°28'55"W	January 2009	46	4	50	41.8 $\pm$ 3.4(34.5–47.5)
Patagonian2	45°32'13"S/66°6'32"W	January 2009	3	28	31	44.7 $\pm$ 5.2(36.5–58.2)
Patagonian 3	46°1'48"S/65°33'7"W	January 2009	4	28	32	40.5 $\pm$ 3.3(34.0–47.0)
Patagonian 4	42°–46°S/63°–66°W	January 2014	20	18	38	38.9 $\pm$ 2.7(34.0–47.0)

### Statistical analyses

Prevalence was calculated according to Bush *et al.* (1997) for each myxozoan species at each sample. Sterne's exact 95% confidence limits were calculated for prevalence using Quantitative Parasitology 3.0 software (QP3.0) (Reiczigel, 2003; Reiczigel and Rózsa, 2005).

The effects of fish total length (L, in cm), year (Y, 2009 and 2014), region of origin (R, Bonaerense -BA- and Patagonian -PAT-) and their interactions on the occurrence of each myxozoan species were evaluated using generalized linear models (GLMs) (McCullagh and Nelder, 1989). Samples Patagonian 1, 2 and 3 (see Table 1), obtained from a research vessel as three different fishing hauls from the same region and year, were pooled in order to give the same treatment to all samples in the analysis. In the case of *Fabespora*, which was absent from Bonaerense samples (see results, Table 2), region of origin was excluded as a predictor variable because the complete separation of samples as a result of a 'too good predictor' produces extreme standard errors (S.E.) that are indicative of poor convergence of a model, resulting in unreliable inferences (Bolker *et al.* 2009).

Data on the exact geographical coordinates of capture were available only for hakes caught during the research cruise (January 2009), so analyses at a small spatial scale were only possible for these samples. GLMs were repeated excluding the variable year and replacing region of origin by haul position (Patagonian 1, 2 and 3) to evaluate its effects on the presence of each myxozoan species.

The variable sex was not included in all former analyses because not all combinations of year (2009 or 2014) and region of origin (Bonaerense or Patagonian) had equivalent female:male ratios (Table 1), resulting therefore in collinearity between sex and year/region of origin combinations. Nonetheless, the characteristics of the sample Patagonian 4 in particular (sample size, comparable sex proportions and parasite prevalence values) allowed the evaluation of the effect of sex on the occurrence of each myxozoan species within this

sample. Therefore, new GLMs, including length, sex and their interactions as predictor variables were fitted and analysed (see below).

Following Mazerolle (2006), for each myxozoan species in every analysis, a set of alternative candidate models was selected among all possible models by choosing those combinations of predictor variables and their interactions considered *a priori* as biologically meaningful (see details of candidate models in Tables 3 and 5). Also, null models (those that include no predictor variables) were fitted to assess if any of the input variables has an effect on the parasite occurrence. The presence-absence of the parasite species was the response variable and all models in each set had a binomial error distribution and a logit link function. The fit of each subglobal model, defined as the most complex model in each set, was assessed visually by building logistic regression quantile-quantile plots and partial residual plots for continuous covariate (length) (Landwehr *et al.* 1984; Zuur *et al.* 2009).

The best model of each set was selected using the Information Theoretic (IT) model comparison method (Anderson *et al.* 2000; Burnham and Anderson, 2002), which provides a framework that allows a simultaneous assessment of several, *a priori* competing biological hypotheses (Burnham and Anderson, 2002; Johnson and Omland, 2004). The process of identifying the best model involves trading off model bias against model precision and IT achieves this by using appropriately constructed criteria to compare models (i.e. Akaike Information Criterion) (Burnham and Anderson, 2002). The relative fit of each candidate hypothesis was quantified by mean of second-order Akaike Information Criterion (AIC<sub>c</sub>) which is adjusted for small samples. The AIC<sub>c</sub> was used because it approximates AIC at large sample sizes (Symonds and Moussalli, 2011). The models were then ranked by AIC<sub>c</sub> and the one with the lowest AIC<sub>c</sub> was selected as the best among all specified for each set. Then differences ( $\Delta_i$ ) between the AIC<sub>c</sub> of the candidate models [AIC<sub>c(min)</sub>] and the AIC<sub>c</sub> of the best

Table 2. Prevalence % (P) of myxozoan species found in *Merluccius hubbsi* Marini 1933

Myxozoan species	Microhabitat	Bonaerense 1 P (CI)	Bonaerense 2 P (CI)	Patagonian 1 P (CI)	Patagonian 2 P (CI)	Patagonian 3 P (CI)	Patagonian 4 P (CI)
<i>Kudoa rosenbuschi</i> (Gelormini, 1943)	Skeletal musculature	2.4 (0.1–12.7)	15.0 (4.2–37.2)	24.0 (13.7–37.9)	32.3 (17.2–50.0)	25.0 (12.2–42.3)	28.9 (16.7–45.3)
<i>Myxoproteus meridionalis</i> Evdokimova, 1977	Kidney (plasmodia) and urinary bladder (spores)	64.3 (48.8–77.6)	65.0 (42.4–83.3)	40.0 (26.8–54.0)	41.9 (25.5–59.8)	50.0 (32.6–67.4)	65.8 (48.7–79.2)
<i>Fabespora</i> sp.	Gall bladder	0.0 (–)	0.0 (–)	8.0 (2.8–18.8)	41.9 (25.5–59.8)	31.3 (16.6–50.0)	15.8 (7.0–31.3)

CI, Confidence interval.

models [ $AIC_{c(\text{best})}$ ] were computed, and the Akaike's weight ( $w_i$ ) was calculated to obtain the relative likelihood of each model fitted of being the best among the whole set of candidates (Johnson and Omland, 2004).

In case of observing a high degree of uncertainty regarding the best model (when it is not strongly weighted;  $w_i > 0.9$ ), model averaging was computed on the subset of models whose cumulated weights ( $\text{acc } w_i$ ) were lower than 0.9, to estimate the magnitude of the effect of each predictor explaining most of the variation of the response variable. Moreover, to avoid redundancy, nested models of any model with a lower  $AIC_c$  value were excluded from the averaged subset (Grueber *et al.* 2011). To assess the magnitude of the effect of each parameter in this subset, new Akaike weights ( $w'_i$ ) were recomputed and parameter estimates, unconditional s.e. and unconditional confidence intervals (CIs) were calculated by averaging all models in the subset using full-model averaging method, weighted by the evidence of the respective models (models  $w'_i$ ) (Burnham and Anderson, 2002; Symonds and Moussalli, 2011). In essence, this approach generates weighted average predictions, where the weights are the relative model probabilities. The continuous input variable (length) was standardized by subtracting its mean and divided by two times its standard deviation. Binary input variables (year, region of origin and sex) were coded using binary dummy variables and then were centred prior to multimodel inference analyses; this is strongly recommended when interactions are present because facilitates the interpretation of the relative strength of parameter estimates (Schielzeth, 2010; Grueber *et al.* 2011).

All statistical analyses were conducted with the statistical software R version 3.0.1 (R Development Core Team; [www.r-project.org/](http://www.r-project.org/)). The packages MuMIn (Bartón, 2009), arm (Gelman *et al.* 2015), AICmodav (Mazerolle, 2015) and the base package implemented in R were used.

## RESULTS

Three species of myxozoans were recorded in *M. hubbsi*: *K. rosenbuschi*, *M. meridionalis* and a previously undescribed species of *Fabespora* (Table 2). Infections with *A. merluccii* or by *M. baueri* were not detected.

### Geographical patterns of infection

*Kudoa rosenbuschi* was found in all samples, with higher values of prevalence in Patagonian samples; *M. meridionalis* was almost evenly distributed among all hake samples; while *Fabespora* sp. was only observed in Patagonian samples (Table 2).

Model validation plots (logistic regression quantile–quantile plots and partial residual plots) for every subglobal model in each set (see Tables 3 and 5) showed no major departures from model assumptions (graphs not shown).

All *a priori* selected biologically competing hypotheses (those considered as plausible explanations of parasite occurrences across samples in both regions), are identified and shown in Table 3 (13 for *K. rosenbuschi* and *M. meridionalis*, and five for *Fabespora* sp.). Within the set of models fitted for both *K. rosenbuschi* (K1–K13) and *M. meridionalis* (M1–M13), multiple models were well supported (within  $\text{acc } w_i < 0.9$ , Table 3). Consequently, a model-averaging approach was used to incorporate information from these well-supported models in order to estimate parameter coefficients and standard errors (Table 4). In the case of *Fabespora* sp., for which region of origin was excluded from the analyses, the null model  $F_2$  lay within the subset of models whose  $\text{acc } w_i < 0.9$ ; therefore, the variables length and year, as well as their interaction, had no effect on the variability of occurrence of *Fabespora* sp. across hosts.

Particularly for *K. rosenbuschi*, six (K1–K6) out of the 13 models fitted were well supported. Among them, only those providing non redundant

Table 3. Alternative candidate GLMs ranked according to  $AIC_c$  to evaluate the effects of predictor variables (L total length, Y year and R region of origin) on the occurrence of three myxozoan parasites (Kr – *Kudoa rosenbuschi*, Mm – *Myxoproteus meridionalis*, Fsp – *Fabespora* sp.) of *Merluccius hubbsi* in two regions of the South West Atlantic

	ID	Candidate models	$K$	$AIC_c$	$\Delta_i$	$w_i$	$accw_i$	LL	$w'_i$
Kr	K1	<b>R</b>	2	210.32	0.00	0.26	0.26	-103.13	0.47
	K2	<b>Y*R</b>	4	211.07	0.75	0.18	0.44	-101.44	0.32
	K3	Y + R	3	211.51	1.19	0.14	0.58	-102.70	–
	K4	<b>L*R + Y*R<sup>a</sup></b>	6	211.97	1.65	0.11	0.69	-99.78	0.21
	K5	L + R	3	212.12	1.80	0.11	0.80	-103.00	–
	K6	L + Y*R	5	212.66	2.34	0.08	0.88	-101.19	–
	K7	L + Y + R	4	213.52	3.20	0.05	0.93	-102.66	–
	K8	L*R	4	213.94	3.62	0.04	0.97	-102.87	–
	K9	Y + L*R	5	215.04	4.73	0.02	0.99	-101.38	–
	K10	L	2	219.95	9.62	0.01	0.99	-107.95	–
	K11	1 <sup>b</sup>	1	221.68	11.36	0.00	0.99	-109.83	–
	K12	L + Y	3	221.79	11.47	0.00	0.99	-107.84	–
	K13	T	2	223.29	12.97	0.00	1.00	-109.62	–
Mm	M1	<b>Y + R</b>	3	291.88	0.00	0.24	0.24	-142.88	0.38
	M2	<b>Y*R</b>	4	292.31	0.44	0.20	0.44	-142.06	0.30
	M3	Y	2	293.60	1.72	0.10	0.54	-144.77	–
	M4	<b>L + Y + R</b>	4	293.95	2.07	0.09	0.63	-142.88	0.13
	M5	R	2	293.98	2.10	0.09	0.72	-144.96	–
	M6	<b>L + Y*R</b>	5	294.25	2.37	0.08	0.79	-141.98	0.12
	M7	L + Y	3	295.05	3.18	0.05	0.84	-144.47	–
	M8	<b>Y + L*R</b>	5	295.20	3.32	0.05	0.89	-142.46	0.07
	M9	L + R	3	295.93	4.05	0.03	0.92	-144.91	–
	M10	<b>L*R + Y*R<sup>a</sup></b>	6	295.05	4.07	0.03	0.95	-141.77	–
	M11	1 <sup>b</sup>	1	295.24	4.37	0.03	0.98	-147.11	–
	M12	L	2	297.92	6.05	0.01	0.99	-146.93	–
	M13	L*R	4	297.98	6.10	0.01	1.00	-144.89	–
Fsp	F1	<b>L*Y<sup>a</sup></b>	4	184.34	0.00	0.35	0.35	-88.07	–
	F2	1 <sup>b</sup>	1	185.70	1.36	0.18	0.53	-91.84	–
	F3	L + Y	3	185.93	1.59	0.16	0.69	-89.91	–
	F4	L	2	196.00	1.66	0.15	0.84	-90.97	–
	F5	Y	2	186.01	1.67	0.15	1.00	-90.97	–

ID, model identification;  $K$ , number of explanatory parameters in the model;  $AIC_c$ , second-order Akaike Information Criterion;  $\Delta_i$ ,  $AIC_{c(\text{best})} - AIC_{c(\text{min})}$ ;  $w_i$ , Akaike weight;  $accw_i$ , cumulative Akaike weight; LL log-likelihood;  $w'_i$  recomputed  $w_i$  for model averaging; over the dotted line models with  $accw_i < 0.9$ ; in bold non nested models used for multimodel inference.

<sup>a</sup> Subglobal model of each set.

<sup>b</sup> Null model of each set.

information (K1, K2 and K4) were selected for multimodel inference based on its respective model  $w'_i$ . These models consistently included region of origin as a predictor variable for explaining parasite occurrence and the inclusion of the other parameters explained no additional variation; in fact, they introduced additional uncertainty. Furthermore, model averaging showed that only region of origin had a CI 95% not including zero, indicating that this variable had a significant effect on the patterns of occurrence of *K. rosenbuschi*. On the other hand, for *M. meridionalis*, 8 (M1–M8) out of the 13 models were well supported, among which M1, M2, M4, M6 and M8 were not nested with models with lower  $AIC_c$  and, therefore, were used for multimodel inference based on its respective  $w'_i$  (Table 3). After model averaging, year was the only predictor that explained

a significant amount of variation of the occurrence of this parasite species (Table 4).

At a smaller spatial scale (within Patagonian samples caught in 2009), five biologically competing hypotheses were selected *a priori* as plausible explanations of parasite occurrences across samples (Table 5). Neither for *K. rosenbuschi* nor for *M. meridionalis* any predictor variable (year and length) explained the occurrence of these species as the null model (K'1 and M'1) of each respective set (K1–K5 for *K. rosenbuschi* and M1–M5 for *M. meridionalis*, see Table 5) lay within the subset of models with  $accw_i < 0.9$ . Notwithstanding, the results in Table 6 indicate that model F'1 with an Akaike weight of 0.71 is the best of five candidate models, indicating that fishing haul is the only predictor variable that explained the patterns of occurrence of *Fabespora* sp. within Patagonian samples caught in 2009.



Table 4. Parameter estimates ( $\hat{\beta}$ ), unconditional standard errors [ $\hat{se}(\hat{\beta})$ ], 95% confidence intervals (CI) and relative importance (RI) of each predictor calculated under full model averaging methods of the occurrence of two myxozoan parasites (*Kr* – *Kudoa rosenbuschi*, *Mm* – *Myxoproteus meridionalis*) of *Merluccius hubbsi* in two regions of the South West Atlantic

	Parameter	$\hat{\beta}$	$\hat{se}(\hat{\beta})$ (adjusted)	CI	RI
<i>Kr</i>	Intercept	-1.53	0.24	(-2.00, -1.05)	–
	L	-0.21	0.53	(-1.26, 0.83)	0.21
	Y(2014)	0.42	0.55	(-1.51, 0.66)	0.53
	R(PAT)	1.84	0.73	(0.41, 3.27)	1
	L*R	0.66	1.66	(-2.59, 3.92)	0.21
	Y*R	-1.27	1.64	(-4.48, 1.95)	0.53
<i>Mm</i>	Intercept	0.16	0.14	(-0.12, 0.44)	–
	L	0.01	0.19	(-0.36, 0.38)	0.32
	Y(2014)	0.66	0.32	(0.02, 1.31)	1
	R(PAT)	-0.61	0.33	(-1.27, 0.04)	1
	L*R	0.05	0.27	(-0.49, 0.59)	0.07
	Y*R	0.38	0.64	(-0.88, 1.65)	0.42

L, total length; Y, year; R, region of origin; in bold predictor variable with significant effect on the occurrence of each myxozoan species.

Host sex had no effect on the occurrence of any myxozoan species within sample Patagonian 4 as the null model lay within the set of models whose  $acc w_i < 0.9$ .

#### DISCUSSION

The available knowledge on the existence of recognizable stocks of *M. hubbsi* in Argentine waters, as well as on the occurrence of seasonal migrations between areas with different oceanographic characteristics provided the theoretical framework to test the suitability of myxozoans as biological markers of hake distribution. Their usefulness was corroborated, identifying them also as good metrics to detect geographical patterns at smaller geographic scales. Furthermore, with the finding of *Fabespora* sp., the list of myxozoans parasitizing *M. hubbsi* is extended to five species, increasing the potential of this group of parasites as BTs for hake stock discrimination.

*Alatospora merluccii* and *M. baueri*, previously recorded for *M. hubbsi* (MacKenzie and Longshaw, 1995; Kalavati *et al.* 1996), were not found in the present study. Indeed, both appear to have a southern distribution in the Atlantic Ocean, where they have been registered only around the Malvinas (Falkland) Islands (MacKenzie and Longshaw, 1995; Kalavati *et al.* 1996), suggesting that both myxozoans are coldwater species. These myxozoans constitute promising BTs for further studies on hake populations and migrations in that region.

*Myxoproteus meridionalis* was described by Evdokimova (1977) from spores collected in the urinary system of *M. hubbsi* in the southwest Atlantic. Since then, it was recorded in this region in its type host (MacKenzie and Longshaw, 1995; Sardella and Timi, 1996, 2004) and in *M. australis*

(MacKenzie and Longshaw, 1995). In the present study, this species showed no marked variations in prevalence among samples. MacKenzie and Longshaw (1995) also found this species in hakes caught in the region of the Malvinas (Falkland) Islands, although at lower prevalence. Studies comprising more fish are necessary to evaluate whether this species could be considered a suitable BT to assess host population structure at a broader spatial scale, including hakes from the southern boundaries of its distribution. Meanwhile, *M. meridionalis* could not be considered a useful BT for discriminating stocks of *M. hubbsi*.

*Kudoa rosenbuschi* has been frequently reported from *M. hubbsi* (Sardella and Roldán, 1989; MacKenzie and Longshaw, 1995; Sardella and Timi, 1996, 2004; Abollo *et al.* 2005; Whipps and Diggles, 2006), showing an apparent geographical pattern. Indeed, MacKenzie and Longshaw (1995) suggested a northern distribution of this species in samples from central to southern Patagonian waters, whereas Sardella and Timi (1996) observed higher parasite burdens in southern areas when comparing hakes from Bonaerense and central Patagonian waters. Consequently, the central Patagonian waters seem to be the area where *K. rosenbuschi* is more prevalent. In agreement with previous findings, in the present study, *K. rosenbuschi* was found in all hake samples, but parasitizing a significantly larger proportion of fish from central Patagonian waters. San Matías gulf, the region where the higher prevalence has been recorded (Sardella and Timi, 1996), was not included in the present work. As proposed in earlier studies, *K. rosenbuschi* can be considered as a suitable BT for *M. hubbsi*. This parasite possesses the additional advantage that, due to its histozoic condition, with melanine pigmented pseudocysts easily detectable in skeletal muscle, its abundance

Table 5. Alternative candidate GLMs ranked according to  $AIC_c$  to evaluate the effects of predictor variables (L total length and PAT research fishing haul position) on the occurrence of three myxozoan parasites (*Kr* – *Kudoa rosenbuschi*, *Mm* – *Myxoproteus meridionalis*, *Fsp.* – *Fabespora* sp.) of *Merluccius hubbsi* in three sampling stations in Patagonian waters

	ID	Candidate models	$K$	$AIC_c$	$\Delta_i$	$w_i$	$accw_i$	LL
<i>Kr</i>	K'1	1 <sup>a</sup>	1	132.82	0.00	0.58	0.58	-65.40
	K'2	L	2	134.89	2.07	0.21	0.79	-65.39
	K'3	P2 + P3	3	136.30	3.48	0.10	0.89	-65.04
	K'4	L + L*P2 + L*P3 <sup>b</sup>	6	136.93	4.09	0.07	0.96	-62.06
	K'5	L + P2 + P3	4	138.41	5.59	0.04	1.00	-65.02
<i>Mm</i>	M'1	1 <sup>a</sup>	1	156.69	0.00	0.61	0.61	-77.33
	M'2	L	2	158.76	2.07	0.21	0.84	-77.33
	M'3	P2 + P3	3	160.05	3.35	0.11	0.96	-76.91
	M'4	L + L*P2 + L*P3 <sup>b</sup>	6	162.16	5.46	0.04	0.99	-76.90
	M'5	L + P2 + P3	4	166.39	9.70	0.01	1.00	-76.80
<i>Fsp</i>	F'1	P2 + P3	3	116.01	0.00	0.71	0.71	-54.90
	F'2	L + P2 + P3	4	118.10	2.09	0.25	0.95	-54.97
	F'3	L + L*P2 + L*P3 <sup>b</sup>	6	121.68	5.67	0.04	0.99	-54.45
	F'4	1 <sup>a</sup>	1	126.30	10.29	0.01	0.99	-62.13
	F'5	L	2	128.16	12.14	0.01	1.00	-62.02

ID, model identification;  $K$ , number of explanatory parameters in the model;  $AIC_c$ , second-order Akaike Information Criterion;  $\Delta_i$ ,  $AIC_{c(\text{best})} - AIC_{c(\text{min})}$ ;  $w_i$  Akaike weight;  $accw_i$ , cumulative Akaike weight; LL, log-likelihood; over the dotted line models with  $accw_i < 0.9$ ; in bold non nested models used for multimodel inference.

<sup>a</sup> Null model of each set.

<sup>b</sup> Subglobal model of each set.

can be estimated and used in quantitative comparisons. Nevertheless, it has been observed that *K. rosenbuschi* is more prevalent and abundant in juvenile hakes than in adults (Sardella and Roldán, 1989). As an explanation of these patterns, Sardella and Roldán (1989) suggested that *K. rosenbuschi* could be an important cause of host mortality. However, as their sample was composed by hakes scattered in a broad region and caught during 3 years, this hypothesis remains unconfirmed. A decrease of prevalence with host age could be also explained by changes in the infection rate along host ontogeny, probably related to the development of immune responses, as has been suggested for *K. allaria* in *Micromesistius australis* (Agnew *et al.* 2003). The existence of ontogenetic variations in parasite burdens could impose limitations to the use of *K. rosenbuschi* as BT, since only hakes of similar size should be compared reliably. Nevertheless, in the present work, only hakes of similar size were included and, therefore, no effect of this variable was observed.

*Fabespora* sp. was only found in Patagonian samples; therefore it constitutes a promising BT for identifying Patagonian stock. Moreover, as samples were taken in the same year during two respective reproductive seasons of each hake stock, the differential presence of *Fabespora* sp. between regions (Bonaerense and Patagonian) suggests that these populations do not mix, at least not to any great extent.

Apart from large-scale differences, variations were also observed at a lower geographical scale. Indeed,

among Patagonian samples (2009) *Fabespora* sp. infects a significantly higher proportion of fish in both southern samples, which in turn were mainly composed by female hakes (Patagonian 2 and 3) (Table 1). Skewed sex ratios are commonly observed for *M. hubbsi* during the reproductive season (summer) in the Patagonian area, a pattern attributable to local segregation produced by some reproductive behaviour that involves male-competition during fertilization, and to sequential movements of post-spawning females to deeper waters (Pájaro *et al.* 2005; Macchi *et al.* 2007). Moreover, this pattern of sex segregation continues during feeding season (winter) forming 'gender concentration nuclei' along the Patagonian Shelf where *M. hubbsi* is also unevenly distributed by ages (Renzi, 1993). Consequently, it is difficult to discern whether the observed differences are attributable to host sex or to geographical origin.

Sex differences in parasite burdens may be caused by physiological or ecological processes, or by a combination of them (Reimchen and Nosil, 2001). On the other hand, the observed heterogeneity in parasite prevalence between hakes of Patagonian samples and, indirectly between host sexes may be the result of sex segregation during both trophic and reproductive seasons, with differential access to the endemic area of this myxozoan. However, Sardella and Timi (1996) found no significant differences of the parasite burdens between sexes of hakes for none of the parasite species recorded. Similarly, in the present study, no differences between hake sexes were observed for any myxozoan species

Table 6. Parameter estimates ( $\tilde{\beta}$ ), unconditional standard errors [ $\hat{se}(\tilde{\beta})$ ], 95% confidence intervals (CI) and relative importance (RI) of each predictor calculated under full model averaging methods on the occurrence of *Fabespora* sp. on *Merluccius hubbsi* in three sampling station in Patagonian waters

Parameter	$\tilde{\beta}$	$\hat{se}(\tilde{\beta})$ (adjusted)	CI	RI
Intercept	-1.31	0.27	(-1.99, -0.90)	-
PAT2	2.12	0.64	(0.94, 3.49)	1
PAT3	1.65	0.65	(0.44, 3.03)	1

within sample Patagonian 4. As a consequence, the locality effect arises as the main explanatory variable of the observed differences between samples. Therefore, *Fabespora* sp. is proposed as a potential BT that would allow studying hake ecological features at various geographical scales (stock identity and intrastock temporal and spatial movements) in this region.

It is noteworthy that, at least for the promising parasite tags for hake population studies, namely *K. rosenbuschi* and *Fabespora* sp., no temporal patterns were observed for parasite occurrence. Indeed the year of capture, even after a period of 5 years, was not identified as a predictor of parasite burdens, indicating stability of parasite infections over long-term temporal scales. This temporal stability is a desirable feature for a BT and ensures repeatability.

Although the potential value of *A. merluccii* and *M. baueri* deserves further study, the present work confirms that the diversity of myxozoans harboured by hakes offers a set of suitable markers, which can provide evidence on stock discreteness at different spatial scales in the region. These species can be selected as BTs, either individually or in any combination, depending on the geographical extent of the study, constituting tools adaptable to the objectives of further research on hake population structure.

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