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Assessing the role of host traits as drivers of the abundance of long-lived parasites in fish-stock assessment studies

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In order to identify the best tools for stock assessment studies using fish parasites as biological indicators, different host traits (size, mass and age and their interaction with sex) were evaluated as descriptors of cumulative patterns of both parasite abundance and infracommunity species richness. The effect of such variables was analysed for a sample of 265 specimens of *Percophis brasiliensis* caught in the Argentine Sea. The abundances and species richness were modelled using generalized linear mixed models (GLMMs) with negative binomial and Poisson distribution respectively. Due to collinearity, separate models were fitted for each of the three main explanatory variables (length, mass and age) to identify the optimal set of factors determining the parasite burdens. Optimal GLMMs were selected on the basis of the lowest Akaike information criteria, residual information and simulation studies based on 10 000 iterations. Results indicated that the covariates length and sex consistently appeared in the most parsimonious models suggesting that fish length seems to be a slightly better predictor than age or mass. The biological causes of these patterns are discussed. It is recommended to use fish length as a measure of growth and to restrict comparisons with fish of similar length or to incorporate length as covariate when comparing parasite burdens. Host sex should be also taken into account for those species sexually dimorphic in terms of morphology, behaviour or growth rates.

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Key words: age; Argentine Sea; collinearity; length; parasite tags; *Percophis brasiliensis*.

INTRODUCTION

The use of parasites as biological tags for stock discrimination is a well-established and widely used method for management purposes of fishery resources (Timi & MacKenzie, 2015); their application being increasingly recognized as a valuable tool in multidisciplinary studies on stock assessment (Cadrin *et al.*, 2005; Cantatore & Timi, 2015). As a consequence of the increasing use of parasite tags for different organisms and environments and the resulting generation of new knowledge, the guidelines originally proposed for this methodology (Kabata, 1963; Sindermann, 1982; MacKenzie, 1987; Williams *et al.*, 1992) have changed with its history. At present, there is a general

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consensus that the most important criterion for an effective parasite marker for stock discrimination is its long residence time in the fish (Lester, 1990; Lester & MacKenzie, 2009; Cantatore & Timi, 2015). Indeed, it is recommended that parasite tags should have a lifespan or remain in an identifiable form in the host long enough to cover the timescale of the investigation (Mosquera *et al.*, 2003; Lester & MacKenzie, 2009). Therefore, parasites with a lifespan shorter than a year are likely to be of little value for stock discrimination because they are acquired and lost as fishes move geographically (MacKenzie & Abaunza, 1998). On the other hand, long-lived parasites, generally represented by larval stages in fishes, confer temporal stability (*i.e.* seasonal, interannual) to communities when hosts of similar size or age are compared (Timi, 2007). This predictability in parasite burdens is necessary to make reliable inferences on stock structure.

Larval stages of several groups of parasitic helminths use fishes as intermediate or paratenic hosts, constituting major components of parasite assemblages in many fish-parasite systems (Poulin & Valtonen, 2001; Luque & Poulin, 2004; Cantatore & Timi, 2015). These helminths are trophically transmitted (Poulin & Valtonen, 2001) and therefore their loads often increase with the age or size of fishes (Dogiel *et al.*, 1958; Poulin, 2000), resulting in cumulative patterns of parasite abundance as fishes grow. These patterns are reflected in ontogenetic changes in the structure of parasite communities in terms of abundance and species richness, patterns frequently reported in the literature (Poulin, 2000, 2004; Poulin & Valtonen, 2001; Timi *et al.*, 2010; Valtonen *et al.*, 2010; Timi & Lanfranchi, 2013).

Several studies have proposed that larger hosts are able to accommodate more parasite species and sustain a greater absolute number of parasites than small ones. They also display larger surface areas for parasite attachment and can ingest larger quantities of food, resulting in a higher exposure to infective stages (Guégan *et al.*, 1992; Poulin, 2000; Valtonen *et al.*, 2010). Larger fishes can also feed on larger prey items, increasing the number and broadening the set of potential parasites (Timi *et al.*, 2010, 2011). Therefore, fish length must be considered in comparisons among samples from different localities to avoid attributing ontogenetic variability in parasite loads to a locality effect, leading to misinterpretation of spatial patterns and stock structure (Cantatore & Timi, 2015). This is especially relevant when fishes of different length or age are included in the comparisons. Cumulative processes are common to all types of parasites, but are expected to be more pronounced for long-lived ones, because they remain for long periods or for the entire lifespan of their hosts, depending therefore, not only on fish size, but also on age and longevity (Cantatore & Timi, 2015). In most of the published studies on parasite tags that control for fish growth in comparisons, however, host age is not contemplated or is used as a synonym of size.

Probably due to the difficulty of age determination for many fish species, which can be costly, time consuming and requires a certain degree of expertise, fish size is often used as a surrogate of age in parasite tags studies, implying that both variables are assumed to act synergistically on parasite burdens. Their functional relationship, however, is not linear, as exemplified by the widely used von Bertalanffy growth model, as well as by other alternative models (Katsanevakis, 2006). Indeed large changes in age do not represent proportional changes in size, especially in older and larger fishes. This means that fishes assigned to a given size class can belong to different cohorts (Fig. 1). Therefore, due to the cumulative effect of long-lived parasites, a sample can be heterogeneous in terms of parasite abundance, depending on the proportion of fishes

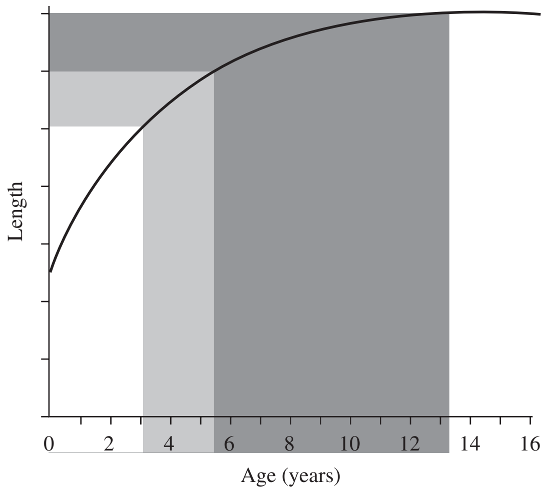

Fig. 1. Hypothetical fish growth curve showing the increasing variability in fish age with length for two consecutive size classes. Despite having similar length ranges, the smaller size class (░) is considerably more homogeneous in age than the larger size class (▓). Consequently there is an increasing probability of assigning fish of different cohorts to the same size class as their length increases.

of different ages. Consequently, assessing the value of different growth descriptors as determinants of parasite abundance is relevant for interpreting differences between samples when they are homogeneous in size, but heterogeneous in age. This assessment could be particularly important for studies on stock discrimination, because different host populations can display different age and size relationships that could be reflected in their parasite burdens (Cantatore & Timi, 2015).

Consequently, the aim of this paper is to evaluate the value of different fish traits (length, mass and age and their interaction with sex) as descriptors of cumulative patterns of parasite abundance and infracommunity species richness, in order to identify the best tools for comparative studies of stock assessment. The Brazilian flathead *Percophis brasiliensis* Quoy & Gaimard 1825 and three of its more common long-lived parasites, previously considered as suitable biological tags for stock assessment in the south-western Atlantic Ocean (Braicovich & Timi, 2008, 2010), are used as a case study.

MATERIALS AND METHODS

FISH SAMPLES, AGE DETERMINATION AND PARASITE INVENTORIES

Two hundred and sixty-five specimens of *P. brasiliensis* caught by the commercial fleet operating at the port of Mar del Plata, Argentina (38° 08′ S; 57° 32′ W) from March 2005 to June 2006 were examined for parasites. Total length (L_T, to the nearest cm), mass (M, to the nearest g) and sex of each fish were recorded. Sagittal otoliths were removed, stored dry in paper envelopes for age determination following Barretto *et al.* (2011). Randomly, one sagittal otolith from the pair was embedded in opaque epoxy resin and sectioned transversely through the core with a microcutter (Maruto MC-201; Maruto Instrument Co. Ltd; http://www.maruto.com/company_en/index.html) to obtain thin transverse sections (thickness

0·5 mm). The opaque bands of these sections were counted by two independent readers under incident and transmitted light using a stereomicroscope at ×40 magnification. Age was determined to the nearest lower year without knowledge of the length and sex of the specimen.

All metazoan parasites were identified and counted. The species richness, including all parasites found in each infracommunity, was recorded. The prevalence and mean abundance (*sensu* Bush *et al.*, 1997) were estimated for larval long-lived species with 95% bootstrap C.I., following Rózsa *et al.* (2000), using Quantitative Parasitology 3.0 (Reiczigel *et al.*, 2013) and the three more prevalent and abundant species, namely *Grillotia carvajalregorum* (Cestoda: Trypanorhyncha), *Corynosoma australe* (Acanthocephala: Polymorphidae) and *Hysterothylacium deardorffoverstreetorum* (Nematoda: Anisakidae) were selected for subsequent analyses. It is noteworthy that, although all larval nematodes of the genus *Hysterothylacium* were morphologically identified as *H. deardorffoverstreetorum* as described by Knoff *et al.* (2012), recent molecular studies have found that larvae of this species are polymorphic, suggesting the possibility of the presence of two distinct species within this taxon (Borges *et al.*, 2012; Pantoja *et al.*, 2015). The selection of the three parasite species was based on previous knowledge on their distribution and value as biological tags for stock delineation in the region. Indeed, these parasites are shared by many fish species inhabiting this region and exhibiting different ecological traits (pelagic, demersal and benthic), being recognised as suitable indicators for many of them (Timi, 2007; Cantatore & Timi, 2015).

STATISTICAL ANALYSES

The number of parasites of each of the three selected species observed for each fish specimen was initially modelled using a generalized linear mixed model (GLMM) with a Poisson distribution and log link function. As all the models were heavily over-dispersed, however, a negative binomial distribution (NB) was preferred and therefore the basic model took the following equation: $\text{Parasites}_{ij} \sim \text{NB}\ (\mu_{ij},\ k)$; $E\ (\text{Parasites}_{ij}) = \mu_{ij}$; $\log\ (\mu_{ij}) = \eta_{ij} = \text{Length}_{ij} + \text{Sex}_{ij} + \text{Sampling\_day}_i$,; $\text{Sampling\_day}_i \sim N\ (0,\ \sigma^2)$, where Parasites_{ij} is the jth observation in sampling day i, and $i = 1, \ldots, 41$, and sampling_day_i is the random intercept, which is assumed to be normally distributed with mean 0 and variance σ^2. The random intercept introduces a correlation structure between all observations from the same sampling day (Zuur *et al.*, 2009, 2013). As fixed covariates, the model included the sex of the fish (categorical with two levels), and the continuous variable length, mass or age. K is the variance parameter from the NB distribution.

A similar model was also applied using species richness as a response variable. As richness values are always within a small range, however, and GLMMs models are hardly over-dispersed, a Poisson distribution with log link function was used in this case.

Initial data exploration indicated that M, L_{T} and age were highly correlated with one another. High collinearity causes large variances for the regression coefficients (Montgomery & Peck, 1992; Zuur *et al.*, 2010; Ieno & Zuur, 2015), therefore, separate models were fitted for each of the three main explanatory variables (*i.e.* M, L_{T} and age) to identify the optimal set of factors that determines the abundance of parasites in *P. brasiliensis*. All continuous variables were standardized prior to inclusion in GLMMs. That means that, for each covariate (M, L_{T} and age), the mean was subtracted from the individual values and the values were divided by its S.D.

Optimal GLMMs were selected on the basis of the lowest Akaike information criteria (AIC), followed by a detailed model validation carried out by plotting Pearson residuals *v.* fitted values and *v.* each covariate in the model (Zuur *et al.*, 2009, 2013). In addition, a simulation study was performed to look at the effect of an individual predictor (M, L_{T} and age). Thus, a large number of datasets were simulated from the model and compared them with the observed data (Gelman *et al.*, 2014). This simulation technique (cross validation) has the potential to assess how well a model is able to predict based on new information. For that purpose, a new NB GLMM model was fitted to 75% of the original data for which Y was observed. Then, using the fitted values from the model, the Y values of the omitted (25%) data were predicted. This process was repeated 10 000 times, each of which stored the Pearson's correlation values between the predicted and omitted data.

All analyses were performed with R (www.r-project.com) using the function glmmadmb from the glmmADMB package (Skaug *et al.*, 2014).

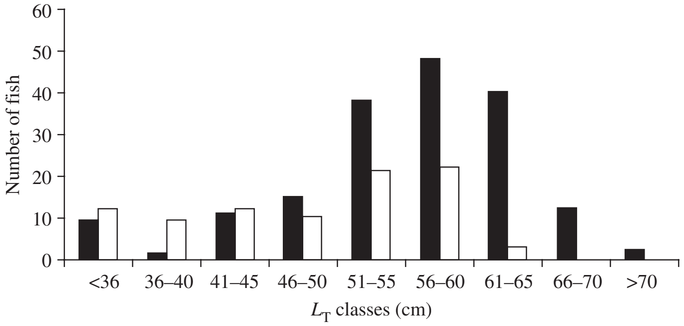

FIG. 2. Frequency of females (■) and males (□) of *Percophis brasiliensis* arbitrarily grouped in 5 cm total length (L_T) classes.

RESULTS

The sample was composed of 176 female and 89 male fish. Females (ranging from 34 to 73 cm and from 132 to 1162 g) were significantly longer and heavier than males (ranging from 28 to 62 cm and from 24 to 1026 g) ($T_{1,263} = 6·62$ and $6·16$, respectively; both $P < 0·01$) (Fig. 2). No differences in mean age were detected between females and males ($T_{1,263} = 1·73$; $P > 0·05$), which ranged from 2 to 14 and 2 to 13 years old, respectively (Fig. 3). High parasite loads were observed for all the three parasite species (Table I). A mean ± S.D. of 6·53 ± 1·69 parasite species per host was found in the entire sample, species richness being 6·83 ± 1·67 and 5·92 ± 1·58 for females and males, respectively.

ABUNDANCE AND SPECIES RICHNESS OF PARASITES AS A FUNCTION OF HOST TRAITS

The nine models that were applied to each parasite species, along with their d.f. and AIC values are given in Table II. Models can be grouped in three sets (M1–M3; M4–M6; M7–M9); within each set, there are three models using a continuous covariate (M, L_T or age) and factor sex, one in which there is an interaction between sex and the continuous covariate, one with both the continuous covariate and factor sex as main terms and one with no sex effect.

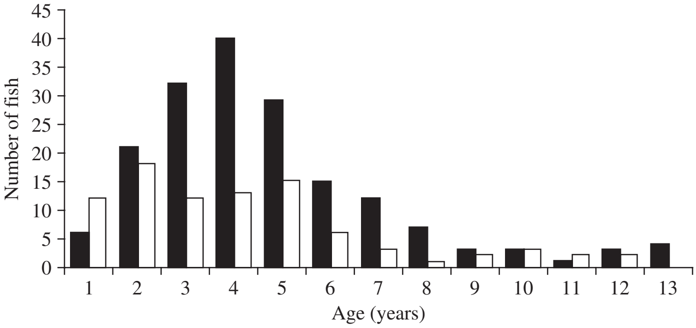

FIG. 3. Frequency of females (■) and males (□) of *Percophis brasiliensis* by age group.

TABLE I. Population descriptors of three selected long-lived parasite species in a sample of 265 specimens of *Percophis brasiliensis*. Values in parentheses are 95% bootstrap C.I. estimated by 2000 bootstrap replicates

Parasite species	Number of parasites	Prevalence (%)	Mean abundance	Range
Grillotia carvajalregorum	233 869	100 (98·6–100)	882·5 (783–1000)	7–5616
Corynosoma australe	35 476	98·1 (95·7–99·4	133·9 (117–156)	0–1186
Hysterothylacium deardorffoverstreetorum	14 451	93·2 (89·5–95·9)	54·5 (45·6–66·3)	0–531

For all the parasite species, GLMMs predicted an increase in parasite abundance with M, L_T or age as well as a significant effect of host sex (Table II and Fig. 4), although the latter varied depending on the species. All covariates used in the models seem to perform well in predicting parasite abundance, but models containing the variable body length showed a marginal preference in explaining parasite abundance as judged by the AICs. The predicted relationships between host length and mean parasite abundance is given by the equation $\mu_i = e^{ni}$, where n_i for each parasite species and host sex are presented in Fig. 4.

Optimal models for *G. carvajalregorum* were those containing L_T with and without sex (M4, M5 and M6), as can be seen by the lowest AICs values (Table II). Model M6 was selected, however, to sketch fitted values as the model containing factor sex showed no residual patterns.

In the case of *H. deardorffoverstreetorum*, AICs values showed that the model consisting of an interaction between L_T and sex (M6) was the optimal one, although model using L_T and sex as additive was also an alternative model.

A more clear preference for the use of the variable L_T over age was observed in GLMMs obtained for *C. australe*. Similarly to *H. deardorffoverstreetorum*, the model consisting of an interaction between L_T and sex (M6) was also the best one.

Results obtained for species richness are shown in Table III and Fig. 5, where the AICs of models M4, M5 and M6 were 1062·02, 1063·97 and 1065·97 respectively, indicating that model M4, consisting of only L_T, was the optimal model.

Selected models confirmed that the underlying statistical assumptions were not violated. No clear non-linear patterns were found that permit the use of, for example, generalized additive modelling. None of the final models were over-dispersed.

Finally, and as part of the model evaluation method, the simulation study carried out for all the species denoted that models containing the covariate L_T or M seem to predict data slighter better compared with those in which age was used. A summary of Pearson's correlation values using the 2·5, 50 and 95% quantiles for each set of 10 000 iterations is given in Table IV.

DISCUSSION

Due to their persistence for long periods in the hosts, as well as to the interrelationships between the considered host traits (L_T, M and age), the abundances of the three long-lived parasite species increased significantly, as expected, with host growth. The

Table II. Results from the model selection using Akaike information criterion (AIC) of generalized linear mixed models with a negative binomial distribution performed for the abundance of three long lived parasite species infecting *Percophis brasiliensis* and fitted separately for standardized mass (M^*), standardized total length ($L_{\mathrm{T}}{}^*$) and standardized age (A^*), where $\log(\mu_{ij}) = \eta_{ij}$, η_{ij} is the predictor function specifying the covariates and random effects, D is sampling day and S is sex. Best models are indicated in bold

Model	Model description	d.f.	AIC
Grillotia carvajalregorum			
M1	$\eta_{ij} = M^*{}_{ij} + D_i$	4	3910·32
M2	$\eta_{ij} = M^*{}_{ij} + S_{ij} + D_i$	5	3908·36
M3	$\eta_{ij} = M^*{}_{ij} + S_{ij} + (M^*{}_{ij}S_{ij}) + D_i$	6	3910·08
M4	$\boldsymbol{\eta_{ij} = L_{\mathrm{T}}{}^*{}_{ij} + D_i}$	**4**	**3900·56**
M5	$\boldsymbol{\eta_{ij} = L_{\mathrm{T}}{}^*{}_{ij} + S_{ij} + D_i}$	**5**	**3900·44**
M6	$\boldsymbol{\eta_{ij} = L_{\mathrm{T}}{}^*{}_{ij} + S_{ij} + (L_{\mathrm{T}}{}^*{}_{ij}S_{ij}) + D_i}$	**6**	**3901·50**
M7	$\eta_{ij} = A^*{}_{ij} + D_i$	4	3952·34
M8	$\eta_{ij} = A^*{}_{ij} + S_{ij} + D_i$	5	3924·04
M9	$\eta_{ij} = A^*{}_{ij} + S_{ij} + (A^*{}_{ij}S_{ij}) + D_i$	6	3923·74
Corynosoma australe			
M1	$\eta_{ij} = M^*{}_{ij} + D_i$	4	2748·16
M2	$\eta_{ij} = M^*{}_{ij} + S_{ij} + D_i$	5	2748·86
M3	$\eta_{ij} = M^*{}_{ij} + S_{ij} + (M^*{}_{ij}S_{ij}) + D_i$	6	2750·32
M4	$\boldsymbol{\eta_{ij} = L_{\mathrm{T}}{}^*{}_{ij} + D_i}$	**4**	**2721·86**
M5	$\boldsymbol{\eta_{ij} = L_{\mathrm{T}}{}^*{}_{ij} + S_{ij} + D_i}$	**5**	**2723·82**
M6	$\boldsymbol{\eta_{ij} = L_{\mathrm{T}}{}^*{}_{ij} + S_{ij} + (L_{\mathrm{T}}{}^*{}_{ij}S_{ij}) + D_i}$	**6**	**2713·94**
M7	$\eta_{ij} = A^*{}_{ij} + D_i$	4	2885·20
M8	$\eta_{ij} = A^*{}_{ij} + S_{ij} + D_i$	5	2846·54
M9	$\eta_{ij} = A^*{}_{ij} + S_{ij} + (A^*{}_{ij}S_{ij}) + D_i$	6	2845·30
Hysterothylacium deardorffoverstreetorum			
M1	$\eta_{ij} = M^*{}_{ij} + D_i$	4	2368·48
M2	$\eta_{ij} = M^*{}_{ij} + S_{ij} + D_i$	5	2367·76
M3	$\eta_{ij} = M^*{}_{ij} + S_{ij} + (M^*{}_{ij}S_{ij}) + D_i$	6	2361·50
M4	$\boldsymbol{\eta_{ij} = L_{\mathrm{T}}{}^*{}_{ij} + D_i}$	**4**	**2330·94**
M5	$\boldsymbol{\eta_{ij} = L_{\mathrm{T}}{}^*{}_{ij} + S_{ij} + D_i}$	**5**	**2323·92**
M6	$\boldsymbol{\eta_{ij} = L_{\mathrm{T}}{}^*{}_{ij} + S_{ij} + (L_{\mathrm{T}}{}^*{}_{ij}S_{ij}) + D_i}$	**6**	**2322·84**
M7	$\eta_{ij} = A^*{}_{ij} + D_i$	4	2388·00
M8	$\eta_{ij} = A^*{}_{ij} + S_{ij} + D_i$	5	2384·66
M9	$\eta_{ij} = A^*{}_{ij} + S_{ij} + (A^*{}_{ij}S_{ij}) + D_i$	6	2385·92

present results, however, suggest the idea that host L_{T} seems to qualify, somewhat, as a better predictor of larval accumulation patterns compared mainly with age and to a lesser extent to M. These findings are based on fit measures (AICs scores), residual analysis and more importantly, on model predictions (cross validation).

The effect of sex was included in most of the models despite the low differences encountered in AICs values when models with and without the factor sex were compared. This decision was plausible as, in most of the cases, the model validation procedures indicated that models containing the effect of sex improved the model fit, albeit the cost of introducing an extra parameter.

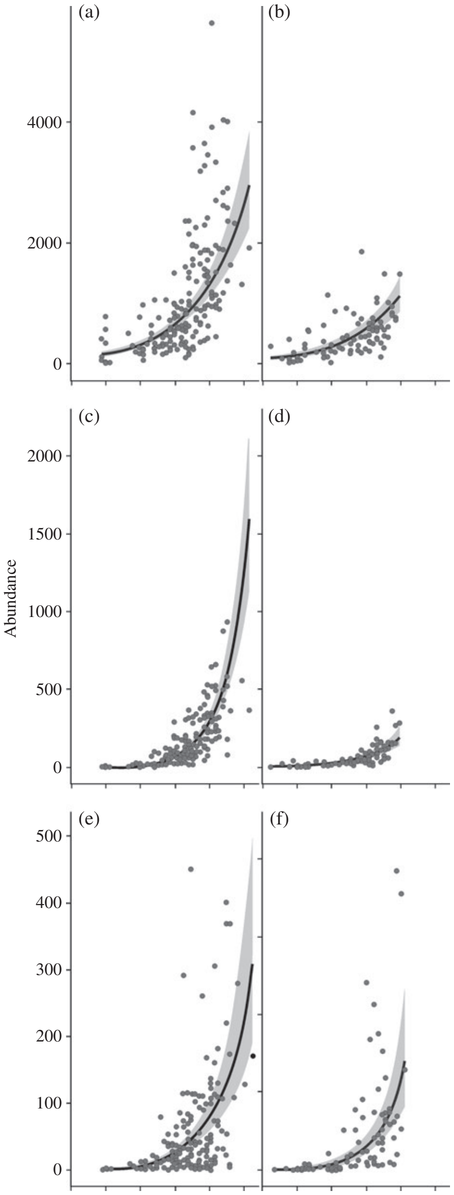

Fig. 4. Model fit of the optimal generalized linear mixed models to (a), (c), (e) female and (b), (d), (f) male *Percophis brasiliensis* showing a positive relationship between parasite abundance and standardized total length (L_{T}*) for three long-lived parasite species: (a) and (b) *Grillotia carvajalregorum* {model M5: females $y \sim \text{NB}[\text{e}^{(6{\cdot}48+0{\cdot}69)L_{\text{T}}{}^{*}j}, 2{\cdot}83]$; males $y \sim \text{NB}[\text{e}^{(6{\cdot}47+0{\cdot}69)L_{\text{T}}{}^{*}j}, 2{\cdot}83]$}; (c) and (d) *Corynosoma austral* {model M6, females: $y \sim \text{NB}[\text{e}^{(4{\cdot}24+1{\cdot}43)L_{\text{T}}{}^{*}j}, 2{\cdot}78]$; males $y \sim \text{NB}[\text{e}^{(4{\cdot}21+1{\cdot}09)L_{\text{T}}{}^{*}j}, 2{\cdot}78]$}; and (e) and (f) *Hysterothylacium deardorffovertorum* {model M6: female $y \sim \text{NB}[\text{e}^{(3{\cdot}27+1{\cdot}13)L_{\text{T}}{}^{*}j}, 1{\cdot}36]$; male $y \sim \text{NB}[\text{e}^{(3{\cdot}72+1{\cdot}14)L_{\text{T}}{}^{*}j}, 1{\cdot}36]$}. –, the fitted curves; ▨, 95% C.I. NB, the negative binomial in the generalized linear mixed model.

TABLE III. Results from the model selection using Akaike information criterion (AIC) of generalized linear mixed models with a Poisson distribution performed for species richness of parasite infracommunities of *Percophis brasiliensis* and fitted separately for each for standardized mass (M^*), standardized total length (L_T^*) and standardized age (A^*), where $\log(\mu_{ij}) = \eta_{ij}$, η_{ij} is the predictor function specifying the covariates and random effects, D is sampling day and S is sex. Best models are indicated in bold

Model	Model description	d.f.	AIC
M1	$\eta_{ij} = M^*_{ij} + D_i$	3	1068·222
M2	$\eta_{ij} = M^*_{ij} + S_{ij} + D_i$	4	1069·892
M3	$\eta_{ij} = M^*_{ij} + S_{ij} + (M^*_{ij} S_{ij}) + D_i$	5	1071·262
M4	$\eta_{ij} = L_T{}^*_{ij} + D_i$	**3**	**1062·028**
M5	$\eta_{ij} = \eta_{ij} = L_T{}^*_{ij} + S_{ij} + D_i$	**4**	**1063·974**
M6	$\boldsymbol{\eta_{ij}} = L_T{}^*_{\boldsymbol{ij}} + \boldsymbol{S_{ij}} + (L_T{}^*_{\boldsymbol{ij}} \boldsymbol{S_{ij}}) + \boldsymbol{D_i}$	**5**	**1065·974**
M7	$\eta_{ij} = A^*_{ij} + D_i$	3	1081·110
M8	$\eta_{ij} = A^*_{ij} + S_{ij} + D_i$	4	1077·986
M19	$\eta_{ij} = A^*_{ij} + S_{ij} + (A^*_{ij} S_{ij}) + D_i$	5	1079·888

Although the relationship between L_T and M for nearly all species of fish follows a potential function, M may show higher temporal variability than L_T. Indeed, M depends on food availability and growth within the weeks prior to sampling, resulting in considerable seasonal and even interannual variations (Schneider *et al.*, 2000). Furthermore, M at L_T can also vary considerably among fish depending on the degree of stomach repletion. On the other hand, a constant increase of L_T occurs, sometimes at different rates as individuals grow, making L_T a better predictor of cumulative abundance of parasites. Temporal variations in M can be also related to gonad development during the reproductive cycle (Schneider *et al.*, 2000). *Percophis brasiliensis* is a multiple spawner with indeterminate annual fecundity that typically spawns from November to April (Militelli & Macchi, 2001*a*) and their condition factor and hepato-somatic and gonado-somatic indices have been shown to vary seasonally in relation to energy allocation needs during the reproductive cycle (Rodrigues *et al.*, 2013). As samples in the present study were caught during a period of 18 months, which includes different phases of the reproductive cycle, M resulted in a less reliable explanatory variable of parasite abundance than L_T.

The discernment between the effects of host length and age is problematic because whereas higher parasite abundance is expected in longer individuals for fish of the same age, the reciprocal situation also applies; indeed, for fish of the same size, higher parasite abundance is expected in older individuals, especially for long-lived parasites. These patterns are due to a higher exposure to infective stages because of broader diets and the consumption of more and larger prey in the first case and to longer periods of exposition of older fish, which have had longer to accumulate parasites than younger hosts in the second case.

The attempt to assess the value of these variables on parasite burdens showed that age of *P. brasiliensis* was overtaken by size when both explanatory variables were modelled individually and then compared. The weaker influence of fish age relative to length on parasite abundance can be a consequence of both methodological and biological causes. In the first case, the discrete temporal scale obtained from otolith ageing

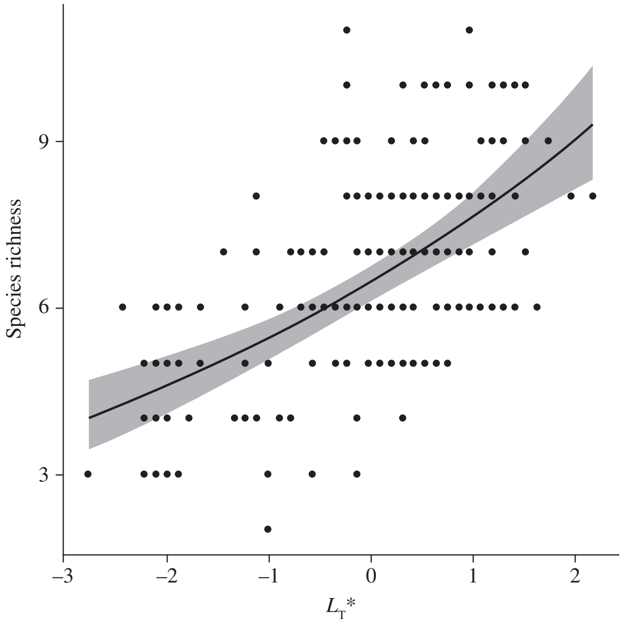

FIG. 5. Model fit of the optimal generalized linear mixed model (M4) showing a positive relationship between species richness of parasite infra-communities and standardized total length (L_T^*) of *Percophis brasiliensis*: $y \sim P(\mu_i)$, $\mu_i = e^{(1{\cdot}86 + 0{\cdot}17) L_T^*}$. –, the fitted curves; ■, 95% C.I.

and measured in years ignores the temporal variability of infection among fish within age classes because any growth beyond a whole year is not accounted for. On the other hand, fish length scaled in cm was more representative of size variability. The relatively low relevance of age regarding length can also be a consequence of the age composition of the samples. Most *P. brasiliensis* examined were adults; only one male and 10 females were smaller than the size at first maturity (28·98 and 38·34 cm, respectively, Militelli & Macchi, 2001*b*) and their size to age relationships fall into the plateau of growth curves of the species (Barretto *et al.*, 2011), where small changes in size can represent large changes in age. Possibly, a more evident effect of host age relative to size could be detected for juvenile fish, for which the size to age relationship is more linear.

Whereas the methodological causes of the observed patterns are related to the host sample (ageing scale and age composition), the biological causes rely on guild of parasites analysed. The three considered parasite species are trophically transmitted, little specific and include paratenic hosts in their life cycles, persisting in trophic webs as larvae. These species are extremely common among fish species in the region (Timi & Lanfranchi, 2009; Rossin & Timi, 2010; Timi *et al.*, 2011) displaying commonly cumulative patterns of abundance with fish size in all host species (Cantatore & Timi, 2015). These characteristics make them highly available for any potential predator, with those preying on larger fish being exposed to higher numbers of infective stages. *Percophis brasiliensis* is an ichthyophagous predator that shows a proportional increase in the prey size as it grows (Milessi & Marí, 2012), a common pattern to many fish species (Scharf *et al.*, 2000). Therefore, any size difference between fish of the same age will

Table IV. Distribution of Pearson’s correlation values using the mean, 2·5, 50 and 95% quantiles in 10 000 simulated data sets from the fitted generalized linear mixed models conducted on *Percophis brasiliensis*. Each set of simulations was carried separately for every parasite species and intracommunity species richness using one continuous explanatory variable at a time. The factor sex effect was included in all models

Dependent variable	Host trait	2·5%	50%	97·5%	Mean
Grillotia carvajalregorum	Length + sex	0·59	0·77	0·88	0·76
	Mass + sex	0·55	0·74	0·87	0·73
	Age + sex	0·47	0·70	0·86	0·69
Corynosoma australe	Length + sex	0·66	0·82	0·90	0·80
	Mass + sex	0·57	0·75	0·89	0·74
	Age + sex	0·51	0·69	0·86	0·69
Hysterothylacium deardorffoverstreetorum	Length + sex	0·41	0·60	0·79	0·59
	Mass + sex	0·31	0·57	0·75	0·56
	Age + sex	0·30	0·57	0·83	0·57
Species richness	Length + sex	0·45	0·61	0·73	0·60
	Mass + sex	0·39	0·57	0·70	0·56
	Age + sex	0·33	0·49	0·63	0·49

result in higher risks of infection for the longer individual eating larger prey. Due to the ubiquity and commonness of these three parasite species in fishes at a regional scale (Timi & Lanfranchi, 2009; Rossin & Timi, 2010; Timi *et al.*, 2011; Braicovich *et al.*, 2012; Cantatore & Timi, 2015) similar processes should be expected at different levels of the trophic web.

On the other hand, a higher contribution of host age to parasite abundance relative to size should be expected for long-lived parasites that are not trophically transmitted, whose abundance is therefore independent of the size and number of prey items in host diet. As an example, metacercariae of digenetic trematodes can persist in their hosts for very long times (Ferguson *et al.*, 2010). Metacercariae of *Prosorhynchus* sp. (Bucephalidae) have been recorded as parasites of *P. brasiliensis* (Braicovich & Timi, 2008, 2010). Cercariae of this family penetrate actively fish skin to form metacercariae which are transmitted by ingestion to other fishes (Cribb *et al.*, 2003). Unfortunately the prevalence and abundance of these metacercariae were too low in *P. brasiliensis* (Braicovich & Timi, 2008, 2010) to perform this kind of analyses.

Host sex also had a marginal effect on parasite abundance of *P. brasiliensis* when included in the optimal models. Female *P. brasiliensis* were more likely to harbour a higher number of *C. australe* than males, whereas the opposite relationship was observed for *H. deardorffoverstreetorum* when fish of similar length could be compared. For both species, the abundance-length relationship changes with sex due to the interaction effect. Finally, modelling results for *G. carvajalregorum*, confirmed an effect of sex with a marginal preference for the females for all length values, although no interaction effect was detected in this case, as can be seen by the parallel slopes drawn in Fig. 4. Physiological, morphological and behavioural gender differences between hosts have been pointed out as possible reasons for dissimilarities of parasite abundance (Poulin, 1996; Šimková *et al.*, 2005; Santoro *et al.*, 2014). Females of *P. brasiliensis* grow faster and reach greater lengths than males of the same age due, probably, to an asynchronism in the sexual maturity (Barretto *et al.*, 2011). This can result in behavioural or physiological differences between sexes. Indeed, the importance of interactions between host length and sex, especially for *C. australe* and *H. deardorffoverstreetorum* indicates that ontogenetic changes in diet composition or feeding rates on some prey can differ between sexes. On the other hand, females have higher nutritional requirements due to their greater reproductive outputs, which can lead to higher feeding rates, accumulating in general more parasites than males.

As in the case of population parameters of parasites, their community structure, measured as species richness, was better explained by host size. This is a predictable result, since an increase in the number of species in larger hosts is a common feature of fish and parasite systems (Guégan *et al.*, 1992; Poulin, 2000; Valtonen *et al.*, 2010; Timi *et al.*, 2011) and because species richness included short-lived species, whose presence depends mostly on fish size rather than age, as suggested by Alarcos & Timi (2012).

In summary, the present findings suggest that, although the three measures of fish growth describe significantly the cumulative patterns of parasites, host length seems to be a slightly better predictor compared with mass or age for both parasite abundance and species richness of long-lived parasites. The use of length has several advantages over age, especially in relation to determination methods, such as low cost, accessibility for rare species and straightforward measurement techniques, which facilitate its use as an explanatory variable in parasitological studies. Therefore, it is recommended to use fish length as a measure of growth and to restrict comparisons to fish of similar length or to incorporate length as covariate in any comparative study.

It is noteworthy that these conclusions are derived from data of trophically transmitted parasites and could not strictly apply to other long-lived parasites with direct transmission. For such guilds of parasites, host age could play a major role in driving their abundance and probably should be considered in comparative analyses. Finally, host sex, often ignored as a determinant of parasite abundance in stock discrimination studies, should be also taken into account for sexually dimorphic species, but particularly for those species with gender differences in growth rates.

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