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Impact of a mouth parasite in a marine fish differs between geographical areas

MARIA SALA-BOZANO[1], COCK VAN OOSTERHOUT[2]† and STEFANO MARIANI[1,3]*†

[1]*UCD School of Biology and Environmental Science, University College Dublin, Belfield, D4, Ireland*
[2]*School of Environmental Sciences, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, UK*
[3]*School of Environment and Life Sciences, University of Salford, Salford, M5 4WT, UK*

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Considerable variation exists in parasite virulence and host tolerance which may have a genetic and/or environmental basis. In this article, we study the effects of a striking, mouth-dwelling, blood-feeding isopod parasite (*Ceratothoa italica*) on the life history and physiological condition of two Mediterranean populations of the coastal fish, *Lithognathus mormyrus*. The growth and hepatosomatic index (HSI) of fish in a heavily human-exploited population were severely impacted by this parasite, whereas *C. italica* showed negligible virulence in fish close to a marine protected area. In particular, for HSI, the parasite load explained 34.4% of the variation in HSI in the exploited population, whereas there was no significant relationship (0.3%) between parasite load and HSI for fish in the marine protected area. Both host and parasite populations were not differentiated for neutral genetic variation and were likely to exchange migrants. We discuss the role of local genetic adaptation and phenotypic plasticity, and how deteriorated environmental conditions with significant fishing pressure can exacerbate the effects of parasitism. *Biological Journal of the Linnean Society*, 2012, ••, ••–••.

ADDITIONAL KEYWORDS: Ceratothoa – connectivity – life history – plasticity – Sparidae – virulence.

INTRODUCTION

Parasites occur in all living taxa and encompass at least one-third of all eukaryotic life on earth (De Meeus & Renaud, 2002), giving rise to an astounding range of adaptations fitting to the vast diversity of host environments (Cornell, Desdevises & Rigby, 1999). Parasites are credited with a vital role in the evolution of their hosts (Boots *et al*., 2009), as the loss in fitness caused by parasite exploitation results in counter-adaptations in the host, ultimately resulting in an 'arms race' that drives evolutionary changes in both interacting organisms (Hochberg, Michalakis & de Meeus, 1992; Møller, Martin-Vivaldi & Soler, 2004; Paterson *et al*., 2010).

Genetic and phenotypic variations in both host and parasite are responsible for the occurrence of coadaptive processes (Gandon *et al*., 1996). The exploitation of the host by a parasite can trigger immunological responses (Simkova *et al*., 2008), which, in turn, can have an impact on the overall metabolic balance and compromise the investment in life history traits, such as growth, survival and reproduction (Møller *et al*., 2001). Genetic variation and immunological responses in the host will, in turn, influence the fitness and the life history traits of the parasite, causing changes in the levels of virulence (defined as the amount of damage a parasite causes to its host). However, importantly, ecological circumstances can also govern virulence evolution (Day & Proulx, 2004), and genomic interactions between host and parasite can occur in environmental contexts that may dramatically change both spatially (Thompson & Cunningham, 2002; Gandon & Nuismer, 2009) and temporally (Brooks & Hoberg, 2007). Therefore, the analysis of the impact of different environmental scenarios on interacting species is key to our understanding of host–parasite coevolution.

*Corresponding author. E-mail: s.mariani@salford.ac.uk
†These authors contributed equally to this work.

Theoretical studies have shown that host–parasite coevolution can be highly dynamic, depending, for example, on the migration rates of both hosts and parasites (Gandon & Michalakis, 2002). Given that gene flow and selective factors can vary considerably across geographical ranges, host–parasite associations are typically heterogeneous across their spatial distribution (Thompson, 1999; Gomulkiewicz *et al.*, 2000; Gandon & Nuismer, 2009), generating a dynamic adaptive landscape that depends on the interaction of selection, gene flow and drift, which can result in a geographical mosaic of coevolutionary hotspots and coldspots (Thompson & Cunningham, 2002). The majority of these studies have focused their attention on the parasite's capability to harm its host and evolve rapidly in response to the defence mechanisms generated by the host, increasing with this the parasite's virulence and the ability of the parasite to harm its host (Nuismer, Thompson & Gomulkiewicz, 2000; Gandon & Nuismer, 2009).

Several empirical investigations have focused on the effects of parasites on the life history and fitness of hosts, especially in fish (van Oosterhout *et al.*, 2007; van Oosterhout, 2008; Fogelman, Kuris & Grutter, 2009; Blanchet, Rey & Loot, 2010), but these have seldom been contrasted with explicit environmental scenarios (but see Wolinska & King, 2009). The aim of this study was to examine the life history effects of a mouth-dwelling, blood-feeding isopod parasite, the cymothoid *Ceratothoa italica* (Schioedte & Meinert, 1883), on two Mediterranean stocks of a coastal benthopelagic teleost, the striped sea bream (*Lithognathus mormyrus* L.). The stocks show indistinguishable gene pools and parasite communities (Sala-Bozano, Ketmaier & Mariani, 2009), but are found under very different environmental pressures. We examined differences in life history descriptors (weight and length at age) and a fitness-related trait that is associated with metabolic activity (hepatosomatic index, HSI) in the host populations, in relation to the parasitic infection and other interacting factors, such as age and sex. We demonstrate temporally stable differences in parasite impact in a scenario of high gene flow, underscoring the importance of environmental factors in host–parasite coevolution.

MATERIAL AND METHODS

Study area and sampling

A total of 626 *Lithognathus mormyrus* were collected during the spawning season, between May and September, in 2006, 2007 and 2008. Sampling areas were representative of the main marine basins in which the species is found (East Atlantic Ocean, ATL; Alborán Sea, ALB; Balearic Sea, BA; Tyrrhenian Sea, TYR; Adriatic Sea, ADR; Aegean Sea, AEG). Four localities were sampled in both 2006 and 2007, a fifth site was only sampled in 2007 (the Adriatic Sea) and a sixth in 2008 (Aegean Sea) (Fig. 1). Our study focuses, in particular, on the BA and TYR fish, which inhabit areas that are climatically comparable. However, the BA fish were caught close to a marine protected area, whereas the TYR fish occur in a habitat with negligible fishery regulation and greater harvesting pressure. Adult fish (> 15 cm) were collected by fishermen using trammel and gill nets, and juveniles (< 15 cm) were collected by anglers using hook and line. Fishermen and anglers donated dead adult fish and juveniles to this study; hence, no special licences were required.

Infection and life history parameters

Fish were measured to the nearest millimetre (standard length, L_S) and weighed (wet weight, W_W) to the nearest gram. They were dissected to identify their sex, and their livers were removed and weighed to the nearest 0.01 g. To assess the physiological condition of the fish, HSI (Lloret & Planes, 2003) was calculated as $HSI = W_L/W_W$, where W_L is the wet liver weight.

Scale reading was used to age the fish (Suau, 1970; Kraljevik *et al.*, 1996). For each fish, a minimum of five scales was removed from above the lateral line and used for reading under a stereomicroscope with $\times 400$ magnification. Two independent readers examined five scales per fish, showing high repeatability in age estimates between observers (correlation coefficient $r > 0.99$, $P < 0.001$).

The buccal cavity and gill arches of each fish were examined to detect the presence of *C. italica*. No *Ceratothoa* species possess a planktonic larval stage, and hence newly released juveniles can only swim short distances, 'hopping' among individual hosts and briefly between the substrate and hosts (Horton, 2000). Juveniles then usually enter from the gill cavity and crawl to settle on the tongue of the host. The first individual to settle grows and matures into a large female, which is fertilized by one or more small males, infecting the host after the female. In the case of death of the female, the largest male undergoes a sex change to replace it (Horton & Okamura, 2003). The number of adult *C. italica* was recorded for each infected individual, and the prevalence (infected fish/total number of fish) of parasite infection was calculated for each infected stock.

Genetic differentiation of *L. mormyrus*

Fin tissue was used to isolate DNA using a modified salt/chloroform extraction protocol (Miller, Dykes & Polesky, 1988; Petit, Excoffier & Mayer, 1999). All

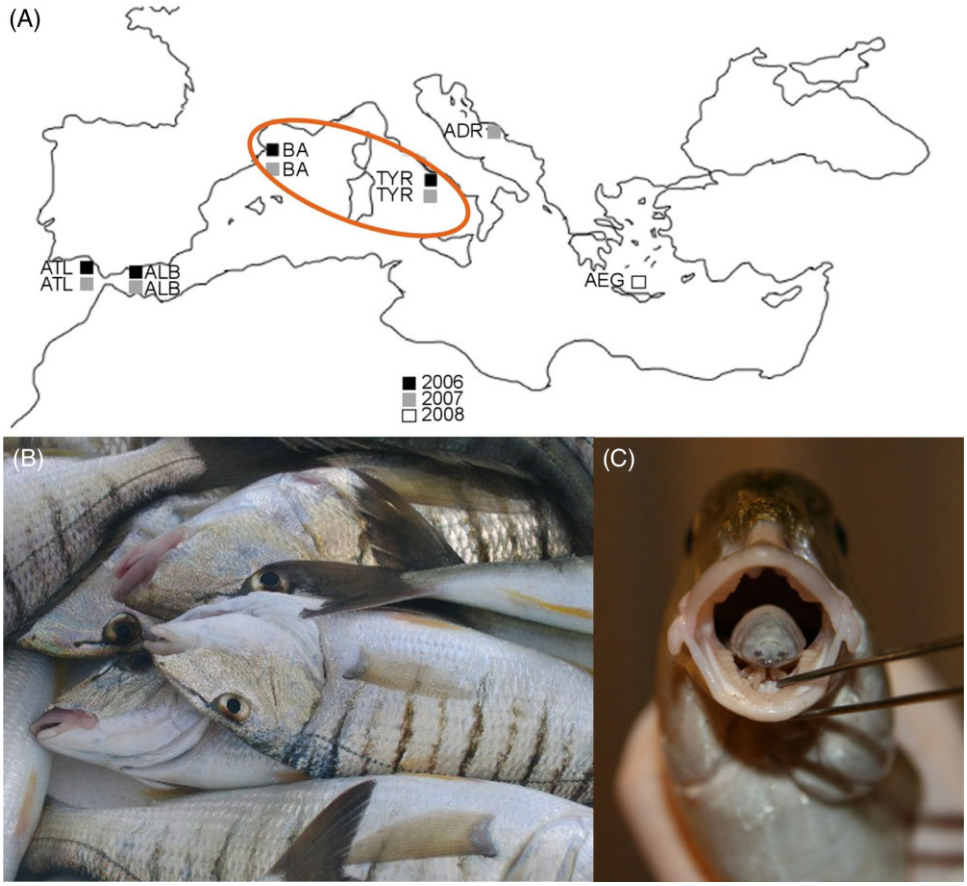

Figure 1. Sampling locations and species of interest: A, map indicating the six sampling locations; the ellipse circumscribes the populations infected by *Ceratothoa italica*; B, the striped sea bream, *Lithognathus mormyrus*; C, a live *C. italica* inside the mouth of its host. ADR, Adriatic Sea; AEG, Aegean Sea; ALB, Alborán Sea; ATL, East Atlantic Ocean; BA, Balearic Sea; TYR, Tyrrhenian Sea.

individuals were polymerase chain reaction (PCR) amplified and genotyped at nine polymorphic microsatellite loci: Lm68, Lm72, Lm19, Lm86, Lm12, Ad05, Ad66, SaL15 and Sal19 (Brown *et al*., 2005; Franch *et al*., 2006). Samples were processed in two multiplex reactions (see Sala-Bozano, Tsalavouta & Mariani, 2008 for details). Genotyping of individuals was performed by allele sizing on an ABI 3130*xl* Genetic Analyser (Applied Biosystems) using forward primers labelled with NED, PET, FAM and VIC dyes and an internal size standard labelled with LIZ 600 (Applied Biosystems). The software GENEMAPPER version4x (Applied Biosystems) was used to score alleles. The data were checked for the presence of null alleles, large allele drop out, errors caused by stutter peaks and possible scoring errors using MICROCHECKER (van Oosterhout *et al*., 2004).

Expected and observed heterozygosity (H_E and H_O), allelic richness (A_R) and linkage disequilibrium were calculated using FSTAT version 2.9.3 (Goudet, 2001). FSTAT was also used for F_{ST} analysis, and pairwise F_{ST} values were tested for their significance with 10 000 permutations. A multidimensional scaling (MDS) ordination, inferred from the pairwise F_{ST} matrix, was employed to visually represent the genetic relationships among population samples.

Historical effective population sizes (N_e) and rates of gene flow were estimated only for infected stocks using a Bayesian approach implemented in the software MIGRATE-n version 2.4 (Beerli & Felsenstein, 1999, 2001). This software estimates theta (θ), which is equal to four times the effective population size multiplied by the mutation rate μ ($\theta = 4N_e\mu$), and a migration rate parameter M, which is equal to the immigration rate m divided by the mutation rate. M quantifies the number of new alleles introduced into the population by immigration relative to mutation (Beerli & Felsenstein, 1999, 2001).

The θ values were translated into N_e estimates using a microsatellite mutation rate of 5×10^{-4}, which is the most frequently employed rate in fish (Barson, Cable & van Oosterhout, 2009). Ten short chains,

each with 500 generations and a sampling increment of 200 generations, and three long chains, each with a total of 5000 generations and a sampling increment of 20 generations, were run. The chains visited a total of 100 000 and 1 000 000 genealogies, respectively (recorded steps multiplied by the sampling increment). The first 10 000 genealogies were discarded (burn-in). MIGRATE was run three times, until N_e and N_em estimates were consistent between runs. The first run used F_{ST}-based estimates as the starting point. Subsequent runs used the results of the previous run as starting values.

Genetic variation in *C. italica*

Genetic differentiation between *C. italica* parasite populations was examined by analysing a portion of the mtDNA cytochrome oxidase subunit I (COI) gene. Sixteen individuals per population were sequenced. Genomic DNA was extracted using the same protocol as for *L. mormyrus* from single legs of ethanol-preserved specimens. PCR amplifications of a 592-bp COI fragment were carried out using universal invertebrate primers (Folmer *et al*., 1994). The PCR recipe and conditions were similar to those of Ketmaier *et al*. (2008). In our study, however, more stringent conditions were used, by increasing the annealing temperature by up to 14 °C and by using high-fidelity *taq*-polymerase (Invitrogen, Platinum).

Products were sequenced in both directions by Macrogen Inc. (Seoul, South Korea). Chromatogram contigs were edited and assembled in Sequencher 4.2 (Gene Codes Corporation, Ann Arbor, MI, USA); sequences were aligned in MEGA-4 (Tamura *et al*., 2007). Nucleotide (π) and haplotype (H) diversity were calculated using DnaSP 4.10.9 (Rozas *et al*., 2003). The software ARLEQUIN version 3.5 (Excoffier, Laval & Schneider, 2005) was employed to calculate haplotype frequencies and to estimate variance in haplotype frequencies between samples, using 10 000 permutations to test for statistical significance.

COI sequences were employed to construct a median-joining network in Network 5.5.1 (Bandelt, Forster & Röhl, 1999) using the default settings in order to illustrate graphically the relationship between the two populations.

Parasite load

We used ordinal logistic regression to examine the factors explaining the variation in parasite load between individual *L. mormyrus*. In this model, the 'number of parasites' was used as response variable, and the population of origin ('Pop'), sex and age were employed as predictor variables. The logit link function was used to calculate the mean odds ratios (the odds of the infection occurring in one group to the odds of it occurring in another group) and their 95% confidence intervals (CIs). The log-likelihood from the maximum likelihood iterations and the G statistic were used to examine whether all the slopes were significantly different from zero. The logit variable z, which expresses the total contribution of the independent variables used in the model, was also noted.

Testing parasite impact

Size (L_S) and weight (W_W) at age, and HIS, were used as estimates of the biometric and metabolic condition of the fish. The primary objective was to examine whether there was a difference between the effects of *C. italica* infection on the two host populations. Thus, we first quantified differences in age structure and sex ratios between populations, in order to take into account the variation in size and HSI explained by these factors, and to assess the effect of the parasite on the residual variance.

Differences in size (L_S) and weight (W_W) between the two infected stocks were analysed with a general linear model (GLM). In the GLM, log-transformed L_S and log-transformed W_W were used as response variables, sex, infection and 'Pop' were used as fixed crossed factors and age was employed as a covariate. We discarded data of fish older than 4 years as there were too few data points in the data from TYR. The same approach was also used to analyse whether size differences existed between groups of fish without *C. italica* infection. For this analysis, we used the standard length and weight of the uninfected samples only ($N = 138$ and $N = 36$ for BA and TYR, respectively) as response variables.

Differences in the metabolic condition of parasitized fish were tested by examining the variation in HSI as response variable, and sex, age and 'Pop' as explanatory variables. The explanatory variables were crossed in two- and three-way interactions. In this model, age was used as a covariate. In all GLMs, we used a backwards elimination of nonsignificant two-way and three-way interactions, and the minimum adequate model is presented in Table S1 (see Supporting Information). Standardized residuals were calculated and used in regression analyses to examine whether residual variation in HSI could be explained by parasite load, and to test whether the impact of parasites on HSI differed between stocks. All statistical analyses were performed using MINITAB 15 (Software Minitab Inc., State College, PA, USA).

RESULTS

General infection characteristics

Two hundred and twenty-eight adult *C. italica* were recovered from 110 fish. The only stocks infected were

Table 1. Sample information for the infected localities Balearic Sea (BA) and Tyrrhenian Sea (TYR)

Locality	Code	Basin	GPS coordinates		N	H_E	A_R
			Latitude	Longitude			
L'Estartit 06	BA06	Balearic	42°02′10.18″N	3°12′22.03″W	95	0.837	12.095
L'Estartit 07	BA07	Balearic	42°02′10.18″N	3°12′22.03″W	99	0.835	11.775
Foce Verde 06	TYR06	Tyrrhenian	41°24′0.0″N	41°24′0.0″W	40	0.829	11.269
Foce Verde 07	TYR07	Tyrrhenian	41°24′0.0″N	41°24′0.0″W	50	0.830	12.453

GPS coordinates, sample size (N) and genetic diversity indices inferred from microsatellites (H_E, expected heterozygosity; A_R, allelic richness) are provided.

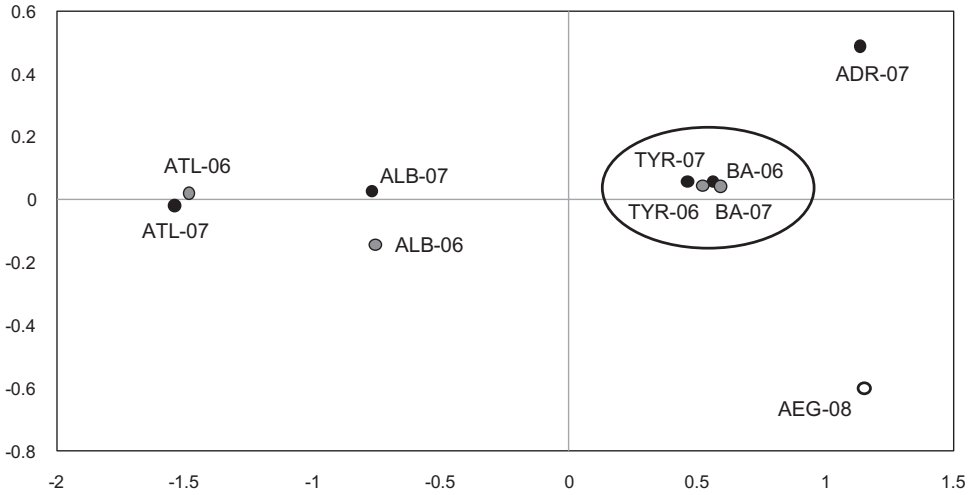

Figure 2. A multidimensional scaling (MDS) plot of F_{ST} pair-wise values among population samples screened for microsatellite variation. Location codes are as in Figure 1. The ellipse highlights the temporally stable genetic identity of TYR and BA.

within the western Mediterranean, in the BA and TYR basins (Fig. 1, Table 1). Parasite prevalence was significantly higher in the TYR (47%; 52 infected fish of a total of 90) than in the BA (30%; 58 of 194 fish) population sample ($\chi^2 = 20.139$, d.f. = 1, $P < 0.0001$).

Genetic differentiation

Global F_{ST} over all populations examined was strong and highly significant (0.036; CI, 0.009–0.045) (Fig. 2). However, the overall genetic differentiation of the two infected *L. mormyrus* stocks, TYR and BA, over 2 years was low and not significant ($F_{ST} = 0.002$; $P = 0.62$), with none of the pairwise comparisons significantly different from zero. The allelic richness (A_R) and expected heterozygosity (H_E) were also similar (Table 1), with no significant departures from Hardy–Weinberg equilibrium or deviations from linkage equilibrium. This pattern was congruent with the results from MIGRATE: the number of migrants per generation (N_em) going from the TYR population into BA was 1.2 (95% CI, 1.04–2.17), and the number of migrants going from BA into TYR was 13.1 (95% CI, 12.28–17.04). The historical effective population size (N_e) for BA was 295 (95% CI, 290–325) and for TYR was 280 (95% CI, 275–330).

Only four nucleotide sites of the 597 bp sequenced in 32 *C. italica* individuals were polymorphic, corresponding to only three unique haplotypes (Fig. S1, see Supporting Information). Despite the fact that more specific DNA isolation and more stringent PCR conditions were used, a stop codon in position 481 was invariably found in all sequences. This particular stop codon was also found, in the same position, in another species of the same genus (*Ceratothoa oestroides*) by Mladineo, Šegvić & Grubišić (2009), who also showed that this did not affect the usefulness of the marker. Overall haplotype frequency did not differ between TYR and BA ($F_{ST} = 0.024$, not significant). Haplotype frequencies for haplotypes I, II and III did not differ

Table 2. Ordinal logistic regression

Predictor	Coefficient	SD	z	P value	Odds ratio (and 95% CI)
'Pop'	–1.0914	0.3111	–3.51	< 0.001	0.34 (0.18–0.62)
Sex	0.9609	0.3268	2.94	0.003	2.61 (1.38–4.96)
Age	1.6793	0.2280	7.36	< 0.001	5.36 (3.43–8.38)

The number of parasites was used as the response variable and the population of origin ('Pop'), sex and age were used as independent factors. Significant variation in individual parasite load is explained by the independent variables in the model: log-likelihood = –197.911; test that all slopes are zero: $G = 151.97$, d.f. = 3, $P < 0.001$. The negative z value and the odds ratio below unity for 'Pop' indicate that the risk of parasite infection is higher in the Tyrrhenian (TYR) than Balearic (BA) population. Similarly, males and younger fish have a higher infection risk than females and older fish, respectively. CI, confidence interval; SD, standard deviation.

between TYR and BA ($\chi^2 = 0.051$, d.f. = 1, $P = 0.821$, $\chi^2 = 0.031$, d.f. = 1, $P = 0.858$ and $\chi^2 = 0.025$, d.f. = 1, $P = 0.873$, respectively).

Factors explaining parasite load

Ordinal logistic regression showed that significant variation in parasite load was explained by 'Pop', sex and age (Table 2). The odds ratio revealed that the parasite load was reduced significantly with age, that male fish had between two to three times higher parasite load than females, and that fish in TYR had an almost three times higher load than those in BA (Table 2).

Effects of infection on host traits

There was a significant male-biased sex ratio in both samples (BA, male : female = 106 : 81; TYR, male : female = 57 : 27). Although this skew did not differ significantly between groups ($\chi^2 = 2.570$, d.f. = 1, $P = 0.109$), the male-biased sex ratio was more prominent in TYR (binomial probability: $P = 0.0007$) than in BA (binomial probability: $P = 0.039$).

Size (GLM $F_{1,266} = 126.03$, $P < 0.001$) and weight (GLM $F_{1,265} = 117.69$, $P < 0.001$) were significantly lower in infected fish (see Fig. 3). The model showed that, although age and 'Pop' explained significant variation in fish size and weight, there was no difference in size or weight between the sexes when accounting for age differences (Table 3). Furthermore, the impact on weight caused by *C. italica* infection appeared to be more severe in TYR than in BA (Fig. 3), which explains the significant interaction term 'Pop' × infection (GLM $F_{1,265} = 5.63$, $P = 0.018$) (Table 3).

Next, we analysed and compared the size of the uninfected fish between samples. This showed that, irrespective of infection, TYR fish were significantly smaller than their BA counterparts of the same age

Table 3. General linear model (GLM) with natural log-transformed wet weight as response variable, infection, population of origin ('Pop') and sex as fixed factors, and age as covariate

Factor	d.f.	MS	F	P
Age	1	31.840	117.69	< 0.001
Sex	1	0.103	0.38	0.539
'Pop'	1	5.774	21.34	< 0.001
Infection	1	34.523	127.60	< 0.001
'Pop' × infection	1	1.522	5.63	0.018
Error	265	0.271		
Total	270			

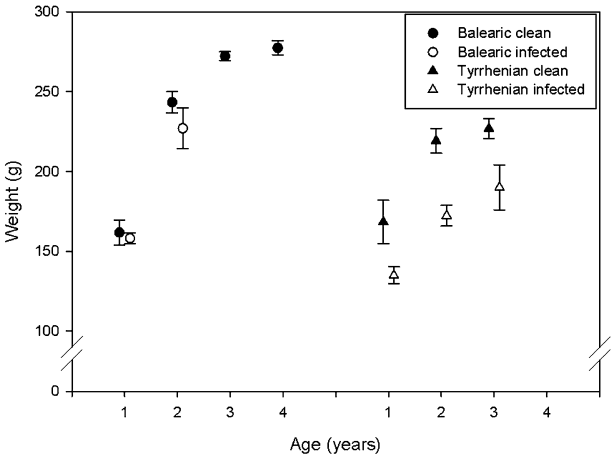

Figure 3. Mean (± SE) wet weight across year classes of clean (filled symbols) and infected (open symbols) Balearic (circles) and Tyrrhenian (triangles) fish. Fish infected by *Ceratothoa italica* parasites are significantly smaller than clean fish, and this effect is significantly more pronounced in the Tyrrhenian (see main text and Table 3 for details on statistical analysis).

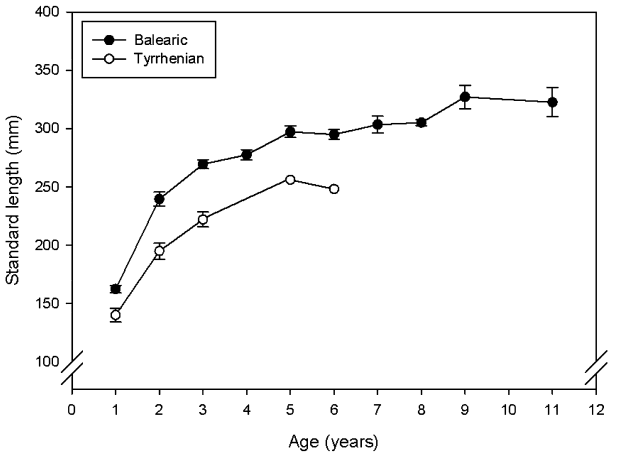

Figure 4. Mean (± SE) standard length across year classes of uninfected Tyrrhenian (open symbols) and Balearic (filled symbols) fish. The Tyrrhenian fish are significantly smaller than fish from the Balearic (see main text for details).

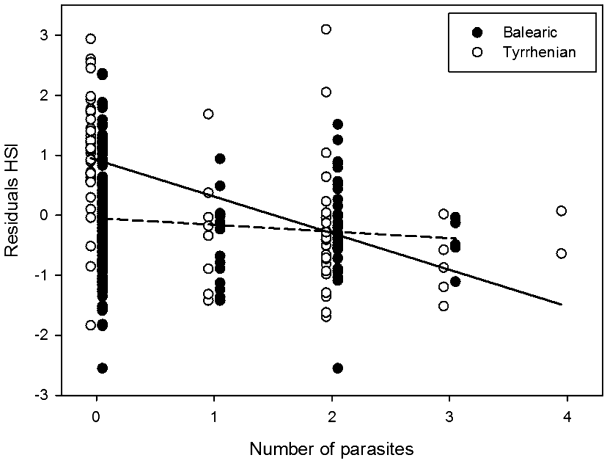

Figure 5. Standardized residuals of hepatosomatic index (HIS) regressed against the number of parasites per individual for the Tyrrhenian (open symbols, full trend line) and Balearic (filled symbols, broken line) fish. Only for the Tyrrhenian fish is there a significant decline in HSI with increasing parasitic load (see main text for details).

Table 4. General linear model (GLM) with hepatosomatic index (HIS) as response variable, age and parasite load as covariates, and sex and population of origin ('Pop') as fixed factors

Factor	d.f.	MS	*F*	*P*
Age	1	0.0001792	10.12	0.002
Sex	1	0.0007773	43.87	< 0.001
'Pop'	1	0.0006453	36.42	< 0.001
Parasite load	1	0.0006317	35.65	< 0.001
'Pop' × parasite load	1	0.0003364	18.99	< 0.001
Error	265	0.0000177		
Total	270			

The significant interaction between 'Pop' and parasite load shows that the effect of parasite infection on HSI differs significantly between populations.

(GLM $F_{1,164} = 9.17$, $P = 0.003$) (Fig. 4), confirming that BA is a more favourable environment than TYR for fish growth.

Finally, we analysed the impact of parasitism on HSI. GLM showed that age, sex, 'Pop' and parasite load all explained significant variation in HSI, and that there was a highly significant 'Pop' × parasite load interaction (Table 4). Similar to the analysis of parasite infection on weight, the effects of *C. italica* on HSI appeared to differ significantly between stocks. To examine this further, we omitted parasite load and the interaction term from the model and calculated the standardized residuals, which were subsequently regressed against the parasite load. In TYR, the parasite load explained 34% of the residual variation in HSI after removing the effects of age, sex and population (regression: $r^2 = 34.4\%$, $F_{1,82} = 43.01$, $P < 0.001$), but, in BA, this was reduced to an insignificant 0.3% (regression: $r^2 = 0.3\%$, $F_{1,185} = 0.64$, $P = 0.424$) (Fig. 5).

In other words, *C. italica* parasite infection appears to have a significantly worse physiological impact on TYR fish.

DISCUSSION

This study examined the infection prevalence and impact of the parasite *C. italica* in the striped sea bream, *L. mormyrus*. The two host populations found to be infected by the mouth-dwelling isopod were genetically indistinguishable on the basis of neutral markers and overall parasitic fauna (Sala-Bozano *et al*., 2009), but the prevalence of *C. italica* infection, parasite load and impact on host weight were significantly greater in TYR than in BA. In addition, the parasite load differed as a function of the host sex and age. Most importantly, the parasite's impact was greater in TYR, and 34.4% of the variation in HSI of fish from this area was explained by the *C. italica* parasite load, whereas parasite infection had no discernible effect on fish in the BA stock. The striking spatial variation in parasite effects between two apparently similar populations could be explained by both local genetic adaptation and phenotypic differences caused by environmental factors, and we evaluate the evidence for both explanations here.

The lack of genetic differentiation at neutral markers between BA and TYR fish is in stark contrast

with the levels of genetic differentiation observed previously among all other *L. mormyrus* populations that were analysed with the same set of genetic markers (Sala-Bozano *et al.*, 2009). This suggests that the lack of differentiation is unlikely to be an artefact of the microsatellite markers used (e.g. size homoplasy; Estoup, Jarne & Cornuet, 2002), but rather reflects actual connectivity between these fish stocks (Fig. 2). Analysis with the software MIGRATE showed that the numbers of migrants per generation (N_em) being exchanged between TYR and BA is considerable ($N_em = 1.2$ for TYR to BA, and $N_em = 13.07$ from BA to TYR). Evidence for high genetic exchange was further corroborated by the mtDNA data of the parasite, which showed that the *C. italica* BA and TYR populations have virtually identical haplotype frequencies. In addition, *C. italica* is an obligate blood-feeder with poor swimming ability (Horton & Okamura, 2003), which depends on its hosts to disperse. Hence, the observation that – at least on *L. mormyrus* – *C. italica* was exclusively present in both focal stocks and was not observed in any of the other four populations surveyed, further supports that both stocks are likely to be demographically connected by migration.

A high rate of gene flow does not necessarily preclude the existence of local genetic adaptation, as reviewed recently in marine fish (Nielsen *et al.*, 2009). However, local genetic differentiation and adaptations are more likely to be eroded under medium/high levels of migration (Hendry, Day & Taylor, 2001; Räsänen & Hendry, 2008). Assuming a common host–parasite coevolutionary model, this is particularly the case at immune and virulence genes, given that the effective migration rate is considerably higher for genes under balancing selection (Schierup, Mikkelsen & Hein, 2001; Muirhead, Glass & Slatkin, 2002). The divergence of such genes requires genetic isolation and strong genetic drift (Miller, Allendorf & Daugherty, 2010), and this does not seem to be supported by our genetic data of both the host and parasite.

Environmentally induced differences in fitness between host populations can also affect parasite tolerance (Thompson & Cunningham, 2002; Blanchet *et al.*, 2010; Kohler *et al.*, 2010). For instance, different environmental circumstances can induce plasticity in the host's condition and growth (Wild, Costain & Day, 2007), which consequently can result in a more severe impact of the parasite on the host. Parasite virulence can also be phenotypically plastic in response to variability in the life history of the host (Nagasawa, 2004; Frank & Schmid-Hempel, 2008). The parasite's virulence is expected to increase with reduced life expectancy of the host, as this will shorten the period of time that parasites can exploit their host (Day, Gaham & Read, 2007) and reproduce before its death (Frank, 1996). This may be particularly relevant to *Ceratothoa* species, which exhibit a relatively long life cycle (Garrey & Maxwell, 1982).

Evidence for environmentally induced differences between BA and TYR stocks was detected when comparing the size and growth rate of noninfected fish. TYR fish were smaller and lighter than those from BA, even when they were not infected, indicating a population under the influence of environmental stresses, beyond the parasitic infection. Moreover, fish from TYR have been shown to mature at significantly smaller sizes than those from BA (Sala-Bozano & Mariani, 2011). Smaller size and earlier maturation in TYR fish are consistent with the existence of compensatory trade-offs between growth and development and reproductive output in a scenario of increased extrinsic mortality (Kuparinen & Merila, 2007). The studied BA and TYR areas are climatically comparable, but are under different local conditions. BA fish were caught close to a marine protected area (Medes Islands), the establishment of which has been shown to greatly benefit the local fish populations (Goñi *et al.*, 2008). TYR fish, however, are native to a more heavily impacted area, with negligible fishery regulation and greater harvesting pressure. This is also supported by FAO catch data (FAO, 2009), which show that the total catches of *L. mormyrus* in Italy have exceeded those in Spain in recent years. Greater fishing pressure may have had an impact on the condition, growth, maturation and sex change (Sala-Bozano & Mariani, 2011) of TYR fish, which could have exacerbated the pathogenic effects of *C. italica* parasite infection. This explanation is in agreement with previous studies which have found that the fitness costs of tolerance and/or resistance are dependent on the environmental conditions (Sandland & Minchella, 2003; Zibiden, Haag & Ebert, 2008), and that these costs can be significantly higher under stressful conditions (Raymond, Sayyed & Wright, 2005; Gassman *et al.*, 2006; Schwarzenbach & Ward, 2006).

Overall, our data appear to be most consistent with the hypothesis that human-induced environmental differences affect the fitness and life history of host populations with cascading effects on parasite impact. Thus, with some caution, by pinpointing the environmental differences that drive life history variation, it may become possible to predict the direction and rate of evolution (Mennerat *et al.*, 2010). As well as providing additional empirical evidence of geographical variation in host–parasite coevolutionary dynamics (Gandon *et al.*, 1996; Thompson, 1999; Gandon, 2002; Thompson & Cunningham, 2002; Gandon & Nuismer, 2009), this study also warrants a more judicious assessment of the environmental context of biological interactions, especially in the case of

exploited resources facing increasingly perturbed natural habitats.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Network representing cytochrome oxidase subunit I (COI) sequences for *Ceratothoa italica*. The size of the pies is proportional to the frequency of the haplotype they represent: black, samples from the Balearic (BA) population; grey, samples from the Tyrrhenian (TYR) population. Small black circles represent the number of mutational steps between haplotypes.

Table S1. General linear model (GLM) of hepatosomatic index (HIS) with age, sex and population of origin ('Pop') as explanatory variables. This model was used to calculate the residuals of HSI, which were then regressed against the parasite load.

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