

Aggregation patterns of macroendoparasites in phylogenetically related fish hosts

J. F. MARQUES^{1*}, M. J. SANTOS^{2,3} and H. N. CABRAL^{1,4}

¹ Universidade de Lisboa, Faculdade de Ciências, Centro de Oceanografia, Campo Grande, 1749-016 Lisboa, Portugal

² Universidade do Porto, Faculdade de Ciências, Departamento de Biologia, Rua do Campo Alegre, s/n, FC4, 4169-007 Porto, Portugal

³ CIMAR – Laboratório Associado/CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental, Rua dos Bragas, 177, 4050-123 Porto, Portugal

⁴ Universidade de Lisboa, Faculdade de Ciências, Departamento de Biologia Animal, Campo Grande, 1749-016 Lisboa, Portugal

(Received 29 November 2009; revised 1 February 2010 and 26 February 2010; accepted 27 February 2010; first published online 26 May 2010)

SUMMARY

Macroparasites are generally aggregated within their hosts with infection and aggregation levels resulting from a continuous arms race between maintaining high mating probability and host mortality low for which host and environmentally related factors contribute to some extent. Here, infection and aggregation patterns of the macroendoparasites infecting the flatfish *Citharus linguatula*, *Arnoglossus laterna*, *Lepidorhombus boscii*, *Scophthalmus rhombus* and *Platichthys flesus* in 3 areas along the Portuguese coast were analysed. Of the 21 macroendoparasite species found only 1 infected all hosts and most were host or area exclusive. For each host-parasite system, values of the indices varied between areas and macroendoparasites were not always aggregated; in fact, some macroendoparasites were generally uniformly distributed, which can be related to specific density-dependent regulation mechanisms. No general pattern was found for infection or aggregation levels of the 3 species infecting more than 2 hosts along the Portuguese coast, i.e. *Lecithochirium rufoviride*, *Nybelinia lingualis* and *Anisakis simplex* s.l., suggesting that regulation mechanisms are not species specific but are locally determined, with host ecology playing a significant role.

Key words: aggregation, endoparasites, flatfish, mean abundance, prevalence.

INTRODUCTION

The distribution of macroendoparasites among their hosts is accepted to be highly aggregated, i.e., most hosts in a given population present low parasite burdens and only a few are highly parasitized. This is, in fact, the first general law of parasite ecology (Crofton, 1971; Shaw and Dobson, 1995; Poulin, 2007) and several authors have devoted their attention to understanding the effects aggregation patterns might have in the density-dependent regulation of both host and parasite abundance (e.g. Anderson and May, 1978; Anderson and Gordon, 1982; Shaw and Dobson, 1995; Rosà and Pugliese, 2002; Newey *et al.* 2005). Moreover, the levels of aggregation observed are not static (Anderson and May, 1978; Anderson and Gordon, 1982; Rosà and Pugliese, 2002) instead resulting from a continuous arms-race between host and parasite density-dependent

regulation mechanisms: if aggregation is too high it may lead to parasite-induced host mortality but too low levels decrease the parasites' mating probability (Morand and Krasnov, 2008) ultimately leading to mortality or extinction.

Even though aggregation of parasites has been investigated in several taxa (e.g. Anderson and May, 1978; Anderson and Gordon, 1982; Shaw *et al.* 1998; Rosà and Pugliese, 2002; Krasnov *et al.* 2006), studies comparing patterns of aggregation and infection amongst different parasite taxa infecting a single host population in a given time and place are scarce (e.g. Newey *et al.* 2005; Matthee and Krasnov, 2009) and those comprising different host populations infected by the same parasite species are even rarer (e.g. Krasnov *et al.* 2004, 2006). Although it is generally accepted that population density does not vary substantially among the different populations making up a species (Poulin, 2006), abiotic conditions can regulate the survival and transmission success of infective parasite stages (Pietrock and Marcogliese, 2003) which, together with host traits such as size, diet and habitat, lead to inter-population variation in infection levels within a given parasite species, as shown for several parasite taxa infecting fish in

* Corresponding author: Centro de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal. Tel: +351 217 500 000 (ext. 22575). Fax: +351 217 500 207. E-mail: jimarques@fc.ul.pt

different areas or periods (e.g. Luque *et al.* 2004; Durieux *et al.* 2007; Luque and Poulin, 2008).

Given that the factors controlling infection levels are likely to vary spatially the degree of aggregation is also expected to do so but this has rarely been analysed (e.g. Krasnov *et al.* 2004, 2006). Although they do not contribute to an increase in the knowledge on universal patterns of aggregation and infection, such studies allow the investigation of the relative importance of host and/or parasite factors that contribute to infection and aggregation patterns or to the exceptions to general rules. Moreover, most studies focusing on aggregation patterns have been conducted on ectoparasites of terrestrial mammals (e.g. Newey *et al.* 2005; Krasnov *et al.* 2006; Matthee and Krasnov, 2009) which is probably related to the extensive datasets available for these host-parasite relationships, resulting, in some cases, from more than 50 years of investigation (e.g. Krasnov *et al.* 2004) or from the importance that these parasitoses might have on human activities and economics (e.g. Newey *et al.* 2005). Nevertheless, abundant information for host-parasite relationships on fishes also exists in some regions, particularly for endoparasites of commercially important marine fish species that can be used as tags to identify stocks (e.g. Timi *et al.* 2005; Abaunza *et al.* 2008; Santos *et al.* 2009). Fish endoparasites are transmitted through the food web and thus reflect the local availability of their intermediate hosts (MacKenzie and Abaunza, 1998), host diet and habitat related features, enabling the analyses of the importance of these factors in aggregation patterns.

In the present study, the infection and aggregation patterns of endoparasites infecting 5 flatfish species along the Portuguese coast – the Atlantic spotted flounder *Citharus linguatula* (Linnaeus 1758), the scaldfish *Arnoglossus laterna* (Walbaum 1792), the four-spotted megrim *Lepidorhombus boscii* (Risso 1810), the brill *Scophthalmus rhombus* (Linnaeus 1758) and the flounder *Platichthys flesus* (Linnaeus 1758) – were analysed in order to (1) investigate how the endoparasite taxa found are distributed within their hosts and (2) evaluate differences in aggregation levels between parasite populations infecting the same host in different areas and between parasite populations infecting different hosts within the same area. Such information should provide insight into whether aggregation levels are a true species character, independent of the study scale, host ecology or environmental characteristics. If indices values consistently vary less between parasite populations than between parasite species then they are characteristic of the parasite species, since variation due to differences in the hosts' immune/defence mechanisms are expected to be low compared to differences in that variation due to the parasites' infectivity, given that the hosts are closely related.

MATERIALS AND METHODS

Sample collection

A total of 1568 flatfish were obtained seasonally, between January 2003 and June 2005 in 3 coastal areas (north – 41°10'N, 8°50'W to 40°00'N, 8°80'W; centre – 39°20'N, 9°10'W to 38°00'N, 8°80'W; and south – 37°05'N, 8°40'W to 37°10'N, 7°25'W) off the Portuguese coast (Table 1). Area definition was based on geomorphological, physical and biological characteristics: whereas the northern and central areas are characterized by average sea surface temperatures (SST) of 14–16 °C, the southern area is characterized by average SST of 18–20 °C; and these areas have distinct faunal assemblages. Whilst the northern area is more related to northern Europe, the southern area is comprised of many species of subtropical origin and the central area represents the northern or southern distribution limit of many marine species.

The 5 species considered in the present study (*Citharus linguatula*, *Arnoglossus laterna*, *Lepidorhombus boscii*, *Scophthalmus rhombus* and *Platichthys flesus*) are commercially important and frequent in landings of several fishing fleet components (beam trawl, trammel nets and gill nets). Despite belonging to 4 phylogenetically close families – Citharidae, Bothidae, Scophthalmidae and Pleuronectidae – these species differ in their life-history patterns and ecological preferences, which are well known in the studied area. *P. flesus* is, amongst the 5 selected, the only species that spends its early life in estuaries and inhabits coastal areas in its adult stage, whereas *S. rhombus* and *A. laterna* are distributed at about 50 m and 100 m deep, respectively, and *C. linguatula* and *L. boscii* are mainly found at greater depths (>200 m). Although they all eat Crustacea, *P. flesus* and *L. boscii* also feed on Polychaeta. *C. linguatula*, *L. boscii* and *P. flesus* include Mollusca and Echinodermata in their diet and all except *A. laterna* eat small Teleostei. Differences in prey item numbers (diet richness) have also been registered for these species along the Portuguese coast with *C. linguatula* and *A. laterna* presenting the more variable diets (ca. 40 different prey items) and *L. boscii* the least variable (14 different prey items) (Teixeira *et al.* 2010).

All fish were measured (nearest mm), sexed and then examined for internal macroendoparasites. All internal organs and mesenteries were carefully inspected under a stereomicroscope and the endoparasite specimens collected, counted, preserved and subsequently identified to the lowest taxonomical level possible, depending on the maturation stage and number of individuals available.

Data analysis

The effect of host sex on parasite burden was evaluated through Mann-Whitney tests and that of

Table 1. Prevalence (%) and mean abundance (in parentheses) of the endoparasite species infecting the five Pleuronectiformes within each area along the Portuguese coast

(Parasite life stage and site of infection, host sample size (*n*), average total length (AvTL) (mm) and its standard deviation (s.d.) are also shown. Stage: A, adult; EM, encysted metacercaria; EP, encysted plerocerci; P, plerocerci; L3, 3rd stage larva. Site: D, digestive tract; M, mesenteries.)

Host	<i>Citharus linguatula</i>			<i>Arnoglossus laterna</i>		<i>Lepidorhombus boscii</i>			<i>Scophthalmus rhombus</i>			<i>Platyichthys flesus</i>	
	Citharidae			Bothidae		Scophthalmidae						Pleuronectidae	
Host family													
Area	North	Centre	South	North	Centre	North	Centre	South	North	Centre	South	North	Centre
AvTL (s.d.)	187(29)	173(12)	150(12)	162(42)	107(20)	162(13)	210(18)	209(23)	267(42)	239(72)	312(42)	244(22)	253(54)
N	165	161	160	71	88	161	199	79	59	108	56	160	101
Endoparasite (Stage)	Site												
Digenea													
<i>Derogenes varicus</i> (A)	D												
<i>Lecithochirium rufoviride</i> (A)	D	23 (0.42)	—	—	1 (0.10)	—	9 (0.19)	1 (<0.01)	—	83 (7.69)	1 (0.01)	—	9 (0.25)
<i>Helicometra fasciata</i> (A)	D						1 (0.01)	2 (0.03)	—				1 (0.02)
<i>Macvicaria soleae</i> (A)	D												4 (0.53)
<i>Proctoeces maculatus</i> (A)	D												6 (0.38)
<i>Proctoeces maculatus</i> (A)	D												1 (0.01)
<i>Zoogonus rubellus</i> (M)	D												4 (0.39)
<i>Zoogonus rubellus</i> (M)	D												4 (0.71)
Cestoda													
<i>Bothriocephalus andresi</i> (A)	D	30 (0.30)	29 (0.33)	21 (0.21)									
<i>Bothriocephalus barbatus</i> (A)	D									17 (0.29)	3 (0.04)	27 (0.36)	
<i>Bothriocephalus clavibothrium</i> (A)	D				3 (0.03)	8 (0.02)							
<i>Bothriocephalus scorpii</i> (A)	D						3 (0.03)	—	—				—
<i>Progrillotia dasyatidis</i> (P)	D						1.9 (0.02)	10.6 (2.79)	—				2 (0.04)
<i>Nybelinia lingualis</i> (EP)	M	1 (0.01)	—	8 (0.13)			1 (0.01)	1 (0.01)	1 (0.01)	2 (0.02)	2 (0.02)	9 (0.30)	
<i>Scolex pleuronectis</i> (P)	D												2 (0.04)
Acanthocephala													
<i>Acanthocephaloides geneticus</i> (A)	D						2 (0.02)	2 (0.04)	—				
<i>Acanthocephaloides propinquus</i> (A)	D						—	3 (0.06)	20 (0.61)	—	6 (0.19)	14 (0.96)	
<i>Echimorhynchus gadi</i> (A)	D						—	4 (0.03)	10 (0.11)				
Nematoda													
<i>Anisakis simplex sensu lato</i> (L3)	M	58 (1.26)	58 (1.20)	15 (0.18)	13 (0.16)	—	13 (0.18)	18 (0.29)	9 (0.19)				
<i>Anisakis typica</i> (L3)	M												1 (0.01)
<i>Cucullanus campanae</i> (A)	D												1 (0.01)
<i>Dychelina minutus</i> (A)	D												14 (2.44)
<i>Hysterothylacium aduncum</i> (A)	D												3 (0.07)

season through Kruskal-Wallis tests conducted for each macroendoparasite species. Prevalence (percentage of infected fishes, P) and mean abundance (mean number of endoparasites per host, M) (Bush *et al.* 1997) were calculated for each endoparasite species infecting a host species within each area and season; variance of abundance (V) was also calculated for each host-parasite pair within each area and season. Significant differences in P and M between seasons and areas were evaluated using Kruskal-Wallis tests. Since all sample sizes were larger than the threshold defined for these hosts in order not to underestimate mean abundance values (>50 individuals per area) (Marques and Cabral, 2007) no effects of sample size in indices' accuracy were expected and, therefore, no corrections for sample size were performed. All test procedures were carried out using SPSS software (SPSS Inc.) with a significance level of 0.05.

Several measures of aggregation have been commonly used to define parasite distributions among their hosts (e.g. Anderson and Gordon, 1982; Shaw *et al.* 1998; Rosà and Pugliese, 2002; Newey *et al.* 2005; Matthee and Krasnov, 2009): the *b* slope of Taylor's power law (Taylor, 1961; Morand and Guégan, 2000), the variance to mean ratio (VMR) of the number of parasites per host (Crofton, 1971) and the parameter *k* of the negative binomial distribution (NBD). The parameter *b* of Taylor's power law, enables the evaluation of parasite aggregation through the relationship between mean abundance (M) and variance of abundance (V), represented by $V = aM^b$, with values larger than unity indicating an aggregated distribution. Because *b* is obtained from the regression analysis of log transformed V on log transformed M, at least 2 host samples are required to be infected by the parasite species in order to calculate *b*. VMR, on the other hand, is directly calculated from V and M values and is an absolute measure of the degree of aggregation allowing direct comparisons between samples with differing prevalence or abundance of infection (Scott, 1987) with values greater than unity also representing an aggregated distribution. The NBD has been extensively used to describe parasite aggregation because of its ease of fit, in most studied cases, and the straightforward calculation of *k* obtained by maximum likelihood techniques applied to the frequency distribution of parasites within a host population. Although samples were collected seasonally in all 3 areas, some parasites were only found in one season within each area and, therefore, *b* could not be calculated for all host-parasite pairs within each area. VMR, on the other hand, could be calculated independently of the number of seasons when the parasite was found. However, given that no significant differences in parasite mean abundance were found between seasons (see Results section), VMR was calculated pooling host individuals from the same

area into 1 sample. For samples showing values of VMR and *b* larger than unity, the fit of the distribution to the negative binomial was tested using the Chi-square test and a significance level of 0.05 in the software XLSTAT (Addinsoft SAR).

To determine whether macroendoparasite prevalence, mean abundance and aggregation vary less among populations of the same parasite species (i.e., among areas) than among different parasite species, values of each index recorded for those parasites infecting more than 2 hosts were correlated with all other values of the same index obtained for that species in all areas and hosts. If values of the same macroendoparasites species are consistent with each other across all areas and hosts, values of the indices can be considered as true species characters and a positive correlation is expected. Pairwise correlations between infection (P and M) and aggregation (VMR) indices were also performed for the same macroendoparasites considering all samples, only hosts within each area and the different areas inhabited by each host to evaluate whether correlations are more similar between different host species inhabiting the same area or between areas inhabited by the same host species. This also allowed the examination of the relative importance of host and environmental factors on parasite infection and aggregation levels.

RESULTS

Parasite assemblages

Twenty-one macroendoparasite species, the majority in the adult stage, were identified from the digestive tract and mesenteries of the 5 Pleuronectiformes analysed in the present study (Table 1). The highest number of endoparasite species was reported in *Platichthys flesus* (11 species) with 8 of them found only in this host. Nevertheless, the other 4 hosts also presented at least 1 'exclusive' endoparasite: *Bothriocephalus andresi* (Porta 1911) in *Citharus linguatula*, *Bothriocephalus clavibothrium* Ariola 1899 in *Arnoglossus laterna*, *Helicometra fasciata* (Rudolphi 1819), *Acanthocephaloides geneticus* (Buron, Renaud et Euzet 1985) and *Echinorhynchus gadi* Müller 1776 in *Lepidorhombus boschii* and *Bothriocephalus barbatus* Renaud, Gabrion et Pasteur 1983 in *Scophthalmus rhombus*.

Lecithochirium rufoviride (Rudolphi 1819) was the only species infecting all 5 Pleuronectiformes and *Anisakis simplex* sensu lato (s.l.) (Rudolphi 1809) and *Nybelinia lingualis* Cuvier 1817 were found in 3 of them (Table 1). Other endoparasite taxa, namely *Radinorhynchinchus* sp. (Acanthocephala) and *Capillaria* sp. (Nematoda) were also found in *C. linguatula* and *P. flesus*, respectively, but given the aims of this study, their levels of infection and aggregation are not reported here as they could not be identified to the specific level. Although most

macroendoparasites were found in more than one area, those infecting *P. flesus* were generally found only in one of the areas inhabited by the host. *Derogenes varicus* (Müller 1784) Looss 1901 and *Bothriocephalus scorpii* (Müller 1776) were found in different areas according to host (Table 1).

Prevalence and mean abundance of infections

Given that no significant differences in the number of macroparasites were found between sexes ($U < 4756$ in all tests, $P > 0.05$) or according to season ($H < 7.19$ in all tests, $P > 0.05$) individuals were pooled within each area and only the indices calculated considering all host individuals from that same area as a sample are therefore reported. Prevalence (P) and mean abundance (M) of each endoparasite species infecting a host varied between areas, with values generally higher in the most southern area where the endoparasite was detected, i.e., central or southern areas off the Portuguese coast (Table 1). Exceptions were *L. rufoviride*, *B. andresi*, *B. scorpii* (higher values in the north) and *A. simplex* s.l. (highest values in the north or central areas). Still, significant differences were only found for *L. rufoviride* and *A. simplex* s.l. infecting *C. linguatula* ($H > 6.82$, $P < 0.05$) and for *L. rufoviride* and *B. barbatus* infecting *S. rhombus* ($H > 1.70$, $P < 0.05$).

Endoparasites infecting more than 1 host usually presented much higher values of prevalence and mean abundance in one of the host species (Table 1): *D. varicus* and *L. rufoviride* infected more *S. rhombus* individuals although a higher number of *D. varicus* per host was found in *P. flesus*; *A. propinquus* was mostly found on *L. boscii* and *A. simplex* s.l. on *C. linguatula* but mean abundance of *A. propinquus* was higher in *S. rhombus*. Although prevalence of *N. lingualis* was not very different between hosts (8% in *C. linguatula* and 9% in *S. rhombus*), mean abundance was almost 3 times higher in *S. rhombus*. Parasites infecting only 1 host species did not present higher values of any of the indices than those presented by non-specific parasites. In fact, the highest values of both indices were attained by the only parasite infecting all 5 host species: *L. rufoviride* (Table 1).

Parasite aggregation within host samples

Values of VMR and *b* (only calculated when the parasite was found in 2 or more seasons) obtained for each endoparasite species within each host sample (Table 2) varied from values lower than 1 for both indices (e.g. VMR = 0.70, *b* = 0.78 in *B. andresi* infecting *C. linguatula* from the north) to 86.48 in VMR (obtained for *B. scorpii* infecting *L. boscii* from the centre) and 3.91 for *b* (obtained for *B. andresi* infecting *C. linguatula* from the centre), indicating

that parasites are not always aggregated within their hosts. This was the case of *L. rufoviride*, *N. lingualis*, *A. typica* and *C. campanae* that were uniformly distributed (values = 1) and *H. fasciata*, *P. maculatus*, *B. andresi*, *B. clavibothrium*, *B. scorpii*, *N. lingualis*, *E. gadi* and *A. simplex* s.l. that were randomly distributed (values < 1) in at least 1 of their host populations. For values of VMR and/or *b* larger than 1 endoparasite distributions were tested to fit the negative binomial model but significant conformity to this distribution was only found in 14 of the 36 samples tested (Table 2).

With the exception of *Macvicaria soleae* (Dujardin, 1845) Gibson and Bray 1982, *B. barbatus* and *A. simplex* s.l., the largest values of the aggregation indices (VMR and *b*) were found in samples presenting the largest values of mean abundance, with correlations between these values generally high and positive ($r = 1$) for most endoparasites within each host across all areas. Nevertheless, low values of correlation were found for *B. andresi* infecting *C. linguatula* ($r = 0.41$) and *B. barbatus* infecting *S. rhombus* ($r = 0.24$) and negative correlations were found for *A. simplex* s.l. infecting *L. boscii* ($r = -0.22$) and *B. clavibothrium* infecting *A. laterna* ($r = -1.00$).

When values of P or M and VMR indices of *L. rufoviride*, *N. lingualis* and *A. simplex* s.l. (endoparasites infecting more than 2 hosts) were correlated across all host and areas all correlations between indices were high and significant in *L. rufoviride* ($r > 0.93$) and *N. lingualis* (except for $r > 0.72$ between all host species in the south) but in *A. simplex* s.l. the only significant correlations were those between all host species within the central and southern areas (Table 3). Generally high correlation values of infection and aggregation indices were found across host populations for each of these 3 macroendoparasite species, the exception was correlation values between both infection indices and VMR in *A. simplex* s.l. infecting *L. boscii* populations.

Whereas for *L. rufoviride* the highest correlations between indices were those between populations of *L. boscii* and *S. rhombus* ($r = 1.00$), in *N. lingualis* identically high correlation values ($r = 1.00$) were found for populations of *C. linguatula* and *S. rhombus* and for host species inhabiting the central area, and in *A. simplex* s.l. the highest correlations were those between host species inhabiting the central and southern areas (Table 3). Nevertheless, correlations considering host species within one area and the different areas inhabited by it were sometimes impossible to perform and others were performed using only 2 samples.

DISCUSSION

The endoparasite fauna reported here for the 5 Pleuronectiformes revealed differences in the

Table 2. Variance to mean ratio of the number of parasites (*VMR*) and slope of Taylor's power law (*b*) obtained for the endoparasite species infecting the five Pleuronectiformes within each area along the Portuguese coast

(The number of samples used to calculate *b* is indicated in parentheses; standard errors are shown in italics below each aggregation value when more than two samples were used. *Significant adjustment of the distribution to the negative binomial ($P > 0.05$).)

Host	<i>Citharus linguatula</i>						<i>Arnoglossus laterna</i>			<i>Lepidorhombus boscii</i>			<i>Scophthalmus rhombus</i>			<i>Platyichthys flesus</i>	
	North		Centre		South		North	Centre	South	North	Centre	South	North	Centre	South	North	Centre
Index Endoparasite	<i>VMR</i>	<i>b</i>	<i>VMR</i>	<i>b</i>	<i>VMR</i>	<i>b</i>	<i>VMR</i>	<i>b</i>	<i>VMR</i>	<i>b</i>	<i>VMR</i>	<i>b</i>	<i>VMR</i>	<i>b</i>	<i>VMR</i>	<i>b</i>	
Digenea																	
<i>Derogenes varicus</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Lecithochirium rufoviride</i>	2.10*	1.11(4)	—	—	—	—	1.00	—	—	—	4.90	1.85(3)	1.00	—	—	—	—
		<i>0.21</i>										<i>0.04</i>					
<i>Helicometra fasciata</i>											0.99	—	2.12	—	—	—	—
<i>Macvicaria soleae</i>																38.74	1.87(3)
																<i>0.03</i>	54.54
<i>Proctoeces maculatus</i>																0.99	—
<i>Zoogonus rubellus</i>																26.71	1.70(3)
																<i>0.25</i>	—
																	1.85(2)
Cestoda																	
<i>Bothriocephalus andresi</i>	0.70	0.78(4)	1.02*	3.91(4)	0.80	0.81(4)	—	—	—	—	—	—	—	—	—	—	—
		<i>0.03</i>		<i>0.18</i>		<i>0.03</i>											
<i>Bothriocephalus barbatus</i>																	
<i>Bothriocephalus clavibothrium</i>							0.99	—	0.94	—	—	—	—	—	—	—	—
<i>Bothriocephalus scorpii</i>											0.98	0.94(2)	—	—	—	—	—
<i>Progrillotia dasyatidis</i>											1.48	—	86.48*	1.69(2)	—	—	2.49
<i>Nybelinia lingualis</i>	1.00	—	—	—	2.19*	—1.49(2)	—	—	—	—	1.00	—	1.66	—	1.00	—	—
<i>Scolex pleuronectis</i>																	1.98
Acanthocephala																	
<i>Acanthocephaloides geneticus</i>											1.48	1.45(2)	2.97	—	—	—	—
<i>Acanthocephaloides propinquus</i>											—	—	2.62	—	8.20	—	—
<i>Echinorhynchus gadi</i>											—	—	0.97	—	1.12	—	—
Nematoda																	
<i>Anisakis simplex sensu lato</i>	2.66	2.13(4)	1.80*	1.16(4)	1.26	1.54(4)	0.88	—	—	—	1.52	1.29(4)	2.27	1.11(4)	4.74	—	—
		<i>0.23</i>		<i>0.12</i>		<i>0.06</i>						<i>0.07</i>		<i>0.41</i>			
<i>Anisakis typica</i>																	1.00
<i>Cucullamus campanae</i>																	1.00
<i>Dychelina minutus</i>																	58.57*
																	1.71(4)
																	<i>0.19</i>
<i>Hysterothylacium aduncum</i>																	2.67

Table 3. Pearson correlation values between infection and aggregation indices obtained for parasite species infecting more than one host and considering (1) different host species inhabiting the same area, (2) the same host species from different areas and (3) all samples

(*P* values are shown in parentheses; correlation values in italics were calculated from only 2 samples.)

	<i>L. rufoviride</i>	<i>N. lingualis</i>	<i>A. simplex</i>
Hosts within area			
North			
P: VMR	0.92* (0.03)	—	0.93 (0.23)
M: VMR	0.93* (0.02)	—	0.94 (0.22)
Centre			
P: VMR	—	<i>1.00*</i> (<0.01)	<i>-1.00*</i> (<0.01)
M: VMR	—	<i>1.00*</i> (<0.01)	<i>-1.00*</i> (<0.01)
South			
P: VMR	—	0.72 (0.49)	<i>-1.00*</i> (<0.01)
M: VMR	—	<i>1.00*</i> (0.02)	<i>1.00*</i> (<0.01)
Areas within host			
<i>C. linguatula</i>			
P: VMR		<i>1.00*</i> (<0.01)	0.79 (0.42)
M: VMR		<i>1.00*</i> (<0.01)	0.82 (0.38)
<i>L. boscii</i>			
P: VMR	<i>1.00*</i> (<0.01)	—	-0.69 (0.52)
M: VMR	<i>1.00*</i> (<0.01)	—	-0.21 (0.86)
<i>S. rhombus</i>			
P: VMR	<i>1.00*</i> (<0.01)	<i>1.00*</i> (<0.01)	—
M: VMR	<i>1.00*</i> (<0.01)	<i>1.00*</i> (0.01)	—
All samples			
P: VMR	0.93* (<0.01)	0.80* (0.02)	-0.03 (0.95)
M: VMR	0.93* (<0.01)	0.92* (<0.01)	0.06 (0.90)

number of parasites species (richness), parasite prevalence and mean abundance between areas within the same host species, as well as between different hosts within the same area. Given that all individuals were adults and no sex differences were found, differences in endoparasite infection levels between host species inhabiting the same area appear to be mainly related to the hosts' ecology, a factor that also might explain differences in endoparasite richness. Environmental factors such as water temperature, oceanic currents and sediment type constrain the type of organisms that can be established in an area and hence the species that can serve as intermediate hosts to the found Digenea, Cestoda, Nematoda and Acanthocephala. Therefore, preferential consumption of prey that are intermediate hosts to the found endoparasite species might explain these differences. However, diet diversity alone cannot explain the differences, given that the lowest number of parasite species ($n=3$) was registered in *A. laterna*, which is known to feed on twice as many prey items ($n=39$) than *L. boscii* that has the lowest diet richness of all 5 Pleuronectiformes in the present study (Teixeira *et al.* 2010). Diversity in prey groups seems to be much more important since the highest parasite richness (11 species) was found on the host that has a more diverse range of prey groups on its diet: *P. flesus* feeds on Crustacea, Polychaeta, Mollusca, Echinodermata and small Teleostei. Moreover, the second highest parasite richness (9 species) was

found on the host species with the second most diverse range of prey groups – *L. boscii* (Teixeira *et al.* 2010). Although differences in host sample size could also be pointed out as a factor explaining differences in richness and infection levels, the present results do not support this: *C. linguatula* was sampled in similar numbers in all 3 areas but more parasite species were found in the north and 2 of them present higher infection levels in this area; twice the number of *S. rhombus* were sampled in the centre than in the south but the an identical number of parasite species was found. Therefore, for these host-parasite systems, once the 40 individuals threshold in sample size that is required not to underestimate parasitological indices has been overcome (Marques and Cabral, 2007), this is not a main factor influencing parasite burden and parasite diversity.

Despite their similar endoparasite richness, *P. flesus* was mainly infected with Digenea and Nematoda whereas *L. boscii* was mainly infected with Cestoda and Acanthocephala. This can be explained by the relative importance of prey items in their diets. Whereas *P. flesus* feeds mainly on Bivalvia, Amphipoda and Decapoda, the most important prey items for *L. boscii* are Decapoda and Teleostei (Teixeira *et al.* 2010). *P. flesus*'s feeding preference for Bivalvia probably explains the higher number of found Digenea, as Mollusca are the first, and sometimes the second intermediate hosts for these endoparasites (Williams *et al.* 1992). However, the habitat

occupied by the host is also important in the completion of a parasite life cycle (MacKenzie and Abaunza, 1998; Poulin, 2006) and, therefore, might also explain the differences found in endoparasite richness and infection levels. Although inhabiting the same geographical area (north, centre or south) these 5 Pleuronectiformes occur at different depths, hence feeding on a different array of prey that constitute intermediate hosts to different parasite species. In contrast, *P. flesus* and *S. rhombus* are usually found in shallow areas (<100m, generally <50 m in *S. rhombus*), *C. linguatula* and *A. laterna* inhabit areas down to about 200 m and *L. boscii* generally occurs in even deeper waters (Froese and Pauly, 2009). In fact, this might be the reason why *A. simplex* s.l., which requires a marine mammal as a final host, was only found in hosts inhabiting deeper waters, and *D. minutus*, that has the coastal Polychaeta *Nereis diversicolor* (Müller, 1776) as an obligate intermediate host (Koie, 2001), was only found in the coastal host that feeds on Polychaeta – *P. flesus*.

Host-related factors are also most likely to be responsible for the finding of most endoparasite species in only one of the hosts included in the present study, although they were observed in other Pleuronectiformes along the Portuguese coast (Marques *et al.* 2006, 2009). Due to the location of the Portuguese coast between the cool temperate and warm temperate North Atlantic and the Mediterranean biogeographical regions (Gubbay, 1995), many species have their limits of distribution here and this might contribute to the differences observed in the number of parasite species between host populations. Differences in host ecology can also explain differences found in infection levels of the endoparasite species occurring in more than 1 host or more than 1 area as, for related host species inhabiting the same area, values of these indices are generally related to the host's ecological preferences (Zander, 2003; Marques *et al.* 2006). Moreover, feeding ecology, ability to enter brackish waters, depth range and geographical distribution have all been recognized to influence macroparasite infection levels on several fish hosts (Luque *et al.* 2004; Poulin, 2006). Thus, parasite data are a good indicator of the host's ecology in a certain area providing further information to that obtained from stomach analysis and artificial tagging studies (Williams *et al.* 1992; Mackenzie and Abaunza, 1998).

The results also revealed that endoparasites were aggregated within most host populations, as it is generally accepted for macroparasite species and corroborated by many studies (e.g. Newey *et al.* 2005; Krasnov *et al.* 2006; Matthee and Krasnov, 2009). However, for the Cestoda *B. clavibothrium* infecting *A. laterna* populations this was not the rule, as the values of VMR were lower than 1. Furthermore, most endoparasite species also presented uniform (VMR = 1) or random (VMR < 1) distributions in at

least one of the host populations, and none of the endoparasites infecting *A. laterna* were aggregated. Although these VMR values were generally associated to very low prevalence (<4% in most samples) and mean abundance (<0.03 in most samples), with the exception of the infection indices presented by *B. andresi* infecting *C. linguatula*, similar low values of the parasitological indices were registered in aggregated parasite populations, suggesting that not all host-parasite interactions are stabilized by preventing the accumulation of large numbers of parasites. Moreover, and although the random distribution of parasites within host populations has been identified as one of the features leading to instability in host-parasite interactions (e.g. Anderson and May, 1978; Tompkins *et al.* 2001; Newey *et al.* 2005), high levels of aggregation can also lead to high mortality if hosts are heavily infected (Morand and Krasnov, 2008). However, losses in host populations due to parasite infections are generally low when few hosts harbour heavy infections (Mosquera *et al.* 2000), which seems to be the case for most of the endoparasites infecting most of the populations of these 5 species along the Portuguese coast. Nevertheless, distribution of parasites in host populations where aggregation appears to be rare (e.g. those infected with *Bothriocephalus* spp.) and where aggregation is extremely high (e.g. *P. dasyatidis* infecting *L. boscii* from the centre; *D. minutus* infecting *P. flesus* from the north) should be monitored as infection indices might change over time leading to deleterious disequilibria in host-parasite relationships. Particular attention should be given to species already representing infection outbreaks in at least 1 population, i.e. $b > 2$ (Morand and Krasnov, 2008), as are the cases of *A. simplex* s.l. and *B. andresi*.

Whereas the low levels of infection and aggregation found in the Cestoda *B. andresi* and *B. clavibothrium* probably result from a compromise between the parasites' long size, restraining the number of individuals that can fit in the host's intestinal lumen, and the maintenance of mating probability (Poulin, 1999), abundance and aggregation of small-sized parasites (e.g. Digenea and larval Cestoda) could be further influenced by intra- and interspecific competition levels within the host. Higher host specificity and larger parasite community sizes are expected to decrease the levels of aggregation as they decrease the probability of randomness in transmission (Krasnov *et al.* 2006; Morand and Krasnov, 2008) but this is not the case for most species infecting these Pleuronectiformes. Lower levels of aggregation were not found in richer communities neither in parasites infecting only 1 host, as can be depicted from the values of VMR in *P. flesus*, the host presenting the richest parasite community and the higher number of 'exclusive' parasites. These data, together with the variation in correlation of infection and aggregation levels between host species and between host

populations, suggest that the degree of aggregation is highly dependent on host factors, with parasite-population-regulation processes more or less intense and host's heterogeneity the prime cause of differences in aggregation (Anderson and Gordon, 1982). In addition to differences in ecology, differences in host susceptibility to infection, whether induced or inherited, are also expected to contribute to the variation found in the levels of parasite infection (prevalence and mean abundance) and aggregation (Anderson and Gordon, 1982). Results obtained here, for this set of related hosts inhabiting the same geographical area (Portuguese coast), revealed that for the same parasite species, infection and aggregation levels and their correlations were not more similar between phylogenetically closer host species (those within the same family, i.e. *L. bosci* and *S. rhombus*) than those between more distant hosts (e.g. *C. linguatula* and *S. rhombus*). Furthermore, the most phylogenetically distant hosts, i.e., *C. linguatula* and *P. flesus* (Azevedo *et al.* 2008) did not present the most different values of infection and aggregation indices and their correlations. Still, and because all indices were also very variable between host populations, studies comparing the magnitude of genetic variation and infection and aggregation levels are needed to evaluate the influence of inherited factors on infection and aggregation levels.

Although not contradicting the conclusion that levels of aggregation and abundance at which population regulation processes start are species-specific (Krasnov *et al.* 2006), the high variability in the values obtained for these indices between parasite species and the fact that aggregation values did not have higher similarities between populations of the same parasite than between parasite species also suggest an important influence exerted by interactions between parasites within the community (Matthee and Krasnov, 2009). Given the distribution of the parasites reported here, with some infecting only 1 population or host species, the effect of this interaction could not be tested. Therefore, and even though some host factors contributing to aggregation have been extensively studied in many host-parasite associations, increased attention should be devoted to understanding the effects of parasite species interactions in shaping the patterns of infection and aggregation.

ACKNOWLEDGEMENTS

The authors would like to thank two anonymous referees for their valuable comments and suggestions to improve the manuscript.

FINANCIAL SUPPORT

This study was partly financed by the Fundação para a Ciência e a Tecnologia (FCT), (J.F.M. grant number

SFRH/BD/8983/2002). This study was also co-funded by the European Union through the FEDER – Fisheries Programme (MARE).

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