

Parasites of fish larvae: do they follow metabolic energetic laws?

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Abstract Eumetazoan parasites in fish larvae normally exhibit large body sizes relative to their hosts. This observation raises a question about the potential effects that parasites might have on small fish. We indirectly evaluated this question using energetic metabolic laws based on body volume and the parasite densities. We compared the biovolume as well as the numeric and volumetric densities of parasites over the host body volume of larval and juvenile-adult fish and the average of these parasitological descriptors for castrator parasites and the parasites found in the fish studied here. We collected 5266 fish larvae using nearshore zooplankton sampling and 1556 juveniles and adult fish from intertidal rocky pools in central Chile. We considered only the parasitized hosts: 482 fish larvae and 629 juvenile-adult fish. We obtained 31 fish species; 14 species were in both plankton and intertidal zones. Fish larvae exhibited a significantly smaller biovolume but larger numeric and volumetric densities of parasites than juvenile-adult fish. Therefore, fish larvae showed a large proportion of parasite biovolume per unit of body host (cm³). However, the general scaling of parasitological descriptors

and host body volume were similar between larvae and juvenile-adult fish. The ratio between the biovolume of parasites and the host body volume in fish larvae was similar to the proportion observed in castrator parasites. Furthermore, the ratios were different from those of juvenile-adult fish, which suggests that the presence of parasites implies a high energetic cost for fish larvae that would diminish the fitness of these small hosts.

Keywords Energetic laws · Biovolume of parasites · Parasite density · Host body volume · Fish ontogeny

Introduction

The energy flux between organisms and the environment has been broadly considered in many species using different techniques. The common goal of many studies has been to understand the macroecological patterns observed in free-living animals (e.g., Glazier 2005, 2008). The results of current studies agree with the metabolic theory of ecology, which is the bridge between the fundamentals of physiology and ecological rules (Brown et al. 2004). Parasites have recently been included in these studies. In general, the abundances of parasites are related to their host body sizes that are in accordance with energetic and ecological rules (George-Nascimento et al. 2004; Hechinger 2015). Parasites always depend energetically on other organisms, i.e., “the hosts” (George-Nascimento et al. 2004; Hechinger et al. 2011; Hechinger 2013). The hosts not only provide food for obligate or temporal parasites but also provide other specific resources. Thus, hosts comprise entire habitats for parasites and attend to all of the needs of parasites. The majority of hosts possess a community of parasites that live in or on them in different abundances and at different stages of the host’s life. Therefore, the energetic dynamics of host-parasite

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systems are complex and are difficult to understand in all of their dimensions. Nevertheless, some methods based on ecological variables have been used to determine the consequences of parasitism on the energetic costs to the hosts (George-Nascimento et al. 2004). Furthermore, other studies have provided specific calculations of physiological parameters based on ecological descriptors to make precise conjectures about the flux of energy between hosts and parasites and the resulting ecological consequences on the hosts (Hechinger 2013).

It is intuitive that if parasites are large or are abundant, then the energetic budget of the host can be decreased. Large hosts have a larger energy flow than small ones so large hosts can harbor more parasites. The energetic requirements of parasites are related to their own body size. As a result, large parasites will require more resources and are normally found on large hosts (George-Nascimento et al. 2004). This relationship is in accordance with universal metabolic energetic laws that relate to the population density of animals and their body mass with a slope close to -0.75 (Damuth's law or the $3/4$ -power law; Damuth 1981). This is explained by the physiological activities of organisms that are also related to body mass with a slope of 0.75 (Kleiber's law; Glazier 2006). However, not all species abide by this rule so another very common relationship between metabolic rate and body mass has been a slope of 0.66 (the $2/3$ -power law) observed in endothermic species (Glazier 2006). Glazier (2005, 2006) has also shown that pelagic organisms have isometric scaling with body mass (slope of ~ 1). Therefore, the slope-value variation depends on the biology of the ectotherm or endotherm species, the pelagic/benthic ontogenetic stage of the organisms, and environmental challenges (Glazier 2005, 2006).

The energetic equivalence law has been applied to parasite communities using the density of parasites versus host body mass as an indirect way of quantifying parasite metabolism (George-Nascimento et al. 2004). This technique is valid because the density of the parasites is a product of the energy deriving from the hosts. This is habitat with a determined and measurable size. In parasites, numeric and volumetric densities are often employed although both parameters are performed differently (George-Nascimento et al. 2004). For different host taxonomical groups, the numeric density of parasites (the parasite abundance per unit host mass) decays with a slope of -0.71 with host body size (George-Nascimento et al. 2004). However, this relationship may be different in the parasites' infracommunities (Poulin and George-Nascimento 2007). The volumetric density of parasites (the biovolume of parasites per unit host mass) does not change with host size, meaning that large hosts may avoid the deleterious effects of parasitism. In other words, hosts have proportional energy budgets for parasites that are similar across different host body sizes (George-Nascimento et al. 2004).

Larval hosts have not typically been used in parasitological studies mainly because they have few parasites (MacKenzie

1974; Herrera 1984a, 1990; King and Cone 2009; Lacerda et al. 2009; Palacios-Fuentes et al. 2012). Most prior studies are descriptive and have reported eumetazoan ectoparasite and/or endoparasite species (e.g., Rosenthal 1967; MacKenzie 1974; Felley et al. 1987; Nielson et al. 1987; Balbuena et al. 2000; Sirois and Dobson 2000; Cribb et al. 2000; Bourque et al. 2006; Skovgaard et al. 2011; Muñoz et al. 2015). In general, the parasitic prevalence in fish larvae can be variable (between 1% and 70%), and the parasite intensity can range between one and nine parasites per host (Lacerda et al. 2009; Muñoz et al. 2015; Palacios-Fuentes et al. 2015). The parasite body size can also vary between 1 and several millimeters in length. For example, pennellid copepods with an average length of 1 mm were found on *Helcogrammoides chilensis* (see Palacios-Fuentes et al. 2012) and larval *Contracaecum* sp. of 6 mm long were found on *Clupea harengus* (see Rosenthal 1967). Thus, parasite size can be large for some fish larvae if we consider that most fish at this stage range between 5 and 20 mm in length. One individual parasite can considerably affect its host's health; similar effects are likely observed in castrator parasites. In such a relationship, castrator parasites first affect the general metabolism, which consequently decreases survival and body growth. This eventually affects the reproduction of their hosts. Therefore, we hypothesize that parasite body sizes are relatively large in larval hosts, and the energetic requirement may be reflected by a large parasite density. This leads to a more negative relationship between parasite density and fish body mass than the relationship observed in juvenile and adult fish. We expect that the body volume of parasites of fish larvae is as large as that of castrator parasites such as bopyrid isopods in decapod crustaceans (e.g., McDermonnt 1991; Muñoz and George-Nascimento 1999), cymothoid isopods in fish (e.g., Adlard and Lester 1994; Fogelman et al. 2009), or larval cestodes in fish (e.g., Mahon 1976), implying large energetic demands on the hosts.

The objective of this study was to determine the relationship between parasite density and host body size in several intertidal fish species in two ontogenetic stages: larval (the pelagic and pre-recruitment phase) and juvenile-adult fish (the benthic and post-recruitment phase). We also include a comparison of the body volume and density of parasites among larval and juvenile-adult fish and castrator parasites (of fish and crustaceans).

Material and methods

Field and laboratory work

Fish larvae were collected from the plankton of the nearshore (<500 m offshore) of central Chile (El Quisco, $33^{\circ} 24' S$, $71^{\circ} 43' W$) during the late austral winter and spring of 2012 and

2013. The samplings were performed during crepuscular times and at nighttime (19:00–23:00 h) onboard an artisanal vessel. Oblique hauls of a Bongo net (60-cm diameter; 300- μ m mesh size) were performed for 10–15 min from a depth of 20 m. The nets then were washed onboard, and all of the zooplankton samples were initially fixed with 5 % formalin buffered with sodium borate and preserved in 96 % ethanol after 12 h.

All fish larvae were separated, counted, and identified into the lowest possible taxon following Balbontín and Pérez (1979), Pérez (1979, 1981), Herrera (1984b), and Herrera et al. (2007). Next, fish were measured using a Moticam 2500 5.0 MPx (Motic Instrument, Inc., Richmond, BC, Canada) connected to a stereomicroscope Olympus SZ-61 (Olympus Corporation, Shinjuku-ku, Tokyo, Japan) with Motic Images Plus 2.0 software (Motic China Group, Co., Xiamen, China). Body length (BL) was measured to the nearest 0.1 cm from the tip of the upper maxilla to the tip of the notochord in preflexion larvae (notochord length) and to the base of the hypurals in flexion and postflexion larvae (standard length). Larval height (LH) and larval width (LW) were also measured vertically at the base of the pectoral fins. Fish body volume was calculated with using the formula for an ellipsoid in cubic centimeters: $[(BL \times LH \times LW)/6] \times 4/3 \times \pi$. In general, an ellipsoid shape represents a fish body well especially when comparing between fish species (George-Nascimento et al. 2004). However, the posterior body of a fish quickly tapers to the end of the tail so an ellipsoid may overestimate the volume of a fish. Thus, for more accurate values, we tested corrections in the formula that yielded minor differences with the real body volume of fish. We multiplied the ellipsoid formula by a factor of 0.75 for most species of larval and juvenile-adult fish with the exception of clingfish species (*Gobiesox marmoratus* and *Sicyases sanguineus*). These are very slender from the half body to the tail, and we thus used a factor of 0.70.

Juvenile and adult fish were collected from the rocky pools of the central zone (33° S) and central-south zone of Chile (36° S) during the late austral winter and spring of 2012 and 2013. The fish were caught using hand nets and anesthetic solution (BZ-20, Veterquímica®) during low tide. The BL, BW, and LH were measured before dissecting the fish to calculate the fish body volume using the corrected ellipsoid formula given above.

We reviewed all of the fish larvae for ectoparasites and endoparasites. The parasites were fixed in 70 % ethanol and then identified according to Muñoz et al. (2015). Juvenile and adult fish were also analyzed for ectoparasites and endoparasites, which were then dissected and fixed in 70 % ethanol and identified following the taxonomical literature of Muñoz and Cortés (2009) and other recently published studies (Muñoz 2010; Muñoz and Bott 2011; Castro and Muñoz 2011). Between 1 and 20 specimens of each parasite species were

measured in length and width (in mm). The geometrical formulas were used to calculate the body volume of the parasites in cubic millimeters according to their body shape: Many parasite species such as acanthocephalan, trematodes, and copepods resemble an ellipsoid; leeches, nematodes, and larval cestodes resemble a cylinder; and monogeneans resemble a flat ellipsoid. We did not apply any correction to the formula to estimate body volume for the parasites because our measurements of their bodies were conducted in the most consistent parts of the body (i.e., excluding the antennae, legs, proboscis, or ovigerous sacs). Therefore, we are unlikely to overestimate parasite body volume.

Data analysis

Only parasitized hosts were considered when calculating parasitological descriptors involving the body volume of parasites and hosts. The descriptors were calculated according to George-Nascimento et al. (2004). The biovolume of parasites per fish was calculated using the abundance of the parasites multiplied by their body volumes. This was expressed as cubic millimeters of parasites. The numerical density was the abundance of parasites divided by the host body volume expressed as the number of parasites per cubic centimeter of host. The volumetric density was calculated as the biovolume of the parasites divided by the host body volume expressed as cubic millimeters of parasites per cubic centimeter of host. The parasite data from five host species—those with larger sample sizes and a large number of parasitized fish—were used to analyze parasite infracommunity data. We employed the average of each parasitological descriptor per host species from all of the parasitized fish species found in this study to analyze the data at the level of the parasite component community.

All of the parasitological descriptors were transformed to \log_{10} to adjust the data to a normal distribution (tested using Kolmogorov-Smirnov analysis) before applying parametric statistics. The parasitological descriptors were compared between larval and juvenile-adult fish using Student's *t* tests. These descriptors were then correlated with host body volume (as a measure of “mass”). We used log-log linear regressions to relate the biovolume, and numeric and volumetric density of parasites with host body volume for all parasitized specimens of larval fish and juveniles of different species. We used analyses of covariance (one-way ANCOVA) to compare the slope and adjusted means between these parasitological descriptors and the host bodies of larval and juvenile-adult fish (Zar 1996).

Information about parasitic castrators related to crustaceans ($n=14$) and fish ($n=6$) was obtained from the literature (Christensen and Kannevorff 1965; Mahon 1976; Wenner and Windsor 1979; McDermott 1991; Lützen and Jespersen 1992; Shields and Earley 1993; Adlard and Lester 1994; Muñoz 1997; Muñoz and George-Nascimento 1999;

Calado et al. 2005, 2008; George-Nascimento and Bustos 2006; Azevedo et al. 2006; Shukalyuk et al. 2005; Cañete et al. 2008; Fogelman et al. 2009; Heins and Baker 2010; Petrić et al. 2010). Only the castrator-host systems with antecedents confirming the castration on the host, total or partial, and with one parasite species were considered in this study. The parasitic castrators in crustaceans were mostly isopods and sometimes rizocephalans. Only females were considered in these host-parasite systems for calculating the parasite body volume because the majority of available information applies only to female parasites. Furthermore, males are very small and are furthermore considered to be parasites of females. Thus, the energetic demand derives mainly from female parasites.

The ratio of parasite biovolume and host body volume (both in cubic millimeters) was compared among larval, juvenile-adult fish and castrator parasites (in fish and crustaceans) using one-way analysis of variance (ANOVA). The ratio between these variables was transformed to \log_{10} to apply ANOVA; we also tested the normal distribution of the data and the homogeneity of variance among the groups using the Kolmogorov-Smirnov and Levene's tests, respectively. Log-log linear regressions were employed to relate biovolume, and numeric and volumetric density of parasites in the following host species: larval fish, juvenile-adult fish, and castrators on crustaceans and fish. A significance level of 0.05 was used in all of the analyses (Zar 1996). Statistical analyses were performed using PAST v. 3.04 (University of Oslo, Norway) and SYSTAT v.11.0 (Cranes Software International Ltd.) software.

Results

A total of 5266 fish larvae from the nearshore zooplanktonic sampling and 1556 fish were collected from the rocky pools. Thirty-one fish species were collected in the whole sample: 29 from zooplankton samplings (all were larvae fish) and 16 from intertidal rocky pools (juveniles and adults). Fourteen species were common between both habitats (Table 1). In terms of the fish larvae, 482 specimens possessed one or two parasites (only parasitic copepods), and 629 juvenile-adult specimens possessed one to seven parasite species (such as copepods, leeches, monogeneans, trematodes, nematodes, acanthocephalans, and cestodes).

Parasite infracommunities

The fish larvae were significantly smaller than the juvenile-adult fish, which is more evident when the fish body volume is considered (Table 2). Furthermore, fish larvae had fewer parasites in terms of richness, intensity, and biovolume than juvenile-adult fish. However, the numeric density of parasites

Table 1 Total number of fish specimens and number of parasitized specimens of all the fish species at two stages (larval and juvenile-adult fish) collected for this study

Fish host species	Larvae		Juveniles-adults	
	Total	Parasitized	Total	Parasitized
<i>Agonopsis chilensis</i>	1	0	0	0
<i>Aplodactylus punctatus</i>	0	0	26	5
<i>Auchenionchus crinitus</i>	209	11	21	14
<i>Auchenionchus microcirrhous</i>	277	35	50	38
<i>Auchenionchus variolosus</i>	96	8	7	5
<i>Bovichtys chilensis</i>	4	0	43	3
<i>Calliclinus geniguttatus</i>	0	0	140	85
<i>Cataetys rubrirostris</i>	1	0	0	0
<i>Chromis crasma</i>	826	0	0	0
<i>Engraulis ringens</i>	51	2	0	0
<i>Gobiesox marmoratus</i>	748	242	174	27
<i>Graus nigra</i>	2	0	11	0
<i>Helcogrammoides chilensis</i>	265	49	267	132
<i>Helcogrammoides cunninghami</i>	669	74	72	22
<i>Hipoglossuna macrops</i>	29	0	0	0
<i>Hygophum bruuni</i>	181	0	0	0
<i>Hypsoblennius sordidus</i>	190	0	77	16
<i>Lapanectus iselinoides</i>	15	0	0	0
<i>Myxodes ornatus</i>	10	8	0	0
<i>Myxodes viridis</i>	199	38	118	50
<i>Ophiogobius jenynsi</i>	23	4	172	49
<i>Pinguipes chilensis</i>	10	0	0	0
<i>Prolatilus jugularis</i>	12	0	0	0
<i>Protomyctophum chilensis</i>	199	0	0	0
<i>Scartichthys viridis</i>	254	0	257	99
<i>Sebastes oculatus</i>	337	0	0	0
<i>Sicyases sanguineus</i>	464	9	120	84
<i>Sindoscopus australis</i>	79	1	0	0
<i>Stomateus stellatus</i>	2	0	0	0
<i>Strangomera bentincki</i>	95	1	1	0
Fish unidentified	18	0	0	0
Total	5266	482	1556	629

was enormous for fish larvae when considering the host body volume in cubic centimeters (Table 2). This is because most fish larvae have a volume of barely 1 cm³. However, in any volumetric unit of the host body, we found that the density of parasites in fish larvae was more than 400-fold greater than in juvenile-adult fish (Table 2). The fish larvae also exhibited a larger average volumetric density of parasites and a ratio of parasite biovolume/host body volume versus the juvenile-adult fish (Table 2).

The biovolume, numeric density, and volumetric density of parasite infracommunities differed among species ($F_{(4, 695)} > 14.78$; $p < 0.001$) and the ontogenetic stage of the

Table 2 Average and standard deviation (SD) of host body size and parasitological descriptors of fish and comparison of these variables, through Student's *t* tests (t = value of the stadigraph, p = probability), between larval and juvenile-adult fish

Variables	Larvae ($N=482$)		Juveniles-adults ($N=629$)		Student's <i>t</i> test (d.f. = 1109)	
	Average	SD	Average	SD	<i>t</i>	<i>p</i>
Host total length (cm)	0.71	0.21	7.82	3.72	41.74	<0.001
Host body volume (cm ³)	0.003	0.01	11.58	19.70	12.89	<0.001
Parasite richness	1.00	0.10	1.58	0.95	13.36	<0.001
Parasite intensity	1.14	0.52	4.36	7.06	10.01	<0.001
Parasite biovolume (mm ³)	0.05	0.07	4.49	13.69	7.08	<0.001
Numeric density of parasites (no. of parasites/cm ³ host)	459.5	340.1	1.19	1.65	-33.79	<0.001
Volumetric density of parasites (mm ³ parasites/cm ³ host)	15.49	17.88	0.62	1.02	-21.78	<0.001

fish ($F_{(1, 697)} > 597.03$; $p < 0.001$). The combined effects of these factors were also observed in the three parasitological descriptors ($(F_{(1, 697)} > 9.59$; $p < 0.001$). The biovolume of parasites increased with fish body volume in some species only (in the larvae of *G. marmoratus* and *H. chilensis* and in the juvenile-adult hosts of *Auchenionchus microcirrhys* and *Myxodes viridis*; Table 3). The slopes of that relationship differed only in *A. microcirrhys* between their ontogenetic stages

($F_{(1, 70)} = 14.05$, $p < 0.001$), and the adjusted means differed in almost all fish species between ontogeny ($p < 0.045$) with the exception of *G. marmoratus* ($F_{(1, 266)} = 1.42$; $p = 0.215$).

The numerical density of parasites decreased with host body volume in both larval and juvenile-adult fish (Table 3). There were no significant differences in the slope of the linear regression applied for four fish species between their ontogenetic stages with the exception of *A. microcirrhys* in which the

Table 3 Linear regressions among three parasitological descriptors (numeric and volumetric density and host body volume) of five fish species at larvae and juvenile-adult stages

	Larvae				Juveniles-adults			
	<i>b</i>	<i>a</i>	r^2	<i>p</i>	<i>b</i>	<i>a</i>	r^2	<i>p</i>
Biovolume of parasites								
<i>A. microcirrhys</i>	-0.290	0.905	0.086	0.087	0.665	-0.351	0.442	<0.001
<i>G. marmoratus</i>	0.160	1.664	0.258	0.012	0.180	-0.981	0.032	0.367
<i>H. chilensis</i>	0.323	2.558	0.104	0.025	0.133	-0.286	0.017	0.129
<i>H. cunninghami</i>	0.108	2.609	0.011	0.361	0.285	-1.130	0.081	0.198
<i>M. viridis</i>	-0.010	2.647	0.001	0.944	0.370	-1.029	0.136	0.008
All fish specimens ^a	-0.190	1.207	0.035	<0.001	0.430	-0.480	0.184	<0.001
Numeric density of parasites								
<i>A. microcirrhys</i>	-0.981	0.072	0.873	<0.001	-0.139	-0.304	0.049	0.184
<i>G. marmoratus</i>	-1.041	-0.067	0.618	<0.001	-0.824	0.041	0.811	<0.001
<i>H. chilensis</i>	-0.898	0.342	0.822	<0.001	-0.956	0.365	0.604	<0.001
<i>H. cunninghami</i>	-0.965	0.117	0.862	<0.001	-0.858	0.086	0.446	<0.001
<i>M. viridis</i>	-0.855	0.421	0.914	<0.001	-0.725	0.101	0.439	<0.001
All fish specimens ^a	-0.967	0.118	0.822	<0.001	0.72	0.238	0.601	<0.001
Volumetric density of parasites								
<i>A. microcirrhys</i>	-0.388	0.092	0.093	0.750	-0.290	-0.351	0.117	0.036
<i>G. marmoratus</i>	-0.526	-0.303	0.069	<0.001	-0.664	-0.982	0.116	0.082
<i>H. chilensis</i>	-0.570	-0.415	0.263	<0.001	-0.837	-0.289	0.284	<0.001
<i>H. cunninghami</i>	-0.581	-0.435	0.142	0.007	0.035	-1.130	0.001	0.715
<i>M. viridis</i>	-0.231	0.524	0.017	0.436	0.439	-1.029	0.014	0.414
All fish specimens ^a	-0.496	-0.217	0.123	<0.001	-0.377	-0.481	0.076	<0.001

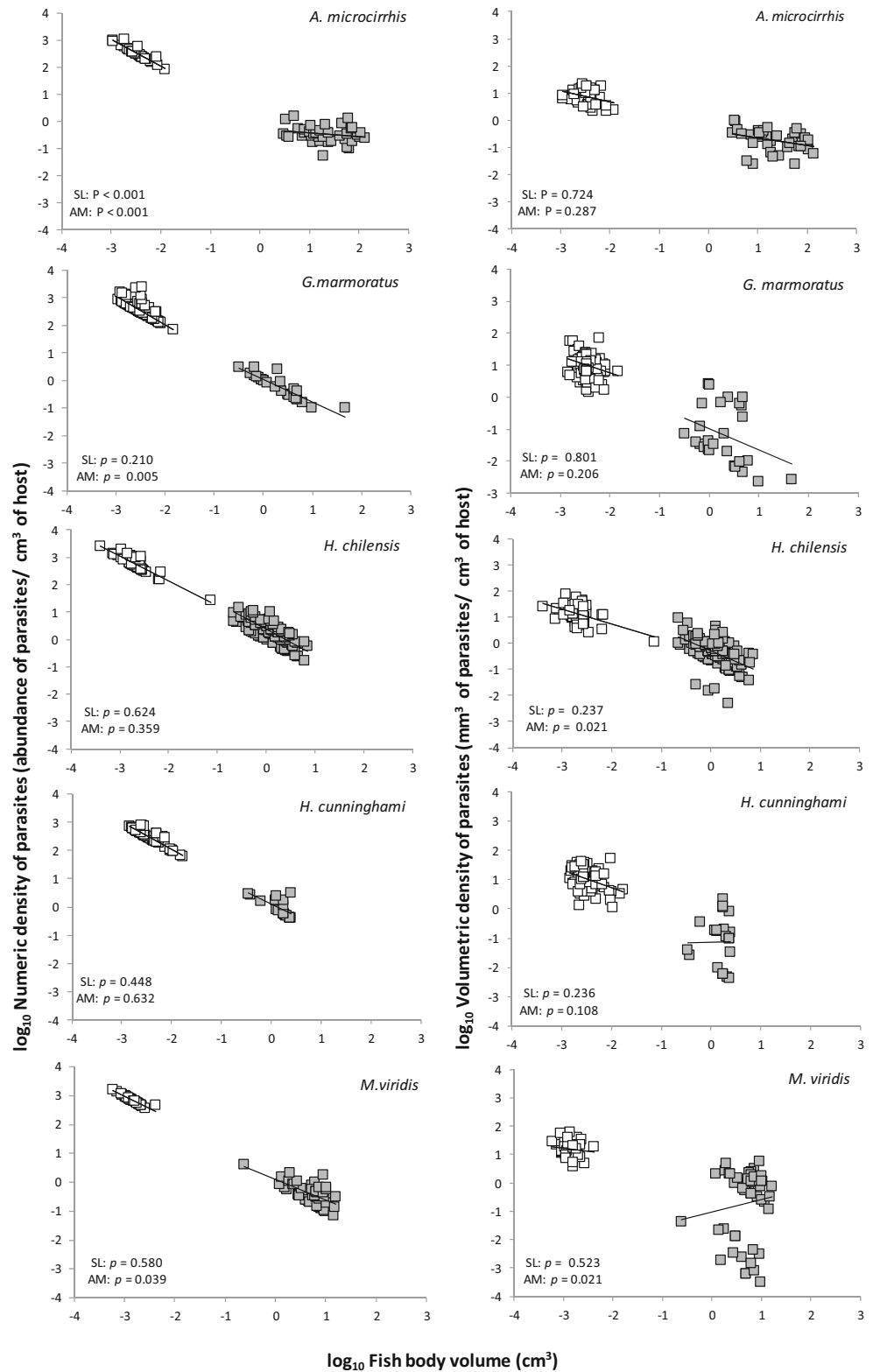
b = slope, *a* = intercept, r^2 = coefficient of determination, *p* = probability

^aThis includes all specimens of 18 fish species which were parasitized (see Table 1)

numeric density of parasites did not vary with body volume of juvenile-adult fish (Table 3 and Fig. 1). The volumetric density of parasites also decreased with fish body volume, but the slope was smaller than that of numerical density versus fish

body volume (Table 3). The numerical density of parasites in the three larval fish species and two juvenile-adult fish species decreased with fish body volume. No differences in the slopes of volumetric density of parasites versus fish body volume

Fig. 1 Relationship between parasitological descriptors—numeric density (abundance of parasites/cm³ of host), volumetric density (cm³ of parasites/cm³ of host)—and host body volume of five fish species at the larval (*white squares*) and juvenile-adult stages (*gray squares*). The statistical probabilities (*p*) from the ANCOVAs that compare slopes (SL) and adjusted means (AM) between both fish groups per each fish species and for both parasitological descriptor are indicated in the graphs



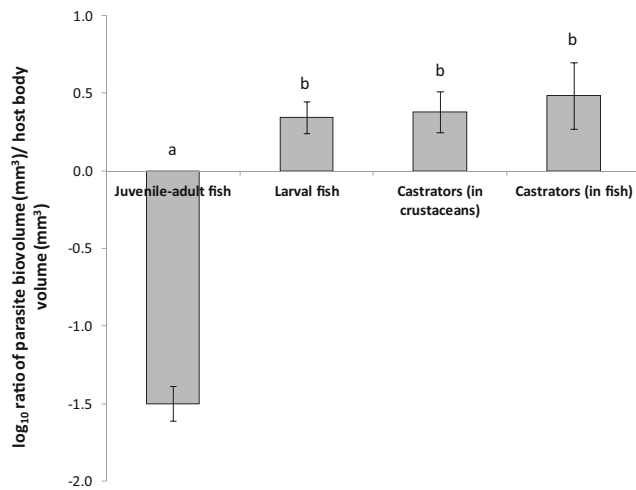


Fig. 2 Ratio between parasite biovolume (mm³) and host body volume (mm³) in four host groups: juvenile-adult fish, fish larvae, castrators in crustaceans and fish hosts. The different letters over the bars indicate significant differences among the groups via one-way ANOVA. The lines over the bars indicate standard errors

were found in any of the fish species between ontogenetic stages, but there were differences in the adjusted means found in some fish species (Fig. 1).

Component community of parasites

The ratio between parasite biovolume and host body volume for four parasite-host groups (parasites in larval fish, parasites in juvenile-adult fish, and castrators in crustaceans and fish) was different ($F_{(3, 43)}=58.78, p<0.001$). However, only parasites of the juvenile-adult fish were significantly different from all of the other groups (Scheffe’s test, $p<0.001$ for juvenile fish versus other groups; $0.93<p<0.99$ for fish larvae and the castrators of fish and crustaceans). Fish larvae have a parasite biovolume per volumetric unit of host body as high as that of castrator parasites (Fig. 2). The percentage of parasite biovolume/host body volume was between 0.4 and 22.0 for castrators in fish, between 0.6 and 17.0 for castrators in crustaceans, between 0.6 and 16.0 for parasitic copepods in fish larvae, and between 0.01 and 0.04 for several parasites in juvenile-adult fish. Juvenile-adult fish were the only group

to exhibit negative values on the logarithmic scale. This clearly differed from the other parasite-host groups (Fig. 2).

Of the four parasite-host systems that we analyzed here, the relationships between parasite biovolume and host body volume were significantly positive in juvenile-adult fish and castrators of crustaceans (Table 4 and Fig. 3). In these two parasite-host groups, no differences were detected in the slope ($F_{(1, 24)}=1.137, p=0.296$), but they did show different adjusted means ($F_{(1, 25)}=24.86, p<0.001$). There was no significant relationship in larvae fish and castrators in fish (Table 4). The numeric density of parasites generally decreased with host body volume, and significant relationships existed between these variables for almost all host-parasite groups with the exception of the fish castrators (Table 4 and Fig. 3). The slope between numeric density of parasites and host body volume differed among the groups ($F_{(2, 35)}=5.31, p=0.009$) after removing the castrators of fish. The juvenile-adult fish group showed the smallest slope between numeric density of parasites and host body volume (Table 4). The volumetric density of parasites did not significantly relate to host body volume in any parasite-host group (Table 4). Even so, the adjusted means differed among the groups ($F_{(3, 42)}=56.29; p<0.001$); only the volumetric density of parasites in juvenile-adult fish had the smallest adjusted mean value versus other parasite-host groups (Scheffe’s test, $p<0.001$ for juvenile fish versus other groups) (Table 4 and Fig. 3).

Discussion

Beside the fact that parasite bodies (or biomass) have been relatively large in fish larvae, their potential effects have been only proposed through indirect observations. Several studies have suggested the deleterious effects that parasites can have on body growth and survival using data such as body size or abundances of fish with or without parasites (Tolonen and Karlsbakk 2003; Santos et al. 2006). Only recently, a study based on otolith analyses provided evidence that ectoparasites on fish larvae negatively affect body growth (Palacios-Fuentes et al. 2012). This suggests that the parasites induce physiological stress on fish larvae.

Table 4 Linear regressions among three parasitological descriptors (biovolume, and numeric and volumetric density) and the host body volume in four host-parasite systems

Host-parasite group	Biovolume					Numeric density				Volumetric density			
	No. of species	<i>b</i>	<i>a</i>	<i>r</i> ²	<i>p</i>	<i>b</i>	<i>a</i>	<i>r</i> ²	<i>p</i>	<i>b</i>	<i>a</i>	<i>r</i> ²	<i>p</i>
Parasites on fish larvae	13	0.467	0.69	0.218	0.107	-0.921	0.277	0.849	<0.001	-0.184	0.565	0.034	0.547
Parasites in juvenile-adult fish	14	0.673	-0.192	0.453	0.008	-0.763	0.211	0.578	<0.002	-0.480	-0.192	0.231	0.082
Castrators in crustaceans	14	0.857	1.474	0.734	<0.001	-0.940	0.225	0.882	<0.001	-0.150	1.443	0.022	0.614
Castrators in fish	6	0.523	2.273	0.273	0.287	-0.450	-0.391	0.20	0.373	-0.781	2.280	0.612	0.065

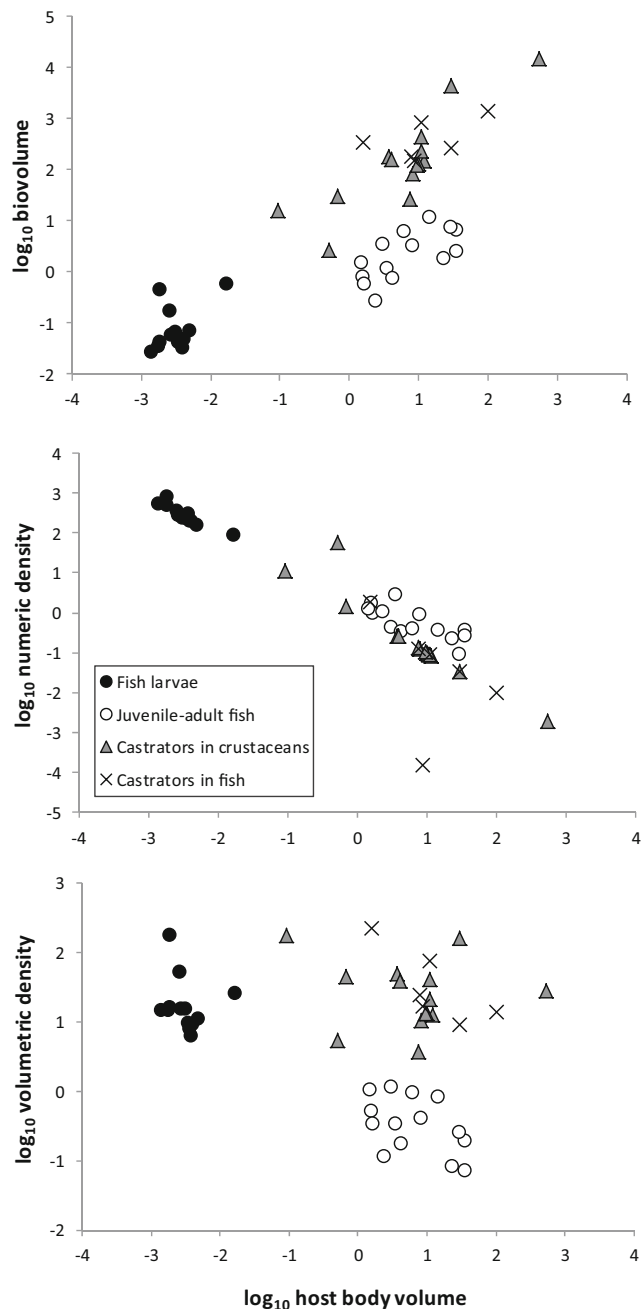


Fig. 3 Relationships between averages of parasitological descriptors: biovolume (mm^3 of parasites), numeric density (abundance of parasites/ cm^3 of host), and volumetric density (mm^3 of parasites/ cm^3 of host) with respect to the body volume of different parasite-host systems, represented by different symbols (shown in the second graph)

The biovolume of parasites was larger in juvenile-adult fish than in larval fish. However, the abundance and biovolume of parasites per volumetric unit of fish (numeric and volumetric density, respectively) were larger in larval fish than in juvenile-adult fish. This means that larval fish have more parasites per host body unit than juvenile-adult fish. This also indicates that parasites likely stress fish larvae. This stress has a significant impact both physiologically and ecologically

on the lives of these small hosts. In fact, the numeric and volumetric density of parasites decreased with host body volume with higher slopes recorded in larval fish than in juvenile-adult fish in both infracommunities (Table 3) and component communities (Table 4). These relationships may result from normal metabolism because pelagic organisms naturally have higher metabolic rates than benthic organisms (Glazier 2005, 2006). However, the impacts of parasites on larval fish may be threefold and not mutually exclusive: (1) detrimental nutritional effects induced by parasites. Parasitic copepods may eat portions of fish larvae such as tissues or mucus; this ingestion may be significant if we consider that parasitic copepods are relatively large in fish larvae. If so, fish larvae maintain the parasites through their own nutritional budget (Anderson and Dale 1981; Astete-Espinoza and Cáceres 2000). (2) The immune response of the hosts. Parasitic infections may trigger an immunological defense in the host to inactivate or remove a parasite, which is a physiological action normally costly in terms of energy to the host (Derting and Compton 2003; Freitak et al. 2003); this cost may be higher in larval fish than in adult fish; and (3) mechanic effects can be caused by parasites via their own body weight. Parasitic copepods may be too heavy for fish larvae which may affect the ability of the fish to swim. There is evidence that a few metacercariae in juvenile *Girella laevis* significantly affect the swimming time of the fish (Rebolledo et al. 2014). We have also recently observed that the average volume of prey eaten by parasitized fish larvae was smaller than in fish without parasites because parasitized fish eat smaller prey and these prey were less mobile (gastropod larvae) than the most frequently ingested prey of unparasitized fish larvae (i.e., copepodites and nauplii) (Janhsen-Guzman et al., unpublished). These antecedents show that the body movements of fish larvae are diminished by parasites (also see Grutter et al. 2010). Fish change their trophic habits to prey on organisms that are easier to catch as a result of having parasites. This evidence also reveals that the amount of food ingested by parasitized larvae is smaller than that ingested by fish free of parasites, which suggests a negative effect on the nutritional budget of the hosts.

Another interesting result was that fish larvae exhibited a large ratio of parasite biovolume to body size similar to castrator parasites (over 0.4 %). This ratio was significantly larger than that of juvenile-adult fish species, and it was also similar to castrator parasites (Fig. 3). However, in this analysis, the volumetric density of parasites did not vary with host body volume in any parasite-host system, which is consistent with the results of George-Nascimento et al. (2004) for component communities of parasites. These authors interpreted this fact as being due to hosts with different body sizes having similarly proportional amounts of resources to defeat parasites. However, juvenile-adult fish do not spend as many resources on parasites as the other host-parasite groups analyzed in this study because they have a lower parasite density (Fig. 3).

Thus, it is possible that the physiological stress of the larval hosts caused by parasites has detrimental effects that are as large as the effects produced by castrator parasites in their hosts.

Castrator parasites have direct or indirect effects on host reproduction. Those parasites that are not in the gonads of the hosts but have large body sizes can induce indirect castration. These parasites use significant nutritional resources of their hosts that can inhibit their development. Castrators first reduce nutritional level parameters in the hosts, which consequently cannot trigger normal hormonal actions to grow or reproduce (Izquierdo et al. 2001). Castrators have a negative effect on the hosts, primarily on survival and body size. Posteriorly, sexual secondary characters and reproduction (totally or partial) of hosts can be affected (Fogelman et al. 2009; Muñoz and George-Nascimento 1999; George-Nascimento and Bustos 2006). Furthermore, the behavior of the hosts (e.g., swimming, alimentary, or sexual performance) may also be affected by castrator parasites. Fish larvae are not in a reproductive stage, and parasites may impact survival, the development of larval stages, behavior, and settlement-recruitment.

Parasitic copepods may use fish larvae as intermediate hosts (Muñoz et al. 2015); larval copepods may detach from their host larvae at some point in their larval life. This detachment facilitates the host's recovery from the effects of the parasitism. However, considering the small body sizes and vulnerable stage of fish larvae, some physiological consequences may remain even after the parasites are no longer present. Therefore, our contribution was to analyze the parasites and their indirect energetic effects using the biovolume and the density of parasites related to the host body size. Such an investigation has not been conducted before on larval hosts. We found that the parasites behave as “castrator parasites” when the parasite biovolume exceeds 0.4 % of the host body size. Fish larvae that have eumetazoan parasites that exceed this ratio impart an important energetic requirement on their hosts that negatively affects different aspects of their host's biology and results in a negative impact on the fitness of their hosts in early development.

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