Variations in the fecundity and body size of digenean (Opecoelidae) species parasitizing fishes from Northern Chile

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Abstract.- Parasite life history and reproductive strategies show considerable variability associated with host identity and/or environmental factors. In this study, we measured parasite body length (PBL) and fecundity of 3 digenean species: Helicometra fasciata, Helicometrina nimia and Neoleburia georgenascimentoi, in their different host species, to evaluate the host influence on these biological traits. Additionally, parasite identifications were confirmed using molecular markers (V4 region of 18S and CO-I genes). From January 2010 to June 2012, 305 fish belonging to Paralabrax humeralis, Acanthistius pictus; Labrisomus philippii, Prolatilus jugularis and Pinguipes chilensis were captured, by diving, off the northern Chilean coastal (24°S). In the laboratory, the parasites were measured and all their parasites collected. The total length and body width of each individual parasite were measured and all eggs were extracted from the parasites’ uterus and counted. Generalized lineal models were used to identify factors affecting fecundity of digeneans. Fecundity of H. nimia was highest in A. pictus and lowest in individuals from L. philippii; fecundity of H. fasciata was highest in P. humeralis and lowest in L. philippii and fecundity of N. georgenascimentoi was highest in P. chilensis. GLM showed that variations in fecundity of digeneans are explained by host fish species and by PBL. Variations in PBL and fecundity are apparently associated with host species sizes: longer parasites (with more eggs per parasite) were recorded in larger fish species (P. humeralis, A. pictus and P. chilensis). However, molecular analyses showed that H. nimia from L. philippii should be considered a distinct species from specimens found in A. pictus and P. humeralis, while N. georgenascimentoi from P. jugularis should be considered a distinct species from specimens found in P. chilensis, which might explain the observed fecundity differences in those host species.

Key words: Helicometra, Helicometrina, Neoleburia, Serranidae, Labrisomidae, Pinguipidae, mDNA
**INTRODUCTION**

Parasite life history and reproductive strategies show considerable variability associated with host identity (Krasnov et al. 2004, Riquelme et al. 2006, González et al. 2012) or environmental factors (Pirotck & Marcogliese 2003, Poulin 2007). There is evidence that the host immunity can affect parasite fitness (Tschirren et al. 2007) and some studies have highlighted its role in controlling helminth populations (Meeusen & Balic 2000). Given that the host immune system represents a strong selective force on parasite success (Morand & Sorci 1998), parasites lacking sufficient adaptations may exhibit depressed fecundity (Trouve et al. 1998).

If generalist parasites (= those infecting several host species) need to develop counter-adaptations against the immune systems of many potential hosts, specialist parasites (= those infecting only one or two host species) can allocate more resources to reproduction or other functions, resulting in a trade-off between the number of host species that can be successfully exploited and parasite success in those hosts (Poulin & Mouillot 2004). Consequently, parasite species with low host specificity may have evolved in less advantageous conditions than those that are more host-specific (Sasal et al. 1999), which could then affect the life-history strategies of each one; and in turn, determine the host-specific levels of parasite abundance (Krasnov et al. 2006). Thus, host specificity takes into account epidemiological indices, such as the percentage of infected host species or the intensity of infection, which may reveal stronger host-parasite interactions (Sasal et al. 1999), and host specificity is recognized as a key factor determining the spread of parasitic diseases (Gemmill et al. 2000).

Parasite identifications are based almost exclusively on morphological characters. However, over the last decade the use of molecular markers has been applied widely as a powerful tool in taxonomy, systematics and phylogeny, allowing to assess whether or not morphological variations correspond to different parasite species or it corresponds only to phenotypic expression of the same species due to environmental variations (Hebert et al. 2004, Radulovici et al. 2010).

Off the northern Chilean coast, fish species such as Brick seabass Acanthistius pictus, Peruvian rock seabass, Paralabrax humeralis (Serranidae) and Chalapo clinid Labrisomus philippii (Labrisomidae) are parasitized by 2 digenean species, Helicometrina nimia and Helicometra fasciata. The serranid species reach similar body length and overlap in habitats associated with seaweeds, e.g., Lessonia nigrescens and Macrocystis spp. (Cisternas & Sielfeld 2008); whereas L. philippii reach lower body length and inhabits demersal habitats (Medina et al. 2004). Similarly, both Pinguipes chilensis and Prolatilus jugularis (Pinguipidae) are hosts to the digenean Neoleburia georgenascimentoi. The latter 2 host species differ in body length and habitat preference (González & Oliva 2008).

In this study, parasite body length and fecundity were measured for these 3 digenean species (H. fasciata, H. nimia and N. georgenascimentoi) in the different host species, to evaluate the influence of the host species on these biological traits and, additionally, parasite identifications were confirmed using molecular markers (V4 region of 18S and CO-I genes). Significant differences in the biological traits among host species can be expected if extrinsic factors are identified as key determinants of reproductive success for these parasites.

**MATERIALS AND METHODS**

**SAMPLING AND DATA COLLECTION**

From January 2010 to June 2012, 305 fish belonging to Paralabrax humeralis, Acanthistius pictus; Labrisomus philippii; Prolatilus jugularis and Pinguipes chilensis were captured, by diving, from northern Chilean coast (Isla Santa María, ISM, 24°S). In the laboratory, the fish were measured and examined, and all parasites were collected using traditional parasitology techniques following González & Poulin (2005). Parasites were sorted, counted, and preserved in 70% alcohol for subsequent analyses prior to being identified based on morphological features, following Bray (2002) and Jones et al. (2005), as Helicometra fasciata, Helicometrina nimia and Neoleburia georgenascimentoi. The number of fish examined by host species, the prevalence, means abundance and means intensity of the digeneans (sensu Bush et al. 1997) in the different host species are summarised in Table 1.

Given that the number of eggs in the uterus can be a snapshot of one continuous process of egg production and release (Herreras et al. 2007), fish samples were obtained at different times during year to avoid potential monthly reproductive peak of the digeneans. The total length was measured from relaxed specimens of the selected digenean parasites using a compound
microscope. Eggs were extracted from the uterus of each of the target parasites and counted. Fecundity was defined as total number of eggs per parasite individual.

**Statistical analyses**

In total, 550 parasites were measured (212 of *H. fasciata*, 228 of *H. nimia* and 110 of *N. georgenascimentoi*). The intensity of infection of these digenean species varied between 1 and 18 parasites per fish. Therefore, the median value was used in statistical analyses when there was more than one individual parasite by fish (Table 2).

Because the data set for *H. nimia* and *H. fasciata* did not meet the requirement of homogeneity of variances, Kruskal-Wallis tests were used to evaluate PBL differences among fish species. For *N. georgenascimentoi*, the difference in PBL (log-transformed data) between host species was evaluated with a Student t-test (Zar 1999). Generalized linear models (GLM) were used to evaluate fecundity (number of eggs per parasite individual) among fish species, including in the models the variables PBL, fish size and intensity of infection. Given that fish size varied significantly among host fish species, a stepwise model was used to evaluate the effect of. The models were performed using distribution normal for response variable (log-transformed), and as normal link function (Quinn & Keough 2002). All analyses were performed in software STATISTICA 7.0.

**Molecular analyses**

Recent literature has shown the presence of cryptic parasite species in different hosts. Hence, molecular analyses were performed using region V4 region of the 18S ribosomal RNA (V4) and these were supplemented with partial cytochrome oxidase subunit I (COI) sequence to confirm the identifications of *N. georgenascimentoi*, *Helicometra fasciata* and *Helicometrina nimia* from the different host species.

For molecular analyses, the parasites were isolated and transferred to a 1.5-ml microcentrifuge tube (one in each tube). DNA extraction was performed according to Leung et al. (2009). The V4 and partial COI gene were amplified by polymerase chain reaction (PCR) using the primers and methodology (including the amplification protocols) described by Hall et al. (1999). The PCR products were visualised on a 1.5% agarose gel and sequenced using an automated capillary electrophoresis sequencer (ABI 3730XL, Macrogen Inc.). The similitude tree reconstruction was performed using the software Mega 5 (Tamura et al. 2011), the algorithm Neighbor-Joining (NJ) (Nei & Kumar 2000), and the evolution model Kimura 2-parameter (K2P) (Kimura 1980). Nodal support was assessed using 1000 bootstrap resamplings (Efron 1982). *Lobatostoma manteri* (L16911.1) and *Aspidogaster conchicola* (DQ482608.1) were used as outgroups for the V4, *Haliotrema aurigae* (EU009803.1) and *Macrogyrodactylus clarii* (GU252718.1) were used as outgroup for COI. These sequences were obtained from Genbank.

1Macrogen Inc., Seoul, Korea <http://www.macrogen.com>
RESULTS

POPULATION PARAMETERS OF THE DIGENEANS

The highest *H. nimia* prevalence was recorded in *L. philippi*, whereas the highest of *H. fasciata* prevalence was in *P. humeralis*. The lowest prevalences of these digeneans were in *A. pictus*. For *N. georgenascim entoi*, the prevalence was higher in *Prolatilus jugularis* than in *Pinguipes chilensis* (Table 1).

PARASITE BODY LENGTH AND FECUNDITY

The number of individuals measured, range of variations of eggs per parasite for each digenean species within their host fish species is summarised in Table 2.

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PBL of *H. nimia* ($H_2\text{df} = 74.19, P < 0.001$), *H. fasciata* ($H_2\text{df} = 9.4, P = 0.009$) and *N. georgenascimentoi* ($t = 2.18, df = 69, P = 0.03$) varied significantly among host fish species. Individuals of *H. nimia* were longest in *A. pictus* and smallest in *L. philippi*, whereas individuals of *H. fasciata* were longest in *H. fasciata* and lowest in *L. philippi* (Table 2). On the other hand, fecundity of *N. georgenascimentoi* was highest in *H. philippi* (Table 2). GLM showed that variation in fecundity of digeneans is explained by host fish species and by PBL (Table 3). Consequently, in those host species where the parasites showed longest body length, they also recorded the highest fecundities.

**Table 2.** Mean parasite body length (PBL, mm), mean number of eggs per parasite and mean intensity of the digenean species in the different host species. In parenthesis, is given standard deviation.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Host fish species</th>
<th>A. pictus</th>
<th>P. humeralis</th>
<th>L. philippi</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. nimia</em></td>
<td>PBL (mm)</td>
<td>5.7 (1.58)</td>
<td>3.3 (1.4)</td>
<td>2.11 (0.5)</td>
</tr>
<tr>
<td></td>
<td>Parasite body width (mm)</td>
<td>1.4 (0.35)</td>
<td>0.8 (0.3)</td>
<td>0.6 (0.1)</td>
</tr>
<tr>
<td></td>
<td>Nº eggs/parasite</td>
<td>326.6 (150.1)</td>
<td>104.9 (85.5)</td>
<td>47.1 (28.1)</td>
</tr>
<tr>
<td></td>
<td>Intensity</td>
<td>8.6 (5.6)</td>
<td>5.4 (5.1)</td>
<td>10.1 (5.5)</td>
</tr>
<tr>
<td></td>
<td>Fish size (cm)</td>
<td>34.3 (3.0)</td>
<td>35.9 (6.9)</td>
<td>26.1 (3.1)</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>40</td>
<td>46</td>
<td>60</td>
</tr>
<tr>
<td><em>H. fasciata</em></td>
<td>PBL (mm)</td>
<td>2.5 (0.6)</td>
<td>2.6 (0.7)</td>
<td>2.1 (0.4)</td>
</tr>
<tr>
<td></td>
<td>Parasite body width (mm)</td>
<td>0.6 (0.1)</td>
<td>0.7 (0.2)</td>
<td>0.6 (0.1)</td>
</tr>
<tr>
<td></td>
<td>Nº eggs/parasite</td>
<td>103.2 (80.4)</td>
<td>150.8 (73.4)</td>
<td>60.2 (43.7)</td>
</tr>
<tr>
<td></td>
<td>Intensity</td>
<td>13.5 (6.4)</td>
<td>12.6 (7.8)</td>
<td>3.95 (1.7)</td>
</tr>
<tr>
<td></td>
<td>Fish size (cm)</td>
<td>35.8 (3.7)</td>
<td>36.2 (6.9)</td>
<td>26.9 (4.0)</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>43</td>
<td>34</td>
<td>19</td>
</tr>
<tr>
<td><em>N. georgenascimentoi</em></td>
<td>P. jugularis</td>
<td>P. humeralis</td>
<td>P. chilensis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PBL (mm)</td>
<td>2.7 (0.6)</td>
<td>3.2 (0.72)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Parasite body width (mm)</td>
<td>0.8 (0.2)</td>
<td>0.8 (0.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nº eggs/parasite</td>
<td>152.9 (120.5)</td>
<td>300.1 (217.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intensity</td>
<td>12.9 (5.4)</td>
<td>5.9 (3.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fish size (cm)</td>
<td>32.8 (4.0)</td>
<td>41.9 (4.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>35</td>
<td>42</td>
<td></td>
</tr>
</tbody>
</table>

Fecundity of *H. nimia* was highest in *Acanthistius pictus* and lowest in individuals from *L. philippi*, whereas fecundity of *H. fasciata* was highest in *P. humeralis* and lowest in *L. philippi* (Table 2). For the V4, a total of 9 sequences from *N. georgenascimentoi* (5 sequences from *P. chilensis* and 4 from *P. jugularis*), 10 sequences from *H. fasciata* (2 from *L. philippi*, 5 from *P. humeralis* and 3 from *A. pictus*) and 9 sequences from *H. nimia* (4 from *L. philippi*, 3 from *A. pictus* and 2 from *P. humeralis*) were obtained. All sequences were 414 bp in length.

For COI, a total of 3 sequences of *H. fasciata*, 3 of *H. nimia* and 8 of *N. georgenascimentoi* were obtained. All sequences were of 737 bp in length. The genetic distance between sequences of *H. fasciata* from *P. humeralis* and *L. philippi* was 0.3% (2 bp difference), both grouped in
the same clade, with 92% bootstrap support. The genetic
distance between the sequences of *H. nimia* from *A. pictus*
and *L. philippii* was 21.8% (161 bp difference),
being located in two separated clades with 86% and 94%
bootstrap support, respectively. The genetic distance
between the sequences of *N. georgenascimentoi* from *P. chilensis*
and *P. jugularis* was 16.7% (123 bp difference),
being located in separated clades with 83% bootstrap
support (Fig. 2). Consequently, analyses using both genes
support that *H. fasciata* is present in all three fish species
studied. However, in the case of *H. nimia*, the genetic
differences among sequences from the fish species
indicate that there is a different genetic group in *L. philippii* (Figs.1 and 2). On the other hand, the analyses for *N. georgenascimentoi*, using COI, show that each host species (*P. chilensis* and *P. jugularis*) harbour a different genetic group (Fig. 2).

**DISCUSSION**

Some studies have demonstrated that parasite biological traits, such as body length and fecundity, vary among host species (Poulin 1996, 1999, Krasnov et al. 2004, Gonzalez et al. 2012). However, in one study based on an acanthocephalan/bird relationship, it was found that the fecundity of 2 digenean species of the genus *Diplostomum*, which shared 2 definitive hosts (birds), did not differ between the host species (Karvonen et al. 2006). In our study, the digeneans *Helicometra fasciata*, *Helicometrina nimia* and *Neolebouria georgenascimentoi*, recorded high fecundity and large body size, in the largest hosts: *Paralabrax humeralis*, *Acanthistius pictus* and *Pinguipes chilensis* respectively. Whereas *P. jugularis* reach small body sizes, respect to the other fish, and had small parasites with low fecundity. It has been demonstrated...
that parasite body size may be constrained by host longevity (Sorci et al. 1999) and host size (Morand et al. 1996). Thus, the high fecundity and body size of H. fasciata, H. nimia and N. georgenascimentoi, could be mainly explained by host body sizes, however, host habitat, host feeding and/or host behaviour may also have some influence (Morand & Guégan 2000, Poulin 2007, Tschirren et al. 2007), for example, P. chilensis lives associated with seaweed (L. trabeculata and Macrocystis spp.), while P. jugularis inhabits sandy areas; these fish also have different diets (Medina et al. 2004), which may indirectly influence the parasite diets, and consequently affect the parasite fecundity (Heimpel et al. 1997).

Inside the host, a parasite is subjected to selective pressures due to food resources (= host nutrients) and host immunocompetence, being suggested that such constraints or advantages could have some influence on the parasite life-history traits (Trouve et al. 1998), which, in turn, might determine host-specific levels of parasite abundances (Sassal et al. 1999, Krasnov et al. 2006). Oliva & Alvarez (2011) studied the relationship between fecundity, worm size, and abundance of the adult digenean Proctoces lintoni (Fellodistomidae) in 4 gastropod hosts (Fissurella spp.) and in the clingfish Sicyases sanguineus from northern Chile. These authors recorded that parasites of the keyhole limpet F. limbata and the clingfish showed the highest mean fecundity, intensity and prevalence; and therefore, they considered that P. lintoni shows the best fitness in those hosts. In our study, H. fasciata showed higher fecundity, and higher mean intensity and prevalence, in P. humeralis, which could suggest that H. fasciata shows the best fitness in P. humeralis. This positive relationship between parasite fecundity and mean abundance/prevalence could be explained by increased availability and/or accessibility of infected prey (= intermediate hosts) to fish population of P. humeralis. Another plausible explanation can be an increased consumption rate of intermediate prey; thereby, an increased likelihood of getting infected prey (increasing parasite abundance/intensity), and in turn, the host could provides a suitable environment (= more nutrients) that promotes the growth of individual parasites with consequent higher fecundity.

Unfortunately, it is not known how many intermediate host species are involved in the life cycle of the digeneans examined, and therefore, we cannot speculate about the role of intermediate host availability in the parasite transmissions.

In the last decades, the use of molecular markers has
been considered a powerful tool in taxonomy, allowing to assess whether or not morphological variations correspond to different species or to phenotypic expressions of the same species due to environmental variability (Hebert et al. 2004, Radulovicci et al. 2010). Our molecular analyses demonstrated that H. nimia from L. philippii and N. georgenascimentoi from P. jugularis and P. chilensis are different species; which could explain their significant differences in parasite body sizes and lower mean fecundity in these hosts (see Figs. 1 and 2, Table 2). Nevertheless, N. georgenascimentoi and H. nimia showed higher prevalence and mean abundance in P. jugularis and L. philippii, respectively, which is concordant with host specificity (Sasal et al. 1999).

The growth and fecundity of helminths can be affected by density-dependence (Shostak & Scott 1993, Tompkins & Hudson 1999, Dezfuli et al. 2002). For example, in an acanthocephalan/fish system, it was observed that egg production by female worms decreased significantly as the infrapopulation size increased, suggesting a density-dependent reduction in female worm fecundity (Hassanine & Al-Jahdali 2008). In our study, generalized linear models supported that biological traits of the digeneans were not influenced by intensity of infection. On the other hand, GLM showed that in the studied digeneans, fecundity was positively correlated with PBL, which is in concordance with reports for other helminth parasites, such as monogeneans, nematodes and acanthocephalans (Sasal et al. 1999, Richards & Lewis 2001, Hassanine & Al-Jahdali 2008).

In summary, body size and consequently fecundity of digeneans varied significantly among host fish species, which is apparently associated with host size species because longer parasites (with more eggs by parasite) were recorded in those longer fish species (P. humeralis, A. pictus and P. chilensis). These results are in agreement with reports for monogeneans (Sasal et al. 1999), copepods (González et al. 2012) and nematodes (Morand et al. 1996, Koprivnikar & Randhawa 2013). However, these results should be cautiously interpreted for the digeneans H. nimia (of L. philippii) and N. georgenascimentoi (of P. jugularis), which correspond to different species as it has been shown with molecular markers. Given the influence that host specificity/generality could have on parasite biological traits; further studies are necessary to advancing in our understanding of the transmission dynamics of the parasites.

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