Geographic and ontogenetic variations in parasite communities of intertidal fish species from the south-eastern Pacific coast

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Abstract

Parasite composition can be affected by physiological and ecological changes during host ontogeny. Intertidal fish do not travel long distances and live in the same area throughout their lifetimes, meaning that parasite communities can differ across geographic ranges. The objective of this study was to analyse the parasite communities of three fish species (*Hypsoblennius sordidus*, *Helcogrammoides cunninghami* and *Scartichthys viridis*) collected from the Chilean coast. The composition of parasite species was compared among host ontogenetic stages (larvae, juveniles and mature fish) and geographic areas. A total of 184 larval, 252 juveniles and 217 mature individuals were collected in the northern area (*c.* 24°S), and 186 larval, 192 juveniles and 112 mature individuals from the central area (*c.* 33°S). Ectoparasites were most prevalent in fish from the central area, whereas endoparasites were most prevalent in the northern area. The parasite species richness varied significantly between geographical areas for *H. sordidus* and *H. cunninghami*, but the parasite composition varied significantly between geographical areas for all fish species analysed. Therefore, the geographical area was the most important factor determining the parasite composition of intertidal fish species. The absence of endoparasites in fish larvae and the increased infestation in juvenile and mature fish may be explained by the shift in habitat from the water column to intertidal pools where prey abundance and availability are higher. On the other hand, hydrographic barriers affecting prey distributions may also offer an explanation as to the differences in parasite composition.

Introduction

Intertidal rocky pools are characterized by temporal and spatial short-term fluctuations in their local environment (e.g. temperature, salinity, radiation) (Hernández *et al.*, 2002). The level of temperature fluctuation depends on the vertical gradient of the intertidal zone and geographic area (Pulgar *et al.*, 2007). For mobile predators such as fish, thermal tolerance and biological interactions (e.g. predation, competition) between ontogenetic stages affect habitat selection (Pulgar *et al.*, 2005, 2007; Ritter, 2017). For example, the increased risk predation and competition at the low intertidal zone may force smaller fish to occupy the high intertidal zone (Hernández *et al.*, 2002).

Morphology, physiology and ecology of fish change throughout their ontogenic development, which may affect the abundance, prevalence and richness of their parasite communities (Flores & George-Nascimento, 2009; Muñoz & Zamora, 2011). Many studies have demonstrated that levels of infection by metazoan parasites increase with longevity and host body size; therefore, an adult fish is more likely to harbour different parasite species than juvenile ontogenetic stages (Poulin, 2007; Muñoz & Zamora, 2011).

Parasite communities of fish from subtidal and demersal habitats show geographic variations that are associated with biogeographical areas known for free-living organisms along the south-eastern Pacific coast (SEP) (González & Moreno, 2005; González & Poulin, 2005; González *et al.*, 2006). Two biogeographic areas are recognized in the SEP: a warm temperate region (Peruvian faunistic province) extending from Peru to 30°S, and a cold temperate region (Magellanic faunistic province) that is influenced by subantarctic currents and extends from 42°S to the southern Chilean coast. There is a transitional area (30–41°S) in which species from northern and southern origins can overlap (Pequeño, 2000; Camus, 2001; González & Moreno, 2005; Hernández *et al.*, 2005). Biogeographical areas are characterized by different chemical and physical characteristics, which may directly or indirectly affect the free-living organisms that are intermediate hosts for parasites. However, no previous studies have compared the parasite fauna of intertidal fish between biogeographical areas along the SEP. Intertidal fish have an extensive geographical distribution along the Chilean coast and inhabit intertidal rocky pools that provide a constant habitat for a variety of resident and transitory

fish (Pulgar *et al.*, 2007), generating micro-environments that could induce differences in the parasite communities between geographic areas.

Intertidal fish *Hypsoblennius sordidus*, *Helcogrammoides cunninghami* and *Scartichthys viridis* are common in the intertidal rocky pools, with extensive geographical distributions along the SEP, from southern Peru to southern Chile (Balbontín & Pérez, 1979; Stepien, 1990; Ojeda *et al.*, 2000; Cancino *et al.*, 2010). There are some parasitological studies conducted on these fish species from the central Chilean coast (Muñoz & Delorme, 2011; Muñoz & Randhawa, 2011; Leiva *et al.*, 2015), but only one study has been published on the species *S. viridis* and *S. gigas* from the northern areas (Flores & George-Nascimento, 2009),

The objective of this study was to describe the parasite fauna in three intertidal fish from the northern and central Chilean coast. Differences in the composition of the parasite fauna would be compared between ontogenetic stages (larvae, juveniles and adults) and the two geographic areas (northern and central area). It was expected that both ontogenetic development and geographical areas would be determinant factors of the parasite composition in these intertidal fish species due to habitat transference (moving from the water column as larvae to intertidal pools as juveniles) and the presence of known hydrographic barriers (e.g. oxygen-minimum zones) that can affect the distribution of prey (intermediate hosts) between geographical regions.

Materials and methods

Fish were collected from two localities on the northern Chilean coast: Isla Santa Maria (23°26′S) and Coloso (23°45′S), Antofagasta; and at two localities on the central Chilean coast: Las Cruces (LC) (33°30′S) and Isla Negra (IN) (33°25′S), Valparaíso. Samplings took place during the years 2006–2009 and 2013–2015 in Valparaíso, and 2014–2015 in Antofagasta, and the larval fish were collected during 2013–2014 (see more details in table 1). Juvenile and mature fish were captured from rocky intertidal pools with anaesthetic solution (0.1%, AQUI-S®, Bayer S.A., Santiago, Chile) and hand-nets. The fish were placed in plastic bags and transported to the laboratory. Larval sampling was conducted from an artisanal fishing vessel in the nearshore region (<500 m offshore), by oblique hauls of a Bongo net (with two conical nets of 60 cm diameter with a 300 μm mesh size) at 20 m depth for 10–15 min. The samples of one net were initially fixed with 5% formalin, buffered with sodium borate and preserved in 96% ethanol after 12 h.

Fish were examined either fresh or frozen (−10°C). A ruler was used to measure (in mm) the body length (BL) of each fish, and the stage of sexual maturity was determined by external observation of the gonads. For each fish species, individual fish were sorted by ontogenetic stage: larvae, juvenile (with small and translucent gonads) and mature (white colour in males, and pink/orange in females). The fishes were identified in the field using the descriptions of Medina *et al.* (2004) and Balbontín & Pérez (1979). The identified larvae were measured in BL (in mm) using a 5.0 MPx Moticam 2500 (Motic Instrument, Richmond, Canada) connected to an Olympus SZ-61 stereomicroscope (Olympus Corporation, Tokyo, Japan), and Motic Images Plus 2.0 software (Motic China Group, Xiamen, China).

The skin, fins, gills (in all three life stages) and opercular cavity (except for the larval stage) were examined to record the presence of ectoparasites using a stereomicroscope Leica M80 (Leica Microsystems, Wetzlar, Germany). The digestive tracts were removed to collect the endoparasites. All organs of juvenile and mature fish were washed with running water and filtered through a 175 mm filter. The retained material was inspected under a stereomicroscope. The collected parasites were fixed in alcohol–formalin–acetic acid solution or preserved in absolute ethanol in individual tubes for subsequent molecular analyses. Ecto- and endoparasites were identified to the lowest taxonomic level possible based on their morphology, following Cribb (2005), Moravec (1998), Jones & Bray (1994) and Petrochenko (1958). Then, the digenean species identifications were supported using the v4 region of the 18S gene (unpublished data). The sequences of each collected species were compared among them and with sequences obtained from GenBank, and the genetic distance was used to discriminate among different species.

For each fish species, population descriptors (prevalence and intensity) and species richness per ontogenetic stage (larval, juvenile and mature) were calculated following Bush *et al.* (1997), and generalized linear models were used to evaluate the effect of host ontogenetic stage (juveniles and mature fish) and geographic area on parasite species richness, with BL as a co-variable. A Poisson distribution was used for the response variable and a log link function (Venables & Ripley, 2002). These statistical analyses were performed using R-Studio v1.1.383 software (RStudio IDE, Boston, USA).

Results and discussion

A total of 1146 fish belonging to *H. sordidus*, *H. cunninghami* and *S. viridis* were examined; 656 from the northern area and 490 from the central area. The BLs per development stage and geographical area for each host species are given in table 1.

In the northern area, a total of six, seven and ten parasite species were recorded in *H. sordidus*, *H. cunninghami* and *S. viridis*, respectively: four ectoparasites (three Copepoda and one Monogenea) and 17 endoparasites (one Acanthocephala, 14 Trematoda and two Nematoda) (supplementary tables S1–S3). In the central area, a total of four, five and 15 parasite species were recorded in *H. sordidus*, *H. cunninghami* and *S. viridis*, respectively: eight ectoparasites (four Copepoda, three Monogenea and one Hirudinea) and 13 endoparasites (two Acanthocephala, eight Trematoda, two Nematoda and one Cestoda) (supplementary tables S1–S3).

For *H. sordidus*, the parasite richness per mature fish was significantly affected by geographic area and BL, but not ontogenetic stage (table 2). For *H. cunninghami*, the parasite richness was significantly affected only by geographic area (table 2). For *S. viridis*, parasite richness was significantly affected by fish ontogenetic stage and BL, but not geographical area (table 2).

Higher levels of parasite intensity and prevalence were found in mature fish. Trematodes were the most common endoparasites in juveniles and mature stages, but were absent in fish larvae. In *H. sordidus* and *H. cunninghami*, opecoelid species were only present in fish from the northern area where no ectoparasites were recorded for these fish species (supplementary tables S1 and S2). The copepod *Trifur* sp. (in the larval stage) was only recorded in larval fish of *H. cunninghami*. In *S. viridis*, *Neobenedenia melleni*, *Hemipera cribbi*, *Lecithaster cf. macrocotyle*, *Monorchimacradena viridis*, *Lepeoptheirus zbigniewi*, *Microcotyle* sp. and Pseudophillydea gen. sp. were most prevalent in the central area (supplementary table S3).

This study evidenced the importance of geographic area in parasite composition of the intertidal fish. Several studies

Table 1. Sample periods (years and seasons), number of examined specimens (sample size) and mean body length ± standard deviation (mm), according to host stage, of *Hypsoblennius sordidus* (HS), *Helcogrammoides cunninghami* (HC) and *Scartichthys viridis* (SV), from the northern (23°S) and central Chilean coast (33°S).

Host	Northern area			Central area		
	Larvae	Juvenile	Mature	Larvae	Juvenile	Mature
HS						
Sample years	2013–2014	2014–2015	2014–2015	2013–2014	2006–2009, 2013–2014	2006–2009, 2013–2014
Sample seasons	WIN–SPR	SPR–SUM	SPR–SUM	WIN–SPR	SPR–SUM	SPR–SUM
Sample size	62	82	136	59	58	31
Body length	3.7 ± 0.9	43.1 ± 4.0	63.9 ± 15.5	3.5 ± 0.8	37.6 ± 7.2	63.5 ± 12.3
HC						
Sample years	2013–2014	2014–2015	2014–2015	2013–2014	2006–2009, 2014–2015	2006–2009, 2014–2015
Sample seasons	WIN–SPR	SPR–SUM	SPR–SUM	WIN–SPR	SPR–SUM	SPR–SUM
Sample size	63	98	74	63	60	67
Body length	4.3 ± 0.8	35.3 ± 4.9	52.4 ± 5.3	7.5 ± 1.3	34.1 ± 5.5	49.7 ± 4.6
SV						
Sample years	2013–2014	2014–2015	2014–2015	2013–2014	2014–2015	2014–2015
Sample seasons	WIN–SPR	SPR–SUM	SPR–SUM	WIN–SPR	SPR–SUM	SPR–SUM
Sample size	61	72	8	64	74	14
Body length	3.2 ± 0.3	63.4 ± 15.2	156.6 ± 53.4	3.2 ± 0.4	55.8 ± 9.9	151.5 ± 22.8

WIN, winter; SPR, spring; SUM, summer.

Table 2. Results of generalized linear models for parasite richness of *Hypsoblennius sordidus*, *Helcogrammoides cunninghami* and *Scartichthys viridis* in the different geographic areas and ontogenetic stages.

Factor	*Hypsoblennius sordidus*			*Helcogrammoides cunninghami*			*Scartichthys viridis*		
	X^2	d.f.	P	X^2	d.f.	P	X^2	d.f.	P
Area	**9.23**	**1**	**<0.01**	**10.80**	**1**	**<0.01**	0.56	1	0.46
Stage	1.31	1	0.25	0.52	1	0.47	**14.37**	**1**	**<0.01**
Body length	**7.91**	**1**	**<0.01**	2.86	1	0.09	**12.38**	**1**	**<0.01**

Statistically significant relationships ($p \leq 0.05$) are indicated in bold.

examining parasites of intertidal fish (juvenile and adults) species from the central south coast of Chile (Muñoz & Delorme, 2011; Muñoz & Randhawa, 2011; Leiva *et al.*, 2017) have not reported the digenean species (opecoelid spp.) present in this study in northern Chile. Additionally, as this is one of the first studies about intertidal fish species from northern Chile, several digenean species reported here could correspond to new undescribed parasite species.

Along the Chilean coast, the three fish species showed differences in parasite community composition between geographic areas (24°S and 33°S). Previous studies (González & Moreno, 2005; González *et al.*, 2006) have shown that only endoparasite species exhibit a biogeographic pattern concordant with known biogeographical areas. In this study, although the host geographical range analysed was more limited (24–33°S), both endo- and ectoparasite species were relatively different between biogeographic areas. For example, digeneans such as opecoelid species were only registered in fish collected in the northern area, whereas ectoparasites such as Piscicolidae gen. sp., *Holobomolochus chilensis* and *N. melleni* were only registered in specimens from the central area.

The life cycles for most of the trematode species recovered are unknown. However, it is possible that the presence of opecoelid and other endoparasite species in the localities of the northern area, and their low prevalence in the localities of the central area (only in *S. viridis*), is a result of geographical variations in prey items or in the availability of infected prey (i.e. the intermediate host). In this context, the discontinuities in hydrography (e.g. oxygen-minimum area) registered at 30°S on the Chilean coast (Camus, 2001; Guiñez *et al.*, 2010) may affect the distribution or availability of intermediate hosts involved in the life cycle for opecoelid species (González *et al.*, 2006). It is known that the species composition of invertebrates and vertebrates tends to vary along the Chilean coast (Ojeda *et al.*, 2000; Valdovinos *et al.*, 2003). On the other hand, ectoparasites have direct life cycles and their distribution is determined mainly by environmental factors and behaviour of their hosts (González & Oliva, 2009). Therefore, the different environmental conditions between the northern and central areas may influence the composition of ectoparasites present in each of the fish species. The existence of a surface water layer known as the oxygen-minimum zone in the northern area (Escribano *et al.*, 2000) could inhibit the

survival of some ectoparasite species. This hypoxia may prevent them from infecting intertidal fish such as *H. sordidus*, but not fish in the subtidal and demersal habitat where several ectoparasite species have been recorded in the northern latitudes of the Chilean coast (Oliva & González, 2005; González & Oliva, 2009).

The higher parasitic richness in mature fish can be explained by a higher exposure time to parasite infection of fish as well as by fish-ontogenetic dietary changes (Muñoz *et al.*, 2002; Muñoz & Zamora, 2011). Fish larvae of *H. sordidus* and *S. viridis* were not infested by ecto- or endoparasites, whereas three larvae of *H. cunninghami* were parasitized by pennellid copepods. Infestation may have been limited to *H. cunninghami* due to differences in larvae; most of the larvae of *H. sordidus* and *S. viridis* were found to be in the small preflexion stage (3.2 ± 0.3 and 3.7 ± 0.9 mm, respectively), whereas larvae of triplefin *H. cunninghami* were rather larger and at the postflexion stage (4.3 ± 0.8 mm). Landaeta *et al.* (2015) determined that larvae of small size and/or early development, as well as reduced abundance in nearshore waters, may preclude copepod infestation in these fish species. Additionally, the absence of endoparasites in fish larvae could be attributed to these larvae inhabiting the plankton for a short period (three months planktonic duration on average; Mansur *et al.*, 2014) and/or feeding of small prey (mostly crustaceans eggs and phytoplankton) that may not harbour parasites. Juveniles and adult fish inhabiting the intertidal zone have a broader trophic spectrum and feed on larger organisms including sessile and/or slow-moving (e.g. molluscs) and active animals (e.g. crustaceans) (Muñoz & Ojeda, 1997). Most endoparasitic species use intertidal organisms such as molluscs and crustaceans as their intermediate hosts (Muñoz & Zamora, 2011; Leiva *et al.*, 2015). The increased consumption of crustaceans and molluscs by juvenile and mature fish, therefore, increases the possibility of acquiring a higher number and diversity of parasites.

In conclusion, our results suggest that geographical area is the main factor influencing the parasite composition of intertidal fish. The differences in the parasite composition could be associated with geographical changes in the diet of the fish, or with the availability of infected prey due to hydrographic barriers affecting prey distribution. This hypothesis could be tested by future studies that focus on the intermediate hosts of the recorded parasite species to advance knowledge of their life cycles.

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