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3 Is ectoparasite **burden** related to host **density**? **Evidence from** nearshore fish larvae off
4 central Chile

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23
24 Running head: **Ectoparasites burden versus host density**

25 **Abstract**

26 Variation in parasite populations may be temporal and/or spatial, related to
27 environmental factors, but it may also be due to differences in host-population density,
28 which is one of the main factors affecting the abundance of directly transmitted parasites.
29 Fish larvae and their ectoparasites were collected by ichthyoplankton samplings in a three-
30 year survey near the coast of central Chile. To estimate the variation of ectoparasite
31 abundance with fluctuations of host density, the prevalence and intensity of ectoparasites
32 (copepods and isopods) were calculated and compared with the density (larval fish
33 abundance standardized to 1000 m⁻³) of six species of nearshore fish larvae, belonging to
34 the families Gobiesocidae, Labrisomidae and Tripterygiidae. Copepods (Pennellidae and
35 Caligidae) and isopods (Cryptoniscidae) were found to be parasitizing the fish larvae. The
36 pennellid copepods were the most prevalent ectoparasite, and clingfish *Gobiesox*
37 *marmoratus* (Gobiesocidae) was the most parasitized fish species (12.81%). The
38 ectoparasite burden of pennellids, caligids or isopods, separately, did not show any
39 correlation with larval densities of four fish species, *Auchenionchus crinitus*,
40 *Auchenionchus microcirrhis*, *Sicyases sanguineus* or *Helcogrammoides chilensis*.
41 Nonetheless, the prevalence and intensity of the pennellid copepods showed a significant
42 and positive correlation with the density of a gobiesocid species (*G. marmoratus*), whereas
43 the prevalence of pennellid copepods (5.10%) showed a significant but negative correlation
44 with the density of larval tripterygid fish (*Helcogrammoides cunninghami*). Ectoparasite
45 abundance is a result of a species-specific relationship with their hosts, but the evidence
46 found suggests no correlation between ectoparasite burden and host density in larval fishes
47 from coastal environments.

48 Key words: Host density, Prevalence, Tripterygiidae, Gobiesocidae, Pennellidae, Caligidae

49 **Introduction**

50 Variation in parasite populations and communities may be temporal or spatial
51 (Kennedy 1975); and **it** can be of high magnitude and without any stable pattern, varying
52 both among and within fish species (Grutter 1994). Another cause of variability is **the**
53 **environment of the parasites, which differs from that of free-living organisms, because it**
54 **has two components: (1) the macro-environment, which is the environment of the host, and**
55 **(2) the micro-environment, which is the host body itself (Rohde 1984). Therefore, both**
56 **environments have an important role in the ecology of parasite communities, by controlling**
57 **the interactions, abundances and diversity of parasites.**

58 **Macro-environment variations can be associated with natural changes in climate,**
59 **seasonal variations and other environmental conditions that can influence parasites through**
60 **direct and indirect pathways (Violante-González et al. 2008; Altman and Byers 2014). For**
61 **example, the prevalence of metazoan ectoparasites of marine fishes is positively and**
62 **strongly related to water temperature (Rohde et al. 1995).**

63 **On the other hand, micro-environment variations,** which include reproductive
64 periods, food availability, mortality rates (Negovetich and Esch 2007) and density (Fellis
65 and Esch 2004) of the host, as well as any biological change in the host, would also affect
66 the abundance of parasite populations (Anderson and May 1978; May and Anderson 1978;
67 Muñoz and Randhawa 2011) leading temporal variations in the dynamics of parasite
68 populations and communities (Muñoz and Randhawa 2011). **The characteristic of the hosts,**
69 **such as host size (Guégan 2005) and host density (Simková et al. 2001), can be important**
70 **for the acquisition and retention of parasites.**

71 Host density usually has a central role on directly transmitted parasites, explaining
72 **species richness,** distribution and abundance (Arneberg et al. 1998; Morand and Poulin

73 1998). Moreover, the probability of the transmission stage (e.g. eggs, larvae) contacting a
74 host (Arneberg et al. 1998) increases with increasing host density, for example, the larval
75 stages of *Caligus rogercresseyi* have a direct relationship with salmon density (Molinet et
76 al. 2011), demonstrating that host density can be more important than oceanographic
77 variables in certain circumstances. Consequently, those species of hosts occurring at high
78 densities should harbor more parasitic species than species occurring at low abundances
79 (Morand and Poulin 1998; Morand et al. 2000).

80 Most research concerning the ecology of marine fish ectoparasites has been carried
81 out on the adult and/or juvenile stages of fishes, but there is scarce information related to
82 ectoparasites on fish larvae. These developmental stages are more vulnerable to the effects
83 of parasites than adult fish (Fogelman and Grutter 2008) due to their fragile nature (Herrera
84 1984; 1990) and considering they are in early stages of immunological and physiological
85 development (Uribe et al. 2011).

86 By using a three-year time series (2010-2012) of plankton sampling in nearshore
87 waters (<500 m offshore) from central Chile, we hypothesize that the variability of the host
88 density (i.e., fish larvae) will influence the ectoparasite burden, considered as prevalence
89 and intensity, in different host species. This will be addressed in the larval stages from three
90 fish families (Gobiesocidae, Labrisomidae and Tripterygiidae) which serve as hosts of the
91 ectoparasites' developmental stages.

92

93 **Material and Methods**

94 *Fieldwork*

95 During the late winter and spring of 2010 (three cruises on 9/2, 9/9 and 10/4), 2011
96 (five cruises on 9/8, 9/15, 10/18, 11/18 and 11/23) and 2012 (five cruises on 11/8, 11/13,

97 11/27, 12/5 and 12/11), nearshore (<500 m offshore) night surveys (1900 to 2300 h) were
98 conducted at El Quisco Bay (33°24'S, 71°43'W), central Chile, on board an artisan vessel.
99 Oblique hauls of a Bongo net (60 cm diameter, 300 µm mesh size) with one TSK flow
100 meter (The Tsurumi-Seiki Co., Ltd.; Tsurumi-ku, Yokohama, Japan) mounted in the frame
101 of the net, were performed for 10-15 min each from a depth of 20 m. Seawater filtered by
102 the net ranged from 13.1 to 437.4 m³ (mean ± one standard deviation: 141.8 ± 102.5 m³).
103 All zooplankton samples ($n = 192$) were initially fixed with 5% formalin buffered with
104 sodium borate and preserved in 96% ethanol after 12 h.

105 Unfortunately, macro-environmental factors, such as sea water temperature, salinity,
106 density and wind strength, could not be incorporated into the study, because the
107 oceanographic and meteorological data were not available in every sampling period.

108

109 *Laboratory work*

110 All fish larvae were separated from the general plankton samples, counted and
111 identified into the lowest possible taxon following the descriptions of Pérez (1979, 1981)
112 and Neira et al. (1998). After this, all fish larvae were observed under a stereomicroscope
113 (Olympus ZS-61; Olympus Corporation, Shinjuku-ku, Tokyo, Japan) to separate and
114 preserve each parasitized larva into individual containers for further analysis. Each
115 ectoparasite was identified to the lowest possible taxon according to their mouth parts and
116 appendages (Castro and Baeza 1986; 1989; Muñoz et al. unpublished).

117

118 *Data analysis*

119 Fish larval abundances were standardised as individuals 1000 m⁻³ for each taxon.
120 From the whole taxocenosis, only three families were selected due to higher prevalence in

121 the samples: Labrisomidae, Gobiesocidae and Tripterygiidae, each represented by two
122 selected species: *Auchenionchus crinitus* (Jenyns, 1841), *Auchenionchus microcirrhis*
123 (Valenciennes, 1836), *Gobiesox marmoratus* Jenyns, 1842, *Sicyases sanguineus* Müller &
124 Troschel, 1843, *Helcogrammoides chilensis* (Cancino, 1960) and *Helcogrammoides*
125 *cunninghami* (Smitt, 1898), respectively (Online Resource 1). These species were grouped
126 into two categories, i.e. non-parasitized larvae (NPL) and parasitized larvae (PL), for
127 calculation of the ectoparasite burden; and the prevalence and intensity were determined
128 according to Bush et al. (1997).

129 Host density, prevalence and intensity for each parasite taxon were tested for
130 normality distribution according to Shapiro-Wilks test. As none of these variables presented
131 a normal distribution (Shapiro-Wilks Test $W > 0.8$; $P < 0.05$) Spearman correlations were
132 carried out to evaluate the existence of a significant relationship between each larval fish
133 host abundance (ind. * 1000 m⁻³) and the ectoparasite burden. All the statistical analyses
134 were run using the STATISTICA 7 package (Statsoft Inc, U. S. A.).

135

136 Results

137 During the study period (2010-2012), a total of 22,671 fish larvae were analysed,
138 consisting of a total of 46 fish larval species. The most parasitized larval fish families were
139 kelpfish labrisomids (*Aucheniunchus crinitus* 3.43%, *A. microcirrhis* 5.51%), clingfish
140 (*Gobiesox marmoratus* 12.81%, *Sicyases sanguineus* 0.52%) and triplefins
141 (*Helcogrammoides chilensis* 5.46%, *H. cunninghami* 5.10%) (Figure 1).

142 Three ectoparasitic taxa belonging to two taxonomic groups (Copepoda, from
143 different chalimus stages, and Isopoda at the larval stage) were recognized in the samples:
144 *Caligus* sp. (Copepoda: Caligidae), *Trifur* spp. (Copepoda: Pennellidae) and Cryptoniscidae

145 (Isopoda) (Figure 2). Prevalence and intensity of the parasite species presented
146 considerably differences in the recorded samples (Table 1). The most prevalent
147 ectoparasites were pennellid copepods, showing the variable intensity range, 1-8 for
148 pennellid copepods, 1- 2 for caligid copepods, and one isopod per parasitized fish. .

149 The prevalence and intensity of ectoparasites were not correlated with the density of
150 most fish larvae ($r_s < 0.7$; $P > 0.05$). However, significant correlations were found in two
151 fish species: pennellid copepods' prevalence (Spearman correlation, $r_s = 0.72$; $P = 0.005$)
152 and intensity (Spearman correlation, $r_s = 0.83$; $P < 0.001$) showed a significant and positive
153 correlation with *Gobiesox marmoratus* density (Figure 3), whereas pennellid prevalence
154 showed a significant but negative correlation with larval *Helcogrammoides cunninghami*
155 density (Spearman correlation, $r_s = -0.76$; $P = 0.021$) (Figure 3).

156

157 Discussion

158 All the species studied showed low and variable ectoparasite prevalence, between
159 0.52% and 12.81%, which is not uncommon for fish larvae. Other studies performed on
160 larval fish of different families have described low prevalence in gobiids (4.4%), *Anchoa*
161 sp. (Engraulidae) (3.6%), *Brevoortia* sp. (Clupeidae) (0.22%) (Felley et al. 1987),
162 *Engraulis ringens* (Engraulidae) (prevalence ranged between 0.9% and 4.7%),
163 *Pomacentrus moluccensis* (Pomacentridae) (4%) (Grutter et al. 2010), and Chilean triplefin
164 *H. chilensis* (Tripterygiidae) (2.7-20.8%) (Palacios-Fuentes et al. 2012).

165 Ectoparasite intensities were also low and variable, mostly between 1-3 parasites
166 per host (the median value was 1 ectoparasite per fish larva), and higher values were rarely
167 found (e.g. up to eight pennellid copepods in one specimen of clingfish *G. marmoratus*).
168 These results concur with the descriptions given by Felley et al. (1987), in which the

169 majority of parasitized fish larvae in an estuary had a single copepod attached and just four
170 fish larvae (Gobies 0.028%, *Anchoa* sp 0.011%, *Brevoortia* sp. 0.0077%) had two or three
171 parasites.

172 This study is a first attempt to find a relationship between ectoparasite burden and
173 larval fish density. The difficulty of estimating host densities in coastal areas limits the
174 probability of determining significant correlations for the parasite-host relationship in
175 marine fishes. Despite that we were unable to find significant correlations between
176 ectoparasite burdens and host densities for most of the fish species considered in this study.
177 The lack of correlation may be caused by random environmental processes near the coast,
178 such as tides, coastal water advection, lunar cycles, wind-driven turbulence and/or seasonal
179 variability (Narváez et al. 2004). Therefore, macro-environmental factors would affect, in
180 different ways, the parasite transmission to the hosts, which should be taken into account in
181 future analyses.

182 *G. marmoratus* was the only host species that showed positive correlations with
183 pennellid copepod burdens (i.e., prevalence and intensity). This fish species was not the
184 most abundant, but it had the highest prevalence and intensity of pennellids, which suggests
185 that these parasitic taxa might prefer this host species. This relationship may be explained
186 by the life cycle of this host, which has planktonic larval stages that spend about one month
187 in the water column (Contreras et al. 2013); thereafter they locate near the sea floor where
188 the probability of infection by pennellidae may increase, because many parasites develop
189 their life cycles near the benthos where are invertebrates that are intermediate hosts for them
190 (Chambers and Dick 2005; Klimpel et al. 2006).

191 Most parasites are host-specific as a strategy to improve their fitness, because each
192 host species provides different micro-environments for the parasites, i.e., some host species

193 are more suitable for a successful life for a parasite than other hosts (Muñoz and Cortés
194 2009). In fact, a similar result was found in the copepod *Caligus rogercresseyi*, which has
195 shown a positive correlation between their larval abundance and density of salmon hosts
196 (Molinet et al. 2011). This link was generated by host densities positively affecting parasite
197 transmission rates, but also because *C. rogercresseyi* prefers salmon hosts to the native
198 hosts.

199 On the other hand, the prevalence of pennellid copepods showed a negative
200 correlation with the larval density of triplefin *H. cunninghami*. The pelagic larval duration
201 of triplefin lasts approximately four months (Plaza et al. 2013; Mansur et al. 2014;
202 Palacios-Fuentes et al. 2014); during these months they aggregate as dense schools near
203 surface waters (Palacios-Fuentes, pers. obs.). Fishes with schooling behaviour may show a
204 lower rate of parasitism depending on their position in the group (Krause 1994), and exhibit
205 significantly greater distances from their neighbours than uninfected shoal members
206 (Barber and Huntingford 1996). This might be a reason why only a few specimens of larval
207 *H. cunninghami* were infected, in spite of the large densities found in the study.

208 Those characteristics of the host biology that increase self-recruitment may benefit
209 the closure of the life cycle of ectoparasite copepods by increasing the probability of their
210 finding a definite host, and thus favour the dispersal of pennellid ectoparasites (Cribb et al.
211 2000). Therefore, pennellid and caligid copepods could be utilizing meroplanktonic fish
212 larvae as intermediate hosts (Palacios-Fuentes et al. 2012; Muñoz et al. accepted), and they
213 would detached before the fish move to the intertidal zone. This means that the definitive
214 host for penellids and caligids found in the larval fish considered in this study would be
215 adult fish from the demersal and subtidal zones, e.g. *Bovichtys chilensis*, *Merluccius gayi*,

216 and *Sebastes oculatus* (Muñoz et al. 2002; George-Nascimento 1996; Oliva and González
217 2004).

218 This is a first approach to gain an understanding how developmental stages of
219 ectoparasites relate to the early life stages of coastal fishes. Consequently there are several
220 questions that still need to be addressed, and it is important for studies of larval fish
221 ecology to comprehend the effects of ectoparasites on feeding habits, growth, condition and
222 survival rates of fish larvae.

223

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232

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352

Tables

353

354 Table I. Prevalence and intensity range of the three parasites families found on six different larval fish species from nearshore waters

355 of central Chile.

356

357

Taxa	Sample size (n)	Total density (ind*1000 m ⁻³)	Pennellidae		Caligidae		Cryptoniscidae		Total	
			Prevalence	Intensity	Prevalence	Intensity	Prevalence	Intensity	Prevalence	Intensity
<i>Auchenionchus microcirrhis</i>	936	7877.698	4.515	[1-2]	0.948	[1-2]	0.043	[1]	5.506	[1-2]
<i>Auchenionchus crinitus</i>	770	5930.722	1.197	[2]	1.642	[1]	0.592	[1]	3.43	[1-2]
<i>Gobiesox marmoratus</i>	1349	13785.591	12.161	[1-8]	0.648	[2]			12.81	[1-8]
<i>Sicyases sanguineus</i>	619	6867.016	0.435	[1]	0.081	[1]			0.52	[1]
<i>Helcogrammoides chilensis</i>	1846	15071.746	5.269	[1-5]	0.126	[1]	0.067	[1]	5.46	[1-5]
<i>Helcogrammoides cunninghami</i>	1124	11927.750	4.109	[1-2]	0.993	[1]			5.10	[1-2]

358

Figure captions

359

360 Figure 1. Three mayor parasitized fish families. Labrisomidae: A. *Auchenionchus*
361 *microcirrhis*, B. *Auchenionchus crinitus*; Gobiesocidae: C. *Gobiesox marmoratus*, D.
362 *Sicyases sanguineus* and Trypterigiidae: E. *Helcogrammoides chilensis*, F.
363 *Helcogrammoides cunninghami*. Scale bars = 1 mm length.

364 Figure 2. Ectoparasites present during the study period. A. Cryptoniscidae B. Pennellidae
365 C-D. Caligidae.

366 Figure 3. Correlations between: A. *G. marmoratus* abundance and prevalence, B. *G.*
367 *marmoratus* abundance and intensity, and C. *H. cunninghami* abundance and prevalence.

368

Supplementary material

369 Online Resource 1

370 Number of specimens for each species through the study period (2010-2012). The value in parenthesis corresponds to the species

371 partial density (ind*1000 m⁻³) on each sampling date.

Sampling date	Taxa					
	<i>A. microcirrhis</i>	<i>A. crinitus</i>	<i>G. marmoratus</i>	<i>S. sanguineus</i>	<i>H. chilensis</i>	<i>H. cunninghami</i>
2010-09-02	111 (695.81)	57 (367.49)	61 (347.87)	17 (81.40)	96 (547.91)	0
2010-09-09	312 (3588.17)	254 (2572.7)	137 (1907.27)	48 (631.45)	539 (5421.66)	25 (84.58)
2010-10-04	52 (330.57)	77 (412.22)	71 (429.24)	27 (180.10)	259 (1330.84)	4 (30.42)
2011-09-08	325 (2094.67)	50 (344.59)	151 (1011.38)	55 (307.38)	373 (3200.94)	148 (733.40)
2011-09-15	60 (168.52)	176 (502.88)	132 (383.97)	42 (121.41)	324 (916.96)	3 (8.06)
2011-10-18	10 (119.25)	7 (107.31)	8 (138.96)	2 (13.91)	34 (678.57)	22 (269.72)
2011-11-18	5 (93.39)	7 (100.43)	2 (36.48)	1 (20.42)	5 (139.34)	12 (229.34)
2011-11-23	10 (99.59)	1 (10.80)	5 (49.92)	2 (20.56)	27 (259.71)	229 (2037.41)
2012-11-08	32 (281.97)	66 (506.55)	305 (2584.68)	132 (2057.68)	62 (417.90)	226 (1869.05)
2012-11-13	6 (90.34)	18 (258.11)	135 (1976.41)	49 (740.41)	36 (641.80)	241 (3636.06)
2012-11-27	3 (35.26)	41 (490.41)	113 (1378.13)	124 (1529.33)	48 (766.35)	174 (2183.11)
2012-12-05	4 (105.27)	14 (215.39)	30 (545.91)	26 (529.91)	21 (376.38)	4 (118.95)
2012-12-11	6 (118.91)	2 (33.22)	199 (2973.82)	94 (1588.40)	22 (373.39)	36 (706.12)

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