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Parasite infection and immune and health-state in wild fish exposed to marine pollution



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ABSTRACT

Association between parasitism and immunity and health-state was investigated in wild *Sebastes oculatus* after having determined that pollution exposure is associated with altered immune and health-state parameters. Given the importance of the immune system in antiparasite defense we predicted: (i) parasite infection would be higher in pollution-exposed than in control fish and (ii) fish with lower immune and health-state parameters would show higher parasitism than fish in better condition. Metazoan parasite fauna was compared between pollution-exposed and non-exposed fish and parasitic indices were correlated with integrated measures of immunity and health-state. Results provided little support for the predictions; some parasite taxa increased, some decreased, and some were not affected in pollution-exposed fish despite their altered health and immunity. Furthermore, there was no link between individual immune and health-state parameters and parasitism. These findings highlight the complexity of host-parasite-environment interactions in relation to pollution in natural marine ecosystems.

Aquatic pollution is a major problem in many freshwater and marine environments around the world. Several studies have focused on the effects of pollutants on the physiology and immune function of fish (Halpern et al., 2008), documenting alterations that range from subtle changes in morphology and physiology (Guardiola et al., 2015; Cazenave et al., 2009) to increases in susceptibility to disease and even mortality (Al-Ansi et al., 2002; Arkoosh et al., 1998). Furthermore, pollution exposure can affect the dynamics of host-parasite interactions in several ways (Dunier, 1996), including direct toxic effects on free-living and/or parasite stages of parasitic organisms, alterations in the populations of intermediate hosts, and a reduction in the ability of fish to defend themselves against invading parasites (Hoole, 1997). Of central importance in the latter case is the effect of pollution on the immune response of the host. Studies simultaneously assessing the effects of pollution on immune function and parasite loads are rare. In fact, few studies have assessed the link between immune function and general health-state to parasitism in natural fish populations (Poisot et al., 2009; Vainikka et al., 2009; Rohlenová et al., 2011).

In this respect, an interesting study system is provided by rockfish *Sebastes oculatus* Valenciennes, 1833 (Family Scorpaenidae) inhabiting reefs along the shore of the Atlantic Ocean in Patagonia, Argentina.

Individuals occupying areas exposed to anthropogenic pollutants present alterations in immunological and health-state parameters when compared to control rockfish from reference sites (Sueiro and Palacios, 2016). Here we evaluated the hypothesis that rockfish exposed to anthropogenic pollution might show higher susceptibility to parasite infection due to their impacted health and immune function. Specifically, we investigated (i) whether parasite infection differs between pollution exposed and non-exposed fish and (ii) whether fish immune and health-state parameters are related to parasite infection at the individual level. We predicted that (i) parasite prevalence, mean abundance, and/or mean intensity would be higher in pollution exposed than in control fish and (ii) fish with lower immune and health-state parameters would show higher parasite abundance and/or intensity than fish in better immune and health condition.

We obtained novel data on the metazoan parasites of the same rockfish individuals (150–328 mm, $N = 63$) sampled for the study of Sueiro and Palacios (2016), collected from polluted and reference (control) sites during winter and summer. Heavy metals (Gil et al., 1999), hydrocarbons (Commendatore and Esteves, 2007), and other toxic substances such as endocrine disruptors (Bigatti et al., 2009) have been registered at the polluted site. On the other hand, reference sites

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do not receive urban, industrial, or other wastes and previous studies suggest they can be considered clean waters (Bigatti et al., 2009; Lezcano et al., 2015). Data on aspects of innate immunity (i.e., differential leukocyte counts, bactericidal capacity of plasma and bacterial agglutination by plasma) and general health-state parameters (i.e., hematocrit and body condition factor) were available for these individuals (Sueiro and Palacios, 2016). The leukocyte profile (percentages of the different cell types) provides an assessment of general immune function that can be altered in fish infected by parasites (Jones, 2001). Bactericidal capacity and bacterial agglutination are mediated by plasma proteins, including complement, lysozyme, and agglutinins such as natural antibodies and lectins, which target a broad array of pathogens and can change in response to parasite infections in fish (Alvarez-Pellitero, 2008). Hematocrit constitutes a physiological index of condition (Fair et al., 2007) and provides an estimate of aerobic capacity (Beldomenico et al., 2008); a reduction in this index, for instance, has been associated with parasites that may cause anemia (Reza Hayatbakhsh et al., 2014). Finally, the body condition factor is considered a global indicator of fish health status (Fulton, 1940) that can be altered by the presence or abundance of certain species of parasites (Guidelli et al., 2011).

Each rockfish was dissected and the parasites were recovered from the body surface, fins, eyes, gills, branchial and body cavities and viscera under a stereoscopic microscope; the musculature was observed on a transparent visor with under light. The ectoparasites (Monogenea, Copepoda, and Isopoda) and endoparasites (Digenea, Cestoda, Acanthocephala, and Nematoda) were determined to the lowest possible taxonomic level. Parasite prevalence, mean abundance, and mean intensity were calculated following Bush et al. (1997). A total of 10 different taxa of parasitic fauna (7 endoparasites and 3 ectoparasites) comprising 1352 individuals were identified on 63 specimens of *S. oculatus* sampled from Nuevo gulf (Table 1). Six of the 10 taxa consist of new records for this fish in the coasts of Patagonia: *Microcotyle* sp., Bucephalidae gen. et sp. (metacercaria), *Scolex polymorphus* (larvae), *Pseudoterranova* sp. (larvae III), *Dichelyne* sp. (adult) and Gnathidae gen. et sp. *Clavellotis sebastidis* had been described by Castro and González (2005) for *S. oculatus*, whereas *Ascarophis* sp., *Anisakis* sp. and *Corynosoma* sp. had been reported by González et al. (2006), but were erroneously attributed to its congener *Sebastes capensis* (Gmelin, 1789), a species not distributed in the coasts of Argentina. Statistical analyses were performed for the four parasitic taxa showing the highest prevalences: the ectoparasites Monogenea and Copepoda and the endoparasites Acanthocephala and Nematoda. Analyses performed on parasite intensity provided the same results and conclusions as those obtained for parasite abundance, thus only the latter are presented.

Acanthocephala were more prevalent at pollution-exposed than at reference sites ($X^2 = 6188$, $p = 0.003$, Fig. 1a), whereas prevalence of the remaining taxa did not vary between sites ($X^2 = 0.13$ – 0.64 , all $p > 0.05$, Fig. 1a). Prevalence of the four parasite groups did not differ between seasons ($X^2 = 0.09$ – 2.47 , all $p > 0.05$, Fig. 1c). Mean abundances of the two ectoparasites (Monogenea and Copepoda) were not affected by site, season, or fish total length, an index of age (Table 2, Fig. 1 b,d). Regarding endoparasites, mean abundance of Acanthocephala was not affected by site or season, but increased with fish total length, whereas mean abundance of Nematoda was significantly higher in reference sites, during the summer, and in larger fish (Table 2, Fig. 1 b,d). At the individual level, no significant relationships were found between the abundance and intensity of parasite taxa considered (i.e., Monogenea, Copepoda, Acanthocephala, and Nematoda) and any of the immune and health-condition parameters considered (lymphocytes, neutrophils, monocytes, bactericidal capacity of plasma, bacterial agglutination of plasma, hematocrit, and condition factor) (Additional Material).

The higher prevalence of Acanthocephala in rockfish exposed to anthropogenic pollution is in line with the prediction that parasitism would be higher in pollution-exposed fish and provides some support to

the hypothesis that these fish, with their altered immune and health-state parameters (Sueiro and Palacios, 2016), would be more susceptible to infections. This result is in accordance with several studies linking pollutants, immunosuppression, and parasite infection susceptibility (Sures, 2008; Khan, 2012). Conversely, the lower abundance of Nematoda in pollution-exposed fish does not support the hypothesis and could be suggesting a direct toxic effect of pollutants on this particular parasite group, which has been consistently proposed as environmental indicator due to its generally high sensitivity to pollution (MacKenzie, 1999, but see Blanar et al., 2009). Contrary to findings for endoparasitic taxa, the most prevalent ectoparasites identified showed no differences in infection prevalence, abundance, and/or intensity between rockfish from pollution-exposed and non-exposed sites. Given that ectoparasites are in constant direct contact with the external environment, it has been argued that in the course of evolution they might have developed higher flexibility and resistance to environmental changes (MacKenzie, 1999). Nevertheless, contrary to our findings and the proposed explanation, a meta-analysis by Blanar et al. (2009) suggests that directly exposed parasitic taxa (e.g., ectoparasites and free-living stages of endoparasites) are overall more vulnerable to contaminants than indirectly exposed taxa (i.e., endoparasites), supporting the hypothesis that the latter would be somewhat protected from pollutants through homeostatic mechanisms of their hosts. Overall, studies suggest that the relationship between parasites and pollution can be highly variable with positive, negative, or non-effects on parasite abundances depending on the type and intensity of pollutants even within a certain parasite group or species (Lafferty, 1997; Blanar et al., 2009; Vidal-Martínez et al., 2010). Interestingly, the study by Blanar et al. (2009) also revealed that parasites from marine systems are overall less vulnerable to pollution than those from freshwater systems, perhaps due to the higher complexity and more open nature of marine ecosystems, which could contribute to our results.

Contrary to the prediction that individuals with lower immunity and health condition would show higher parasitism, no relationships were found between immune and health-state parameters and the abundance and intensity of the most prevalent parasite groups found in rockfish, even after correcting for other effects (site, season, and/or total body length) when indicated (data not shown). These results suggest that host immune and health condition are not significant predictors of parasite infection in this system, at least for the physiological parameters and parasite types assessed. The finding of few (or no) significant associations between fish immune and health parameters and parasitism seems to be a common pattern in studies performed under field conditions (e.g., Poisot et al., 2009; Vainikka et al., 2009; Rohlenová et al., 2011). Thus, the absence of relationships between immune and health parameters and parasite infection of rockfish in the context of anthropogenic pollution is in line with the notion that host-parasite interactions in natural ecosystems can be highly complex. Factors such as seasonal changes in abiotic and biotic environmental components, abundance, density, and condition of hosts, host age and behaviour, among others (Bagge et al., 2004), can contribute to variation in parasitism, making it difficult to detect the potential effects of altered immunological defenses of hosts by pollutants in natural systems. In addition, hosts could have also evolved parasite tolerance strategies to minimize fitness costs of parasitism (Baucom and De Roode, 2011), thus potentially contributing to the lack of relationships between parasite loads and indices of general health and condition. A combination of field experiments and more controlled environments in the laboratory are likely to be necessary to complement studies in the wild in order to tease apart the different effects of pollutants without losing ecological relevance.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.marpolbul.2017.04.011>.

Table 1
Prevalence (P), mean abundance (MA) and mean intensity (MI) with standard deviations (SD) for all parasite taxa found in the rockfish *Sebastes oculatus* from pollution-exposed (EXP) and reference (REF) sites sampled in two different seasons.

	ST	L	Winter						Summer					
			EXP (n = 8)			REF (n = 18)			EXP (n = 16)			REF (n = 21)		
			P (%)	MA ± SD	MI ± SD	P (%)	MA ± SD	MI ± SD	P (%)	MA ± SD	MI ± SD	P (%)	MA ± SD	MI ± SD
Endoparasites														
Trematoda digenea	M	E	0.0	0.0	–	0.0	0.0	–	6.0	0.1 ± 0.3	1 ± –	19.0	0.3 ± 0.7	1.5 ± 1.0
Bucephalidae gen. et sp.														
Cestoda	Pl	I	13.0	0.1 ± 0.4	1 ± –	6.0	0.1 ± 0.5	2 ± –	0.0	0.0	–	0.0	0.0	–
<i>Scolex polymorphus</i>														
Nematoda	LIII	Me, Mu	50.0	1.3 ± 2.4	2.5 ± 3.0	50.0	2.7 ± 4.9	5.3 ± 5.9	75.0	1.8 ± 1.7	2.3 ± 1.5	71.0	4.7 ± 7.1	6.5 ± 7.7
<i>Pseudoterranova</i> sp.	LIII	Me, Mu	13.0	0.1 ± 0.4	1 ± –	33.0	0.5 ± 0.9	1.5 ± 0.8	6.0	0.1 ± 0.5	2 ± –	14.0	0.2 ± 0.5	1.3 ± 0.6
<i>Anisakis</i> sp.	LIII	Me, Mu	75.0	2.4 ± 2.7	3.2 ± 2.6	56.0	2.1 ± 3.2	3.8 ± 3.6	88	8.0 ± 9.3	9.1 ± 9.4	91.0	14.2 ± 14.5	15.7 ± 14.4
Anisakidae gen. et sp.	A	I	13.0	0.3 ± 0.7	2 ± –	0.0	0.0	–	6.0	0.1 ± 0.3	1 ± –	0.0	0.0	–
<i>Dichelyle</i> sp.	A	S	0.0	0.0	–	0.0	0.0	–	25.0	0.4 ± 0.7	1.5 ± 0.6	19.0	0.7 ± 1.8	3.8 ± 2.8
<i>Ascaropsis</i> sp.														
Acanthocephala	C	Me	50.0	1 ± 1.4	2.0 ± 1.4	39.0	1.3 ± 2.1	3.4 ± 1.9	69.0	2.4 ± 3.4	3.5 ± 3.7	38.0	0.8 ± 1.6	2.1 ± 2.1
<i>Corynosoma</i> sp.														
Ectoparasites														
Monogenea	A	G	50.0	1.8 ± 2.2	3.5 ± 1.7	50.0	2.7 ± 3.5	5.3 ± 3.2	63.0	2.0 ± 2.6	3.2 ± 2.7	71.0	4.4 ± 5.6	6.1 ± 5.8
<i>Mitrocoryle</i> sp.														
Copepoda	A	G	75.0	10.4 ± 14.3	13.8 ± 15.1	39.0	4.5 ± 8.3	11.6 ± 10.1	44.0	3.8 ± 7.7	8.7 ± 9.9	52.0	6.3 ± 10.7	12.0 ± 12.3
<i>Clavellotis sebastidis</i>														
Isopoda	Pla	BS, F	25.0	0.4 ± 0.7	1.5 ± 0.7	0.0	0.0	–	0.0	0.0	–	5.0	0 ± 0.2	1 ± –
Gnathiidae gen. et sp.														

Stage (ST): A = adult, M = metacercaria, Pl = plerocercoid, LIII = larvae III, C = cystacanth, Pla = pranzia larva; Location (L): G = gills, BS = body surface, E = eyes, I = intestine, Me = mesenteries, Mu = muscle, F = fins,

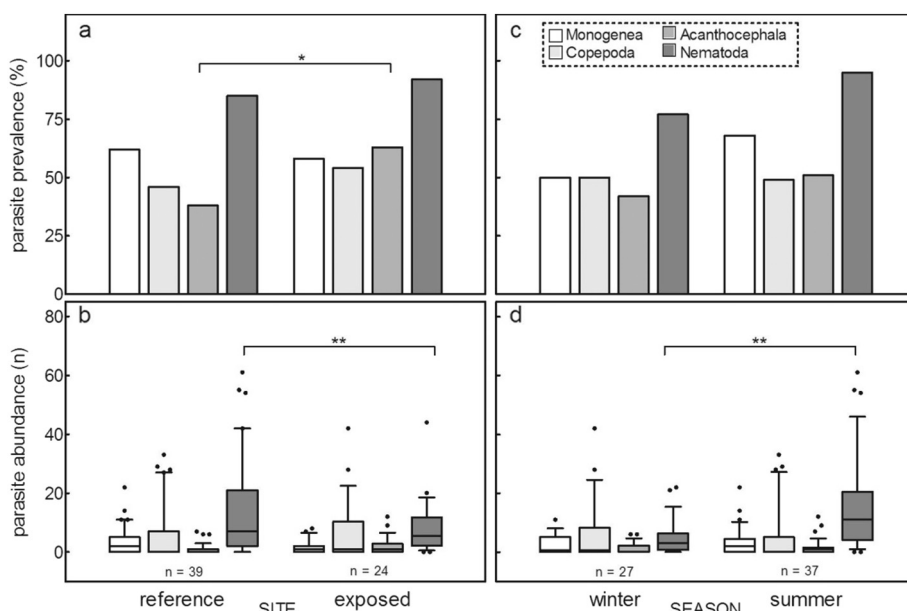


Fig. 1. Effects of site (left panels) and season (right) on the prevalence (top row) and abundance (bottom row) of the four parasitic groups with highest prevalences found in rockfish *Sebastes oculatus*. Significant effects from Chi-Square (prevalence) and Mann-Whitney (abundance) tests are indicated by asterisks (* = $p < 0.01$, ** = $p < 0.001$). In bottom panels, boxplots depict medians (horizontal lines inside boxes), 25 and 75 percentiles (edges of boxes), 10 and 90 percentiles (whiskers), and outlying points included in the analyses (dots). Sample sizes (n) for each group are shown in bottom panels.

Table 2

Generalized linear models (negative binomial distribution and log link) for mean abundances of parasite taxa of rockfish *Sebastes oculatus* sampled from reference and pollution-exposed sites during winter and summer seasons. The models take into account fish total length (TL) as an index of age. Significant effects are indicated in bold.

Effect	Monogenea		Copepoda		Acanthocephala		Nematoda	
	Z value	p	Z value	p	Z value	p	Z value	p
Site	0.522	0.602	0.370	0.711	1.045	0.296	3.986	<0.001
Season	0.157	0.876	0.972	0.331	1.235	0.217	4.736	<0.001
Site x Season	0.608	0.543	0.762	0.446	-1.752	0.080	0.492	0.622
TL	0.626	0.532	1.328	0.184	2.926	0.004	5.916	<0.001

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