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Immunological and health-state parameters in the Patagonian rockfish *Sebastes oculatus*. Their relation to chemical stressors and seasonal changes

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ABSTRACT

We present the results of a field study that evaluates whether exposure to anthropogenic pollution impacts immunological and health-state parameters of wild marine fish during the breeding and non-breeding periods. We assessed aspects of innate immunity (bactericidal capacity, bacterial agglutination, and leukocyte profile) and general health-related parameters (neutrophil to lymphocyte ratio, hematocrit, and condition factor) in the Patagonian rockfish (*Sebastes oculatus*) sampled from polluted (exposed) and reference (control) sites during winter (i.e., coolest temperatures and active reproductive period) and in summer (i.e., warmest temperatures and non-reproductive period). Results showed lower bactericidal competence, hematocrit, and condition factor in fish from exposed sites independently of season, whereas lymphocytes were higher and monocytes lower at the exposed site only during summer. Moreover, fish sampled during winter displayed lower bactericidal competence, hematocrit, and condition factor than those sampled in summer independently of site, whereas the opposite pattern was found for bacterial agglutination. These results could be explained by life-history theory, which predicts a re-allocation of resources between reproduction and other physiological functions (including immunity) during the most energetically demanding season. The present results show an alteration in immunological and health-state parameters of wild marine fish exposed to anthropogenic pollution independently of season, which could potentially result in higher susceptibility to disease and in turn population decline.

1. Introduction

Anthropogenic pollution is currently widely recognized as one of the most serious threats to biodiversity [1,2]. Given that human populations are concentrated along coasts, marine ecosystems and especially coastal areas are repositories for enormous amounts of industrial and domestic waste, making these ecosystems some of the most impacted and altered worldwide [3,4]. It has thus become increasingly essential to understand and monitor the effects of aquatic pollutants on wildlife. Considerable scientific evidence indicates that a multitude of environmental contaminants can impair the health and fitness of individuals from many taxa [5], increasing the potential to seriously impact natural populations [6].

Fish inhabiting urban and industrialized coastal areas are often exposed to high levels of complex mixes of anthropogenic pollutants from early stages of development through to adulthood [7]. This chronic exposure to contaminants in the water can lead to decreased disease resistance [8], with immunosuppression hypothesized as the main mechanism by which toxicants mediate this decline [9] Exposure to contaminants has been shown to significantly alter immune parameters of a range of fish species [8,10–12]. However, most of these findings come from studies conducted under laboratory conditions, using model or commercial fish species, and in the Northern Hemisphere (Reviewed in, [13,14,8]). As such, knowledge on immunotoxicity and related health effects caused by environmental exposure to pollutants on free-living fish under natural conditions remains scarce, especially in southern latitudes. This knowledge can be particularly valuable when polluted areas include, or are located nearby, conservation priority

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sites, as is the case of the Península Valdés region in Patagonia, Argentina.

Peninsula Valdes was designated an UNESCO Natural World Heritage Site in 1999 because it encloses important natural habitats for the *in situ* conservation of several threatened species of outstanding universal value, and globally important concentration of breeding sites for marine birds and mammals. South of the Peninsula, in Nuevo Gulf, areas around Puerto Madryn (the only coastal city in this region), are known repositories of industrial sewage. Recent studies in the area have documented the presence of anthropogenic contaminants and the occurrence of morphological and physiological alterations in several invertebrate marine species [15–18]. Nevertheless, the health status and potential immunotoxic effects on fish (or any marine vertebrate) inhabiting this region remains unstudied, highlighting the need to address this environmental concern that could be having larger detrimental consequences on local wildlife than currently known.

In addition to chemical stressors, seasonality and its associated changes (e.g., photoperiod, ambient temperature, food availability), can modulate the entire physiology of organisms, including their immune systems [19–21]. Furthermore, the intensity of negative effects of pollutants on immune function has the potential to vary seasonally as a result of changes in other costly physiological functions (e.g., thermoregulation, reproduction) that individuals undergo during their annual cycles. For instance, chemical exposure and temperature are known to interact to alter immune responses in various fish species [22]. Thus, the possible effects of seasonality should be taken into account in ecoimmunotoxicological studies.

In this context, the primary aims of our field study were to determine whether exposure to anthropogenic pollution impacts immunological and health-state parameters of wild marine fish in the Peninsula Valdes region and whether the marked seasonality at this high latitude site influences the potential impact of contaminants. As our focal species we selected the scorpaenid rockfish *Sebastes oculatus,* a long-lived demersal predator associated with shallow and rocky substrates that displays high site fidelity [23]. Because of their longevity and high position in the benthic food web, their probability of exposure to persistent bioaccumulative contaminants is high. In addition, it is one of the most abundant and widespread fish species inhabiting the Northern Patagonian gulfs of Argentina [24], where it is considered a significant piece of coastal recreational [25] and off-shore commercial trawl fisheries [26]. This viviparous species shows marked seasonality in its reproductive function, producing only one brood per year during early winter to late spring (June–November), followed by a recovery period during summer and fall (December–May) [27,28].

In order to achieve our objectives we sampled fish from polluted and reference (control) sites during winter (i.e., coolest water temperatures and active reproductive period) and in summer (i.e., warmest water temperatures and non-reproductive period). We assessed aspects of constitutive innate immunity of individuals, which provides the first line of defense against invading microorganisms [29,30]: the leukocyte profile, bactericidal capacity of plasma and levels of natural antibodies in plasma. The leukocyte profile provides insights regarding the major cellular components of the immune system [31], including those involved in innate (e.g., neutrophils, monocytes) as well as acquired (i.e., lymphocytes) immunity. Bactericidal capacity of plasma provides a measure of the integrated bacterial-killing ability of plasma proteins, including complement and lysozyme among others [32]. Bacterial agglutination ability is mediated by plasma agglutinins such as natural antibodies and lectins. Natural antibodies can recognize a broad array of pathogens and promote their opsonization (marking for destruction) and phagocytosis [33]. In addition, as general health-related parameters, we measured hematocrit and calculated the neutrophil to lymphocyte (N:L) ratio and a body condition factor. Hematocrit constitutes a physiological index of condition [34] and provides an estimate of aerobic capacity [31], the N:L ratio is considered a reliable index of stress in vertebrates [35,36], whereas the body condition factor is considered a global indicator of fish health status [37].

We predicted that individuals from sites exposed to pollution would show (1) an altered leukocyte profile, (2) lower bactericidal and bacterial agglutination capacity of plasma, (3) lower hematocrit, (4) higher N:L ratio, and/or (5) lower body condition factor compared to individuals from reference sites. Finally, we predicted that any effects of pollution on the measured parameters would be exacerbated during winter, given that it is likely the most energy-demanding season for this species.

2. Materials and methods

2.1. Study sites

The sampled sites were the area around the two piers of Puerto Madryn city (42.45°S; 65.02°W), as the site exposed to anthropogenic pollution (EXP; Fig. 1), and three reference (REF) sites, Punta Este (42.47°S −64.56°W), Cerro Avanzado (42.50ºS; 64.52ºW; only sampled in winter), and Bañuls (42.39°S −64.59°W; only sampled in summer). Puerto Madryn is the only coastal city located in Nuevo gulf and is under rapid population growth and industrial development [38]. Tourism, fisheries, and aluminum production are the most important economic activities resulting in important maritime traffic. The main wastewater discharges into the gulf occur in the area of piers and derive from fishery plant effluents, from the aluminum plant through atmospheric deposition, and from maritime operations including crude oil transport and loading, unloading of petroleum-derived products, fishing operations, bilge waste, and ballast water discharges from ships [38]. The presence of heavy metals [39], hydrocarbons [40,41], and other toxic substances such as endocrine disruptors [16] has been registered in the area around the piers. On the other hand, reference sites do not receive urban, industrial, or other wastes and previous studies suggest they can be considered clean waters [16,18,42].

2.2. Fish sampling, blood collection, and processing

Rockfish (150–328 mm) (N = 74) were captured by spearfishing during the winter (July 2013, at sea temperatures of 9 °C) and late summer (March–April 2014, at 18 °C) from the study sites at Nuevo Gulf. After removing fish from the water, they were anesthetized with benzocaine [43] and blood samples (0.5–2 ml) were drawn from the caudal vein by means of heparinized sterile plastic syringes. One drop of blood was immediately used to prepare a thin blood smear for differential leukocyte counts and a microcapillary tube was filled for hematocrit measurement. The remaining blood was transferred to a sterile tube and kept on ice until arrival at the laboratory. Anesthetized fish were also placed on ice and transported to the laboratory. Once at the laboratory, plasma was separated by centrifugation (1500 g for 5 min) and stored at −80 °C until analyses. Fish were sacrificed and their weight (g) and total length (mm) recorded. All individuals were sexed by internal examination of the gonads, which at that time were removed and weighted (g).

2.3. Immunological measurements

2.3.1. Leukocyte differential counts

Thin blood smears were prepared with a drop of fresh blood, air-

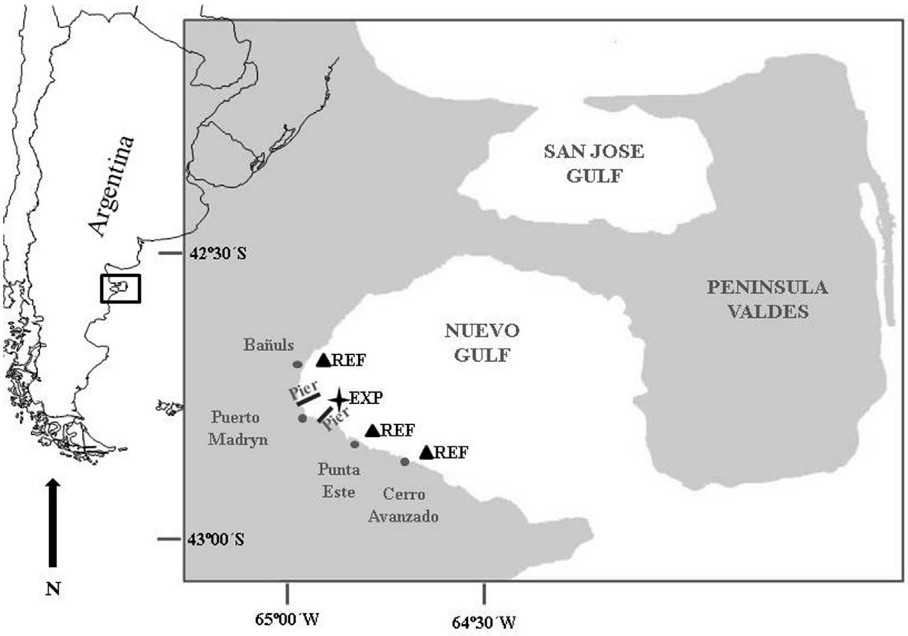

Fig. 1. Map of the study area and sampling sites. EXP: pollution exposed site, REF: reference sites.

dried, fixed with ethanol for 3 min, and stained with Giemsa (Tinción 15, Biopur). Then the relative abundances of the main white blood cell types (i.e., lymphocytes, neutrophils, and monocytes) were estimated from blood smears by classifying the first 200 leukocytes encountered under 1000 × magnification using a compound microscope as described in Palacios et al. [44]. Because there is no available description on white blood cells for *S. oculatus* we identified these cell types on the basis of morphology following published leukocyte descriptions for other fish species [45,46].

2.3.2. Plasma bactericidal capacity

Bacterial killing capacity of fish plasma was determined according to Matson et al. [32], with a few modifications for use in rockfish. Briefly, *Escherichia coli* (ATCC 8739) were, suspended in sterile phosphate-buffered saline (PBS) to produce a working solution containing approximately 200 colony-forming bacteria per 10 μl. All plasma samples were diluted 1:10 with sterile PBS, and sample reactions were prepared by adding 10 μl of the bacterial working solution to 90 μl of the diluted plasma samples. All sample reactions were incubated for 4 hs at 18 °C to provide adequate time for bacterial killing to occur. Three control reactions were prepared by adding 10 μl of the bacterial working solution to 90 μl PBS, and were plated before, midway, and after plating the sample reactions. All sample reactions and controls were plated in duplicate using 50 μl aliquots on 4% tryptic soy agar and incubated overnight at room temperature (~25 °C). The number of bacterial colonies on each plate was then counted, and the percentage of colonies on each plate per the mean number of colonies in control plates was calculated. This percentage was subtracted from 100 to obtain the percentage of bacteria killed.

2.3.3. Bacterial agglutination by plasma

Bacterial agglutination was determined using a plate agglutination technique described previously for other fish species [47,48]. Briefly, bacteria (*E. coli* - ATCC 8739) were grown in sterile tryptic soy (TS) broth and then fixed in 1% formalin overnight at 4 °C. Fixed bacteria were washed three times with PBS and adjusted to a concentration of approx. 1×10^9 bacteria/ml. Plasma samples (20 μl) were added to the first column of a 96-well plate and serially twofold diluted along the rows with PBS. A negative control (PBS instead of plasma) was included in each plate. Then 20 μl of fixed bacteria were added to all wells. Plates were vortexed and incubated at ambient temperature overnight. Agglutination titers were determined as −log2 of the highest dilution showing bacterial agglutination.

2.4. Additional health-related parameters

Hematocrit (i.e., the proportion of red blood cells in whole blood) was determined after centrifuging whole blood in heparinized glass capillary tubes for 5 min. The proportion of neutrophils and lymphocytes obtained through the differential count was used to calculate the N:L ratio for each individual. Finally, the condition factor (CF) was calculated using the equation $CF = (100 \times W)/L^3$, where W is the weight without gonads of the fish in grams (obtained by subtraction of the gonadal weight from the total weight) and L is the total length of the fish in centimeters [49]. The condition factor assumes that the heavier the fish in relation to its length, the better its condition.

2.5. Statistical analysis

The lack of fish at the Cerro Avanzado site (REF site) during summer, likely due to movement of fish to a deeper rocky reef (inaccessible for spear fishers) looking for lower temperatures, forced us to find an alternative sampling reference site (Bañuls) in order to maintain two reference sites per season. To be able to compare data from reference and exposed sites between seasons, we pooled data from both reference sites for each season (i.e., Punta Este with Cerro Avanzado in winter and Punta Este with Bañuls in summer). This was done after having determined that none of the parameters differed significantly between the reference sites we wanted to pool. After this procedure, all immune and health-related endpoints were examined using a two-way analysis of covariance (ANCOVA) that included the fixed effects of site (EXP and REF) and season (winter and summer), their possible interaction, and total length (LT) as a co-variable that is an index of age of the individuals. Variables and model residuals were examined for normality and homoscedasticity. Bacterial agglutination titer, hematocrit, and condition factor met the assumptions for parametric tests. Bactericidal capacity had to be arcsine square-root transformed to meet the assumptions. Lymphocytes, neutrophils, monocytes, and N:L ratio did not conform to the assumptions even after transformation (highly skewed variables) and were therefore analyzed using non-parametric Mann—Whitney tests and site and season were analysed separately. P-values below 0.05 were considered significant in all analyses. Sample sizes differ among variables because not all measurements could be obtained for some individuals. Statistical testing was performed using the software SPSS 15.0.

3. Results

Immunological and health-related parameters of fish from exposed and reference sites in summer and winter seasons are summarized in Table I (supplementary material).

3.1. Immunological parameters

Lymphocytes were the most common white blood cell type followed by neutrophils and monocytes. Basophils and eosinophils were not detected in the sample. Fish from the exposed site showed higher relative abundance of lymphocytes and lower abundances of monocytes than fish from reference sites in summer, whereas no difference between reference and exposed sites was observed in winter (Table 1; Fig. 2A and C). The relative abundance of neutrophils did not vary between sites and seasons (Table 1, Fig. 2B).

Bactericidal capacity, assessed as the percentage of *E. coli* killed by plasma, showed significant effects of site and season (Table 1, Fig. 3A). Fish from reference sites displayed, on average, stronger bactericidal capacity than fish from the exposed site. Fish sampled during the summer season showed, on average, higher bactericidal capacity than fish sampled in winter. Bacterial agglutination by plasma showed an effect of season but no effect of sampling site (Table 1, Fig. 3B). Contrary to the pattern observed for bactericidal capacity, bacterial agglutination was higher in fish sampled during winter. In addition, there was a weak but significant positive effect of total body length on bacterial agglutination titer (Table 1, Fig 3C). Finally, it is worth noting that although the interaction terms between site and season were not significant in the models for bactericidal capacity and bacterial agglutination (Table 1), a trend towards a larger difference between sites during summer than in winter can be observed for bactericidal capacity in Fig. 3A.

3.2. Additional health-related parameters

The effects of site and season on hematocrit were statistically significant (Table 1), with fish from the exposed site showing lower values than fish from reference sites and fish sampled in winter showing lower values than those sampled during summer (Fig. 4A). The N:L ratio showed significant differences between sites only during summer, being lower in fish from exposed sites (Table 1; Fig. 4B). Regarding the condition factor, fish from the exposed site showed lower values than fish from reference sites, whereas contrary to the pattern for hematocrit, values were higher in winter than in summer (Table 1, Fig. 4C). None of the health-related parameters was affected by total length of the individuals (Table 1). Similarly to the case of bactericidal capacity, despite the non-significance of the interaction term, a trend towards a larger difference between sites during summer than in winter can be observed for condition factor in Fig. 4C.

Table 1
Statistical models for immunological and health-state parameters of fish from exposed and reference sites sampled during winter and summer.

Parameter	Analysis	Test	P
Lym	Mann—Whitney U-test	site	
		winter U = 114.5, df = 1	0.835
		summer U = 65.5, df = 1	**0.009**
	Mann—Whitney U-test	season	
		reference U = 206.5 df = 1	0.428
		exposed U = 50.0, df = 1	0.259
Neu	Mann—Whitney U-test	site	
		winter U = 115,5, df = 1	0.864
		summer U = 94.0, df=	0.105
	Mann—Whitney U-test	season	
		reference U = 213.0, df = 1	0.522
		exposed U = 53.5, df = 1	0.329
Mon	Mann—Whitney U-test	site	
		winter U = 109,5, df = 1	0.688
		summer U = 68.5, df=	**0.011**
	Mann—Whitney U-test	season	
		reference U = 211.0, df = 1	0.491
		exposed U = 50.0, df = 1	0.226
BC	2-way ANCOVA	site $F_{1,63} = 4.24$	**0.044**
		season $F_{1,63} = 10.33$	**0.002**
		site x season $F_{1,63} = 1.07$	0.304
		covariate length $F_{1,63} = 0.99$	0.324
BA	2-way ANCOVA	site $F_{1,71} = 0.33$	0.856
		season $F_{1,71} = 10.21$	**0.002**
		site × season $F_{1,71} = 0.85$	0.772
		covariate length $F_{1,71} = 10.76$	**0.002**
Htc	2-way ANCOVA	site $F_{1,61} = 8.47$	**0.005**
		season $F_{1,61} = 6.78$	**0.012**
		site × season $F_{1,61} = 0.00$	0.991
		covariate length $F_{1,61} = 1.45$	0.234
N:L	Mann—Whitney U-test	site	
		winter U = 120, df = 1	1.000
		summer U 84 = , df = 1	**0.05**
	Mann—Whitney U-test	season	
		reference U = 210.9, df = 1	0.479
		exposed U = 52.0, df=	0.291
CF	2-way ANCOVA	site $F_{1,75} = 5.97$	**0.017**
		season $F_{1,75} = 8.87$	**0.004**
		site × season $F_{1,75} = 1.21$	0.275
		covariate length $F_{1,75} = 1.32$	0.254

Note. Bactericidal capacity (BC) was arcsine square-root transformed before analysis. BA, bacterial agglutination; Lym, lymphocytes; Neu, neutrophils; Mon, monocytes; N:L neutrophil to lymphocyte ratio; Htc, hematocrit; CF, condition factor; LT, total length.

4. Discussion

In the present study we investigated the effects of inhabiting sites exposed to anthropogenic pollution on immunological and health-state parameters of wild marine fish in the Peninsula Valdes region of Argentina and whether the marked seasonal changes at this high latitude region can exacerbate these effects. Several of the parameters evaluated showed sensitivity to anthropogenic pollution and/or to seasonal changes. On the other hand, we found only weak support for an interaction between pollution-exposure and seasonality, which was interestingly in the opposite direction to that predicted. Below we discuss in more detail the main findings of this study.

S. oculatus from the exposed site showed lower bactericidal capacity, percentage of monocytes, hematocrit, N:L ratio, and condition factor, but higher percentage of lymphocytes than fish from reference sites. Together these results suggest that fish living at the polluted site have altered immune function and health that could result in a lower ability to fight infection and, more generally, to survive natural environmental challenges (e.g., pathogens, predators, food scarcity).

Fish from the polluted site showed relative lymphocytosis (high

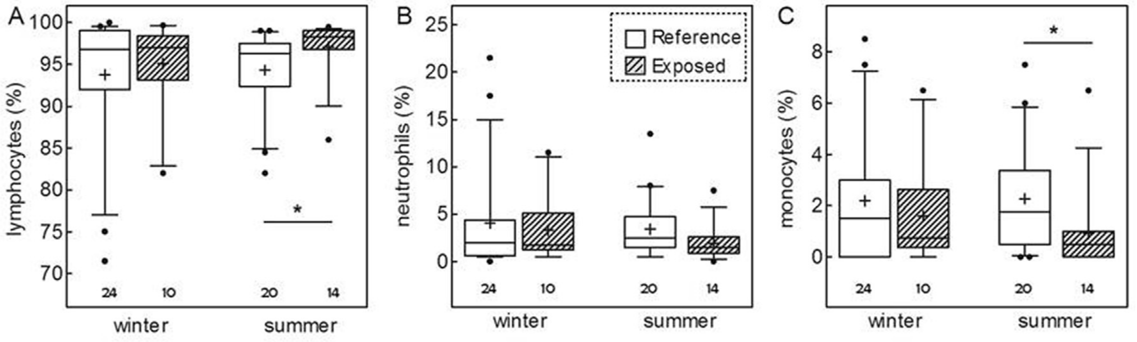

Fig. 2. Box plots of white blood cell counts of the rockfish *Sebastes oculatus* showing effects of site within each season. Significant effects from models in Table 1 are indicated by asterisks (* = $p < 0.05$, ** = $p < 0.01$). Boxplots depict medians (horizontal lines inside boxes), means (crosses), 25 and 75 percentiles (edges of boxes), 10 and 90 percentiles (whiskers), and outlying points included in the analyses (dots).

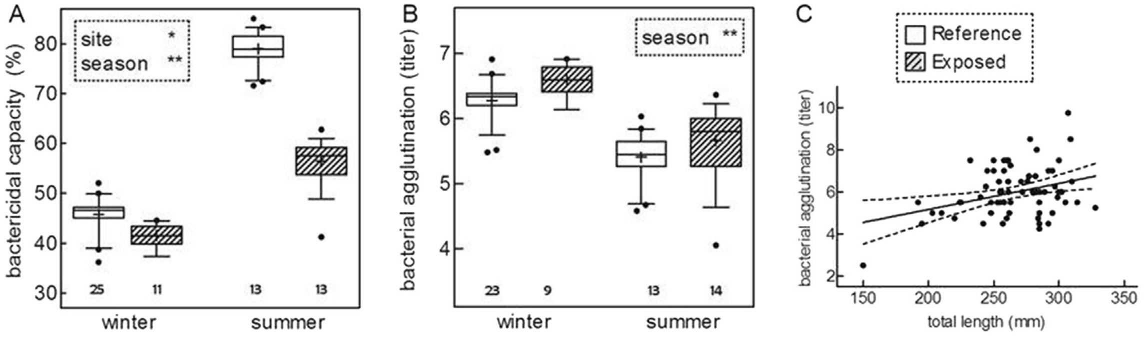

Fig. 3. A,B) Box plots of immune parameters of the rockfish *Sebastes oculatus* showing effects of site, season, and/or their interaction as predicted by models in Table 1. Significance level: * = $p < 0.05$, ** = $p < 0.01$. Boxplots depict medians (horizontal lines inside boxes), means (crosses), 25 and 75 percentiles (edges of boxes), 10 and 90 percentiles (whiskers), and outlying points included in the analyses (dots). C) Partial regression plot between bacterial agglutination titer and total body length.

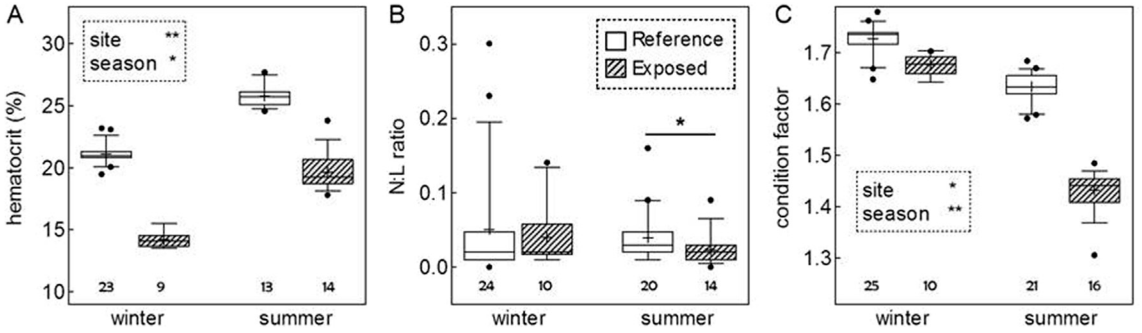

Fig. 4. Box plots of health-related parameters of the rockfish *Sebastes oculatus* showing effects of site, season, and/or their interaction as predicted by models in Table 1. Significance level: * = $p < 0.05$, ** = $p < 0.01$. Boxplots depict medians (horizontal lines inside boxes), means (crosses), 25 and 75 percentiles (edges of boxes), 10 and 90 percentiles (whiskers), and outlying points included in the analyses (dots).

lymphocyte percentages) and monocytopenia (low monocyte percentages) compared to fish from reference sites. Other studies in fish exposed to pollutants have reported lymphopenia and monocytopenia [50,51] or, conversely, lymphocytosis and monocytosis [10,52–56]. Lymphocytes play an important role in adaptive humoral and cell-mediated immunity in fish, while monocytes play important roles in the inflammatory response, which is an essential part of the innate response to pathogens. Increased numbers of lymphocytes combined with decreased number of monocytes, as found in our study, could be indicative of an ongoing infection, metal-induced cell or tissue damage leading to abnormal hematopoiesis, and/or direct stimulation of immune defenses by the presence of pollutants [35]. Any of these alterations could impact negatively on fish survival. We are currently investigating the potential relationship between pollution exposure and parasitic infection in this system.

In agreement with our prediction, bactericidal capacity of *S. oculatus* exposed to anthropogenic pollution was lower than that displayed by fish from reference sites. This suggests a lower capacity of fish exposed to pollution to limit bacterial infections. A few other field studies have also shown reduced bactericidal capacity in feral fish from polluted compared to non-polluted sites [52,57]. In addition, it has been experimentally demonstrated that bactericidal capacity is negatively impacted by anthropogenic chemicals [58–60]. On the other hand, bacterial agglutination by natural antibodies and other agglutinins present in plasma, which provide constitutive protection against pathogens of a broad specificity [61], did not differ between sites. This parameter of wild *S. oculatus* might not be sensitive to anthropogenic contaminant exposure or might need higher levels of pollution to be affected. For instance, Plumb and Areechon [47] and Prabakaran et al. [62] have found that high concentrations of pollutants can suppress this humoral immune response of fish in experimental conditions whereas lower concentrations of the same pollutants have no effect. Bacterial agglutination was the only parameter showing a significant correlation with body length, probably reflecting the ontogeny of this immune component [44,63].

The lowest values of hematocrit and condition factor were measured, as predicted, in fish exposed to anthropogenic pollution. The condition factor is considered a proxy of fish health status, based on the general assumption that, for a given length, heaviest fish are in better condition [64]. In accordance with our results, both field and experimental studies have shown that fish from clean waters display higher condition factor compared to fish from polluted waters [51,65–67] suggesting that this parameter can provide information on potential pollution impacts, especially when measured together with other health-related parameters. Hematocrit, on the other hand, provides an index of the O_2 carrying capacity of blood. Low hematocrit might indicate fish from exposed sites are suffering anemic conditions, which could affect fish motility and ability to hunt or escape predators among other metabolically demanding activities. Anemia could be occurring either by inhibition of erythropoiesis or an increased frequency of erythrocyte death [68]. Also, indirectly, the reduction of hematocrit could be attributed to effects of pollutants on fish growth and food utilization processes [69]. Regarding N:L, we found a pattern contrary to that predicted based on the idea that this ratio serves as a stress index. Fish from exposed sites showed lower rather than higher ratios than those from reference sites. This could be interpreted as exposed fish being less stressed. However, it is worth noting that the decrease in this ratio was caused by an elevated lymphocyte percentage in the exposed site, which, as discussed above, could be caused by an ongoing infection. Distinguishing an alteration in the N:L ratio caused by stress from one caused by infection is a challenge [35]; thus interpretation must be made in context with other parameters measured. In our case, the lower hematocrit and body condition of fish from the polluted site is more in line with interpretation of the low N:L ratio as result of an ongoing infection than of low stress. Future research will benefit from the inclusion of alternative indices of stress such as levels of stress hormones and oxidative stress.

Reproductive cycle of rockfish was taken into account in the present study. This approach is very common in fish ecology, but is still rare in ecotoxicology. We observed that fish sampled during their active reproductive season (i.e., winter) displayed lower bactericidal capacity and hematocrit than those sampled during the non-reproductive season (i.e., summer) independently of site, whereas the opposite pattern was found for bacterial agglutination and condition factor. These results could be explained by life-history theory, which predicts a re-allocation of resources between reproduction and other physiological functions (including immunity) during the most energetically demanding season. Additionally, fish immune responses can also be modulated by a range of environmental conditions that vary with season, such as temperature and photoperiod [20]. In our study area, temperature and photoperiod both decrease during the reproductive season, thus they could also account for the observed decrease in bactericidal capacity and hematocrit in rockfish during breeding, since lower temperatures and photoperiod can negatively affect these parameters [19,20]. An experimental approach would help distinguish between these two non-mutually exclusive hypotheses.

Non-parametric tests by season showed that the effects of site on lymphocytes, monocytes, and the N:L ratio were significant only in summer. Furthermore, whereas ANCOVAs did not show a significant interaction between site and season (perhaps due to insufficient statistical power) graphs of bactericidal activity and condition factor showed a trend towards a greater magnitude in the difference between sites in summer than winter. Thus, there is some evidence suggesting that effects of site were more pronounced during summer than winter, which is contrary to our prediction. At present we do not have an explanation for these trends. Further research is needed to elucidate potential effects of seasonality in this system and their underlying causes.

5. Conclusions

Our results indicate an alteration in the immune and health parameters of wild rockfish associated with living exposed to anthropogenic pollution. This could potentially result in higher susceptibility to disease and in turn population decline. Thus these findings underscore the need to continue and deepen, through field and experimental studies, our understanding of the effects of anthropogenic pollutants on immune and health parameters in wild species of this unique Natural World Heritage Site; expanding the questions to other physiological parameters (e.g., adaptive immunity, stress hormones, oxidative stress), to other key ecological vertebrate species in the area, and from an organismal to a population approach.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.fsi.2015.11.021.

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