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Impaired Health in Flounder, *Paralichthys* spp. Inhabiting Coastal Chile

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Prolonged exposure of fish to a variety of pollutants can induce stress (Adams 1990). These include municipal effluent, PCBs, pesticides, polycyclic aromatic hydrocarbons (PAHs), heavy metals in mine tailings, effluent from pulp and paper mills, etc. Manifestations of stress, which might culminate in a change of homeostasis, include reduced feed intake and impairment of growth and reproductive potential accompanied by immunosuppression. All of these factors could influence survival. Biomonitoring methods have been devised to assess the impact of pollution on fish health by comparing potentially contaminated and reference samples of the same species. These include body and organ indices, histopathological and hematological methods and the prevalence, abundance and diversity of parasites (Adams 1990; Khan and Thulin 1991; Overstreet 1993).

Several contaminants of industrial and municipal origin are discharged without treatment along the coastline of central Chile (Ahumada 1992, Carrasco and Gallardo 1994; Ahumada et al. 1983). A study conducted with sediment originating from Concepción Bay revealed lesions in two species of flatfish, *Paralichthys* species (Leonardi and Tarifeño 1996). This embayment is a repository for effluent originating from two municipalities, a fish meal plant plus petroleum hydrocarbons of unknown origin (Ahumada 1992). Decomposition of organic matter contributes to an anoxia of the bottom waters. A second embayment, San Vicente, receives from a steel mill effluent which includes several heavy metals and also petroleum hydrocarbons from an oil refinery (Ahumada 1992, 1994; Larrain et al. 1998). Further southwards, untreated pulp and paper mill effluent is discharged into the Gulf of Arauco. All three areas provide habitats for several commercial fish species. Rudolph and Rudolph (1999) reported recently elevated levels of a detoxicating enzyme, benzo(a) pyrene hydroxylase, in the liver of a flounder, *Paralichthys microps* (Gunther 1881) taken from Concepción Bay and attributed these to PAHs. In view of this and other reports of pollutants occurring along the coastline of Chile, the present study was initiated to ascertain if some of the above-mentioned biomarkers could detect and priorise differences in pollution effects in two sympatric species of flounder, *P. microps* and *P. adspersus* (Steindachner 1867) originating from Concepción and San Vicente Bays and the Gulf of Aranco. Reference samples were obtained from an area external to Concepción Bay. The results of this study are reported herein.

MATERIALS AND METHODS

Study area: This area is located off the coast of central Chile in the south Pacific Ocean (Fig. I). Concepción Bay is shallow (~45m in depth) with three ports receiving discharges from two municipalities and several industries. Penco, a site

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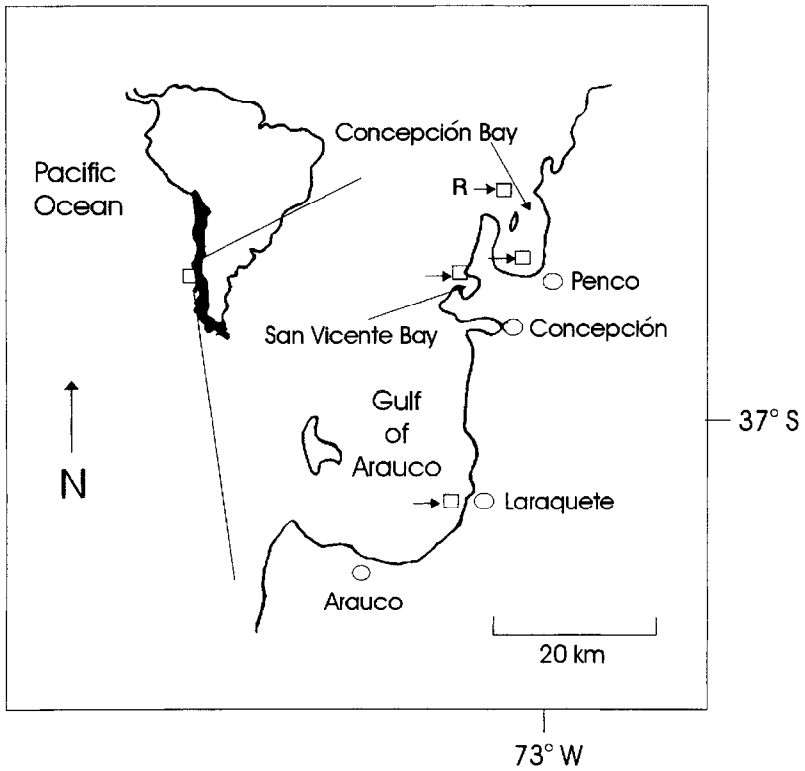

Figure 1. Locations (arrows) where flounder were sampled off the coast of Chile. R is the reference site.

where fish samples were obtained, is contaminated with organic matter originating from a fish meal plant, sewage and unknown sources of petroleum hydrocarbons. Oxygen deficiency occurs in the water column during summer and water temperatures vary from 8- 17°C throughout the year (Ahumada 1992; Carrasco and Gallardo 1994). San Vicente Bay, with a similar depth and water temperature but considerably smaller, receives discharges predominantly from a steel mill, small quantities of domestic effluent and petroleum hydrocarbons via a river. A gyre of water from the continental shelf flushes the bay daily in contrast to the three-day flushing which occurs in Concepción Bay. Laraquete, in the Gulf of Arauco, a third location which is confluent with the Pacific Ocean, receives effluent from the municipality but most important is the discharge from a chlorine-bleaching pulp and paper mill (BKME) and it can be observed as a brownish-turbid surface layer for at least three km along the shoreline (Khan, unpubl. data). Since operations expanded in the 1990s, groundfish catches have been reported to decrease considerably in the southwestern coastal area. Sediment in the area consists of a dark, muddy texture containing bark and decomposing wood derivatives.

Fish sampling: Flounder, *Pardichthys* spp. were captured primarily by gillnet and less often by otter trawl (only San Vicente Bay) at depths of 8- 15 m at Penco, San Vicente Bay and Laraquete during spring in November and December, 1998. A fourth site was considered a reference area because of its location just external to Concepción Bay. The fish were bled from the caudal artery after capture for determination of hemoglobin, total plasma protein and lymphocytic levels (no./1000 erythrocytes) and prevalence of a blood protozoan from Giemsa-stained blood smears (see Khan 1987).

Mean abundance of the parasite was estimated by means of a hemocytometer. Total length, eviscerated body and organ weights were recorded from each fish. Tissues, including gill, liver, spleen, kidney and gonad, were fixed in 10% formalin, processed by conventional histological methods and sections stained with hematoxylin and eosin. Additional cross sections of liver were stained with Sudan black B for lipid and spleen with Perl's Prussian blue for the presence of hemosiderin which was estimated by digital image analysis (Khan and Nag 1993). Identification of the different stages of follicular maturation in the ovary was based on the study by Tanssen et al. (1995). Since inadequate numbers of each species of flounder were captured for examination in San Vicente Bay and the reference site coupled with their similarities in feeding, growth, reproduction and response to contaminated sediment (see Leonardi and Tarifeño 1996), variables of the two species were pooled in the present study. The data, which included k-factor (eviscerated body weight/length^3), organosomatic indices (organ weight/eviscerated body weight) and blood values were compared among the four groups of fish by the ANOVA and subsequently by Tukey's studentized range (HSD) test using the SAS System.

RESULTS AND DISCUSSION

Differences were observed in macroscopic lesions, condition (K) factor, organosomatic indices and blood values among the four groups of fish examined. While external lesions were not apparent in samples from the reference site (CB), the prevalence of fin and epidermal necrosis increased from 42% at San Vicente, to 60% at Penco and 100% from Laraquete. Samples taken from the reference site were significantly greater in length and body weight than from Laraquete while only body weight differed at Penco (Table 1). No differences in length or body weight were observed between the reference site and the San Vicente samples but K-factor was significantly greater at the latter than at other sites. Additionally, K-factor was significantly greater at the reference sites than at Laraquete or Penco. However, hepato-, spleno- and ovariosomatic indices (OSI) were significantly greater in samples at Laraquete and Penco than at the reference site. Higher OSI values were associated with the pre-spawning condition at Laraquete (90% of 20 fish) and Penco (85% of 20 fish) in contrast to the post-spawned states of samples (84% 13 fish) taken at the reference site. Significantly lower hemoglobin, toal serum protein and lymphocyte values were noted in samples from Laraquete, Penco and San Vicente than at the reference site (Table 1). In addition, anisocytosis and the presence of polychromatic erythrocytes were apparent in all samples from Laraquete and Penco whereas only polychromatic erythrocytes were seen in samples originating from San Vicente. None of these anomalies were recorded in fish taken from the reference site.

A blood-inhabiting protozoan, *Cryptobia* sp., was observed in both species of flatfish. Prevalence of infection was 100% at all four sites but the mean abundance was greatest in samples taken from Laraquete with an estimated mean of 500/ml of blood whereas it was low (<50/ml) in fish taken at the other locations.

Pathological lesions were observed in the tissues of the two species of flatfish captured at the four sites in Chile. Anomalies in the gills occurred more often in the sample from Penco than at the other three sites and were least in fish taken outside of Conceptión Bay (Table 2). Hyperplasia was observed primarily in the distal one-third of the secondary lamellae, of which some, from Penco, had fused at the tips. Interlamellar hyperplasia was also noted in samples from three sites, extending up to 25% of the length of the secondary lamellae. Edema, epithelial lifting and telangiectasis were also apparent in some fish. Another prominent feature was the occurrence of mucus cell hyperplasia which was noticed in fish from all four sites

Table 1. Comparison of biological variables of the two species of female flounder, *Paradichthys* spp., sampled at four coastal sites in Chile in 1998

Variable	Sites†			
	CB	L	P	SV
n	12	20	20	12
Length (cm)	35.4±1.4	30.8±0.9*	31.7±0.5	31.5±0.7
Weight (g)	436±66	270±88*	290±12*	386±52
K-Factor ($W/L^3 x10^{-2}$)	1.00±0.03	0.90±0.03*	0.90±0.01*	1.19±0.4‡
HSI ($x10^{-2}$)€	2.21±0.27	3.13±0.16*	3.20±0.19*	2.49±0.25
SSI ($x\ 10^{-4}$)	7.88±0.64	12.0±0.90*	12.77±0.81*	11.17±0.95
OSI ($x10^{-2}$)	3.07±0.65	6.86±0.62*	6.75±0.64*	3.75±1.04
Hemoglobin (g%)	3.72±0.36	2.26±0.22*	2.15±0.26*	2.14±0.27*
Total protein (g%)	14.03±0.77	7.77±0.28*	9.34±0.29*	7.35±0.39*
Lymphocytes/1000 erythrocytes	19.3±1.98	7.15±1.19*	10.7±1.31*	8.08±1.36*

€HSI = hepatic somatic index, SSI=spleen somatic index, OSI=ovarian somatic index
†CB = Concepción Bay, L = Laraquete, P = Penco, SV = San Vicente
‡Significantly different ($P<0.05$) than at other three sites
*$P<0.05$ than at CB

especially in the secondary lamellae and less often in the interlamellar area. Macrophage aggregates, which were more numerous, and less often fibrosis were apparent in the liver of samples from samples in Penco and San Vicente. Fatty degeneration (identified as large vacuoles in hepatocytes and Sudan black B positive for lipid) occurred in fish from all four sites. Moreover, concentration of hemosiderin pigment in the spleen increased progressively from the outer Conceptión Bay sample to significantly greater values than those originating from Laraquete, San Vicente and Penco respectively. Concentration of the pigment was also significantly greater in the kidney of fish taken from the last three aforementioned sties. No anomalies were observed in any of the ovaries sampled in the present study. However, most (75%) of the ovarian samples taken from outer Conceptión Bay were in the post-spawning phase, characterised by post-ovulatory follicles and oocytes in the late perinucleolar stage. In contrast, ovaries of samples originating from the other three sites were either in the advanced vitellogenic stage with a centrally located germinal vesicle in the follicle or in the mature phase, identified by migration of the germinal vesicle from its central location.

Results from the present study suggest that the health of two flounder species, *Paralichthys* spp. off the coast of Chile at Laraquete and Penco was severely impaired but was less pronounced at San Vicente and least at an area external to Conceptión Bay. These findings are based on external macroscopic lesions, condition factor, blood values such as hemoglobin, total serum protein and lymphocytes, as well as pathological changes in the liver, gills, spleen and kidney. Moreover, significantly greater values of HSI, SSI and OSI were probably associated with increased MFO activity, increased hemosiderin deposits in the spleen and delayed spawning respectively. These assumptions are in agreement with a number of studies which have reported that hepatomegaly is related to increase MFO activity following exposure to PAHs and pulp and paper mill effluent (see Jimenez and Stegeman 1990; Munkittrick et al. 1994). Additionally, there is also evidence that prolonged exposure

Table 2. Comparison of the occurrence (%), of pathological lesions in the tissues of two species of female flounder, *Paralichthys* spp., sampled (n = 12/site) at four locations off the coast of Chile in 1998.

Tissue - abnormality	Sites			
	CB*	L	P	SV
Gill				
Secondary lamellar hyperplasia			75	
Fusion of secondary lamellae			50	
Interlamellar hyperplasia		25	25	25
Epithelial lifting			25	
Edema	25	25		50
Telangiectasis		25	25	
Mucus cell hyperplasia - secondary lamellae	50	100	100	100
Mucus cell hyperplasia - interlamellar area		50	25	25
Liver				
Fibrosis			25	25
No. of macrophage aggregates (100x)	2	5	10	8
Fatty degeneration	75	75	75	50
Spleen				
Hemosiderin (%/mm^2)	2.7±0.1‡	7.5±0.2	17.9±0.8‡	11.7±0.5
Kidney				
Hemosiderin (%/mm^2)	1.9±0.1‡	5.6±0.2	4.9±0.2	4.2±0.2

*CB = Concepción Bay, SV = San Vicente, P = Penco, L = Laraquete
‡Significantly different ($P < 0.05$) from other sites

of fish to some contaminants is capable of impairing gonadal development through disruption of estradiol production (Munkittrick et al. 1994). Our results provide additional support for the view that sediment-inhabiting species, such as flatfishes, are useful as bioindicators since they frequent a habitat where some anthropogenic compounds tend to accumulate.

Low blood values such as hemoglobin, total plasma protein and lymphocytes, noted in flounder sampled at the three polluted sites in the present study, might be the result of synergism between the contaminants and the hemoflagellate as no anemia was observed in the infected samples taken at the reference location. Although most species of *Cryptobia* are known to be harmless but one of these, *C. (= Trypanoplasma) bullocki* causes anemia in the flatfish, *Paralichthys dentatus,* and mortality especially in juveniles (Burreson and Zwerner 1984). A study which investigated the interaction of petroleum hydrocarbons and a hemoflagellate, *Trypanosoma murmanensis,* in two species of marine fish, especially a flatfish, *Pleuronectes (Pseudopleuronectes) americanus,* revealed anemia and prolonged patency of the infection in adults (Khan 1987). Moreover, another trypanosome, *T. danilewskyi,* has been reported to inhibit lymphocytic proliferation (see Woo 1987). It is likely, then, that the higher parasitemias in flounder examined at Laraquete, in the present study, might be the result of the combined effects of the pollutant and the parasite on the host's defense mechanism.

Some of the pathological lesions observed in flounder in the present study were comparable to those reported in other species of flatfish following exposure to a variety of pollutants (Haensly et al. 1982; Khan et al. 1994). Haensly et al. (1982) and Khan et al. (1994) also observed gill lesions in flounder following natural exposure to petroleum hydrocarbons and paper mill effluent respectively.

Additionally, hemosiderosis occurs in fish exposed to several pollutants (Khan et al. 1994). However, liver damage in the Chilean samples was minimal compared to fish taken from heavily contaminated habitats (Khan et al. 1994, 1996). It is likely that the sediment in the three embayments in Chile in which the flounder were submerged was not as toxic as in the previously-mentioned habitats or the fish were exposed intermittently. The latter appears to be more likely as flounder, during late spring to autumn, migrate from the bays into deep water to avoid rising temperatures and depletion of oxygen which occurs in the water column especially in Conceptión Bay (Ahumada et al. 1983). In the present study, gonadal maturation was apparently delayed in samples from three impacted sites in contrast to those from outer Conceptión Bay. Pollutants, such as paper mill effluent, have been reported to disrupt gonadal development, affect secondary sexual characters and delay maturity (Munkittrick et al. 1994). Since most of the latter were not observed in the *Paralichthys* spp., it appears that the fish taken from the contaminated areas in Chile were not as severely affected as reported in other regions (Haensly et al. 1982; Khan et al. 1994, 1996).

In conclusion, results from the present study indicate that the health of flounder, sampled from three embayments in Chile, where several anthropogenic compounds of industrial and municipal origin were discharged, was impaired based on macroscopic and microscopic lesions, blood values, condition factor and organosomatic indices in contrast to reference fish. Low lymphocytic values were probably indicative of a compromised immune system and most likely associated with infection by opportunistic bacteria that induced fin necrosis and also the parasitemia of a hemoflagellate infection (see Khan and Thulin 1991). Although the biomarkers used in this study were unable to detect specific responses to any one pollutant, these can serve as a basis for monitoring environmental health along coastal Chile.

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