Astyanax fasciatus AS BIOINDICATOR OF WATER POLLUTION OF RIO DOS SINOS, RS, BRAZIL

SCHULZ, U. H. and MARTINS-JUNIOR, H.

Universidade do Vale do Rio dos Sinos, Unisinos, Centro de Ciências da Saúde, Laboratório de Ecologia de Peixes, C.P. 275, CEP 93022-000, São Leopoldo, RS, Brazil

Correspondence to: Uwe H. Schulz, Universidade do Vale do Rio dos Sinos, Unisinos, Centro de Ciências da Saúde, Laboratório de Ecologia de Peixes, C.P. 275, CEP 93022-000, São Leopoldo, RS, Brazil, e-mail: uwe@cirrus.unisinos.br

(With 1 figure)

ABSTRACT

The effects of an increasing downriver pollution gradient on the reproductive system of *Astyanax fasciatus* were investigated in the Rio dos Sinos, RS. The comparison of mean oocyte diameters, gonadal indices and gonado-somatic relationships of specimens captured in polluted areas with individuals from unpolluted reference sites revealed a significant decrease of these parameters with increasing water pollution. High loads of organic and industrial sewage are considered responsible for these effects. Condition factors showed an inverse relationship, and increased significantly in downriver polluted areas. The declining gonadal indices showed that energy was allocated to somatic growth. The results of the study recommend the use of *A. fasciatus* in biomonitoring essays.

Key words: *Astyanax fasciatus*, biomonitoring, heavy metals, reproduction.

RESUMO

Astyanax fasciatus como bioindicador da poluição da água do Rio dos Sinos, RS, Brasil

Os efeitos da crescente poluição ao longo do rio sobre o sistema reprodutivo de *Astyanax fasciatus* foram investigados no Rio dos Sinos, RS. A comparação dos diâmetros médios de ovócitos, do índice gonadal e da relação gônado-somática de espécimes capturadas em áreas poluídas com os indivíduos de locais sem poluição (área de referência) revelou diminuição significativa desses parâmetros com a crescente poluição da água. As cargas elevadas dos esgotos orgânicos e industriais são consideradas responsáveis por esses efeitos. Os fatores de condição mostraram relacionamento inverso e aumentaram significativamente nas áreas poluídas ao longo do rio. A diminuição dos índices gonadais mostrou que a energia é alocada no crescimento somático. Os resultados do estudo recomendam o uso de *A. fasciatus* em experiências de biomonitoramento.

Palavras-chave: *Astyanax fasciatus*, biomonitoramento, metais pesados, reprodução.

INTRODUCTION

Antropogenic activities have caused profound alterations in physical and chemical characteristics of the aquatic environment. Industrial and municipal sewage containing heavy metals, hydrocarbons, and halogenated hydrocarbons accumulate in aquatic food webs causing chronic or acute effects in fish populations (Herrick, 1991). The reduced fitness and growth of fish occurs at sublethal levels depending on exposure time, toxicity, and concentrations of the chemical substances involved (Lanno & Dixon, 1994). The early life stages of fish, like eggs and larvae are particularly sensitive to contaminants (Fiuman, 1993).

Leatherland (1993) examined the evidence for endocrine, reproductive, and developmental dysfunctional states in Great Lakes fish caused by heavy

metals and halogenated hydrocarbons. These dysfunctions include thyroid lesions, low egg thyroid hormone content, high prevalence of precocious sexual maturation of males, poor expression of secondary sexual characteristics, low concentration of gonadal steroid hormones in females and in males, low egg fertility rates, and high embryo mortality, often associated with embryo deformity (scoliosis, lordosis, conjoined twins, polycephaly, clubbed tail, missing eye and jaw deformities). These pathologic alterations are thought to have contributed to the decline of Great Lake salmonids, particularly Canadian lake trout (*Salvelinus namaycush*).

Von Westernhagen's review (1988) cites numerous studies which show that exposure of mature female fish to contaminants may reduce ovary weight and egg size, and increase teratological and pathological effects on larvae. Early life stage mortality and decreasing reproductive success are of special importance because their occurrence is not easily detected and alterations in population structures and abundance may only be perceived over the long term.

Biological indicators have been used on many occasions to monitor water quality. Ghetti & Ravera (1994) mentioned more than 90 different methods based on physiological, pathological, and structural alterations on the community levels. Fish are considered valuable bioindicators on organismic, population, and community level (Karr, 1981; Wootton, 1990). The US National Research Council (1986) recommends the use of bioindicators because "only biological monitoring can tell us what (toxic) materials are doing to organisms".

Studies by the Environmental Protection Agency (Fundação Estadual de Proteção Ambiental – FEPAM) in Brazil's southernmost state, Rio Grande do Sul, revealed high loads of domestic and industrial sewage in the lower reaches of the Rio dos Sinos (FEPAM, 1999). Heavy metals from leather and metallurgical industries particular concern cause. Since no historical data on fish diversity and abundance are available, a comparison of present and past situations is not possible. However, strong anecdotal evidence exists from fishermen who indicate that fish abundance has declined drastically, since the 1970's, with increased industrial activities.

Astyanax fasciatus was chosen as an indicator species to monitor the influence of water quality on the reproductive potential of fish. *A. fasciatus* is a nonmigratory fish species (Vazzoler, 1992), abundant from Central America to the Río de la Plata delta in Argentina (Nomura, 1984). The species has a central role in riverine food webs (Agostinho *et al.*, 1984). Araújo (1998) and Menni *et al.* (1996) classified *A. fasciatus* as relatively tolerant to environmental degradation. These characteristics are needed in an indicator species as recommended by Landres *et al.* (1988).

The goal of this study is to investigate the influence of water quality on the reproductive potential of mature female *A. fasciatus*, by comparing oocyte diameter, gonadal index, gonadosomatic relationship, and condition factors along a pollution gradient in the Rio dos Sinos, and to test its aptitude as a bioindicator organism.

MATERIAL AND METHODS

Description of the study area and study sites

The Rio dos Sinos is 190 km long. It originates in the municipal district of Caraá, at an altitude of 740 m and flows into Lake Guaíba, near the city of Porto Alegre (Fig. 1). Its watershed of 4,002 km^2 corresponds to approximately 4.5% of the Guaíba watershed and 1.5% of the total area of Rio Grande do Sul State. A total of approximately 1.2 million inhabitants in 29 municipal districts depend on the Rio dos Sinos for their water supply.

The study was carried out in the potamous reaches of Rio dos Sinos. Three sample sites were chosen in the upper (Monjolo), middle (Parobé) and lower (São Leopoldo) potamous portions, following the increasing pollution gradient of the river. Monjolo, the uppermost site, was defined as a reference station. According to FEPAM (1999), water quality at this point was classified from two (on a scale from one, indicating pristine conditions, to four, indicating heavily polluted), with relatively little influence from domestic sewage (Table 1). Industrial effluents are absent in this area. The second site, Parobé, was classified as 3. Agricultural activities are intense, and pesticides were detected (FEPAM, 1999).
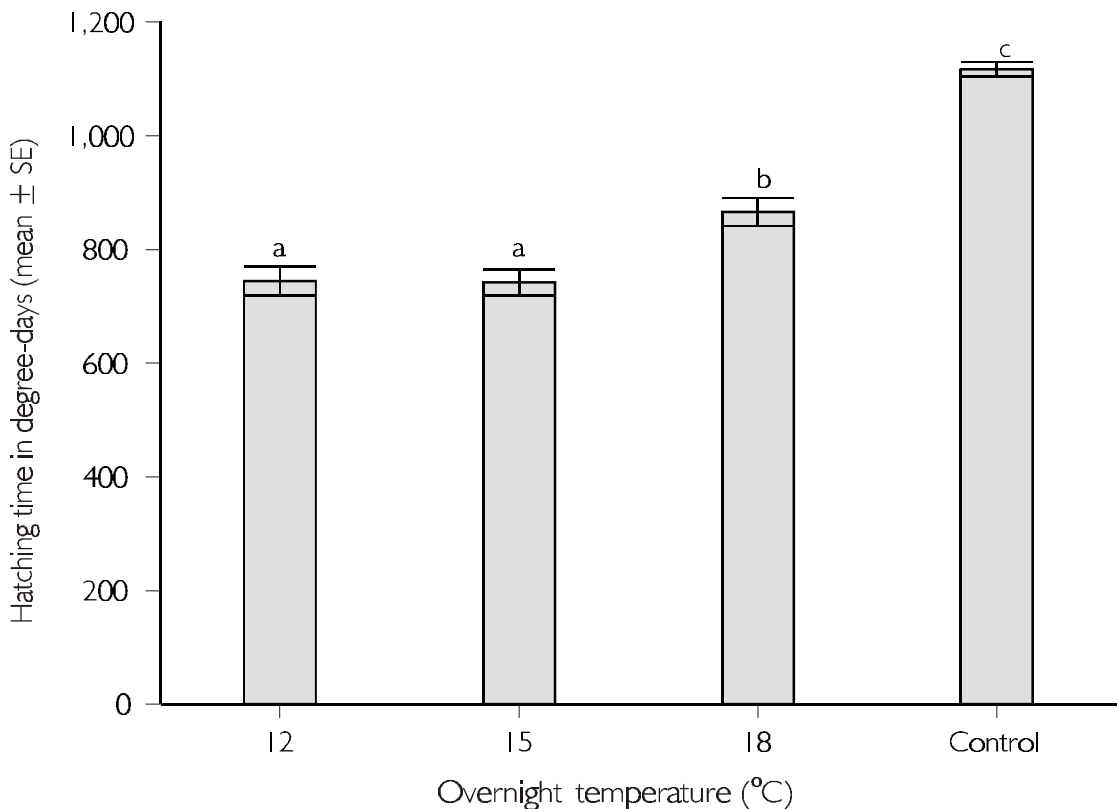
Fig. 1 — Study sites on the Rio dos Sinos (⬇).

The river receives an unknown quantity of sewage from a leaching garbage deposit and smaller amounts of industrial effluents. The most polluted collection site was São Leopoldo with water quality of class four. Municipal sewage from the cities of São Leopoldo (180,000 inhabitants, with approximately 20% of its sewage treated) and Novo Hamburgo (with 225,000 inhabitants, and no sewage treatment) can cause severe oxygen depletion (Table 1). Low pH values and high concentrations of heavy metals are characteristic of metallurgic and leather industry effluents.

Sampling

Collections were conducted four times at each site (seasonally) from November 1998 to December 1999. Fish were captured with gill nets of 15 to 25 mm knot to knot mesh size.

Fifty minutes of electric fishing by boat was also conducted along the river banks, using the aluminum hull of the boat as cathode (at 750 V, max. 4 A, direct current; EFKO, Leutkirch, Germany). Captured individuals were preserved on ice until processing in the laboratory.

Only individuals larger than 10 cm were examined for: total length (± 1 mm), total weight (± 0.1 g), gender (males were not evaluated), and maturity stage of the ovaries [according to criteria established by Nomura (1975) and Vazzoler (1996). Only stage three = mature, were evaluated for: ovary weight (± 0.0001 g), and diameter of 20 oocytes per ovary (± 0.001 mm) and analyzed using graded oculars (ocular micrometer) on a dissecting microscope at 40 x magnification, always obtaining the smallest possible diameter of the cell.

TABLE 1
Physical-chemical parameters of the investigated sites (FEPAM, 1999).

			Sites		
			Monjolo (reference)	Parobé	São Leopoldo
Diss. O$_2$	mg/L	min.	6.6	4.9	0.2
pH		min.	6	6.2	5.2
pH		max.	8.2	7.8	7.6
BOD	mg/L	max.	2.7	3.6	84
Turbidity	NTU	max.	67	135	277
TDS	mg/L	max.	250	230.2	1,090
Cd	mg/L	max.	0.003	0.003	0.040
Pb	mg/L	max.	0.025	0.025	0.110
Cu	mg/L	max.	0.014	0.002	0.034
Cr (hexa)	mg/L	max.	0.060	0.059	0.821
Hg	mg/L	max.	0.178	0.259	0.460
Ni	mg/L	max.	0.016	0.012	0.037
Zn	mg/L	max.	0.050	0.06	0.061
Quality class			2	3	4

Based on these parameters the gonadal index (GI – relation between gonadal weight and volume of the fish), the gonadal-somatic relationship (GSR – relation between gonadal weight and weight of the fish) and the condition factor (K – general measure of well-being) were calculated applying the equations suggested by Strange (1990), Wootton (1990), and Vazzoler (1981):

$$GI = Wo/Lt^b$$
$$GSR = Wo \times 100/Wt$$
$$K = Wt/Lt^b$$

Where: Wo = ovary weight; Wt = total weight; Lt = total length; and b = slope of the regression line of the relation $\log_{10}Wt$ and $\log_{10}Lt$.

Data analysis

In the first step, data per sampling site were separated according to length classes of the fish. The ANOVA was applied to test if the mean oocyte diameter, gonadal index, gonadal-somatic relationship, and condition factor were length-dependent. Out of 12 comparisons per length class, 10 showed no significant differences (p > 0.05), and 2 cases (p = 0.0427 and p = 0.0475) were considered borderline (Table 2). Based on these results, the data of all fish per sampling site were grouped to increase the statistical power of subsequent tests.

The mean value for each parameter per site was calculated. In the second step, the mean of each parameter from the polluted sites at Parobé and São Leopoldo were compared with the means of the Monjolo reference site by applying Student-t-test (p = 0.05 significance level; Statistix Version 1.0).

RESULTS

At all research sites, *A. fasciatus* was a common species at 15% to 22% of total fish abundance. Of 328 captured individuals with a total length exceeding 10 cm, 81 were mature stage 3 females and included in the investigation. Thirty-three specimens were captured at Monjolo (reference site), 19 in Parobé and 29 in São Leopoldo. The greatest mean oocyte diameter was registered in Monjolo, the lowest in São Leopoldo. Oocytes collected in Parobé were of intermediate sizes.

The mean gonadal index and gonadal-somatic relationship displayed the same pattern, decreasing from the reference site to the most polluted site. The mean, minimum, and maximum values for all parameters are given in Table 3. The site-to-site comparisons of the parameters showed that all differences between Monjolo and São Leopoldo were significant (Table 4).

TABLE 2

ANOVA of mean values ($\overline{X}$) of oocyte diameter (diam), gonadal index (GI), gonado-somatic relationship
(GSR), and condition factor (K) per length class and study site.

Length class (cm)	Monjolo (reference)				Parobé				São Leopoldo			
	$\overline{X}$ diam (mm)	$\overline{X}$ GI	$\overline{X}$ GSR	$\overline{X}$ K	$\overline{X}$ diam (mm)	$\overline{X}$ GI	$\overline{X}$ GSR	$\overline{X}$ K	$\overline{X}$ diam (mm)	$\overline{X}$ GI	$\overline{X}$ GSR	$\overline{X}$ K
10-10.9	nd	nd	nd	nd	0.7809	1.1569	16.4560	0.5873	0.7672	0.4671	7.2837	0.5945
11-11.9	nd	nd	nd	nd	0.8215	0.5699	7.5594	0.6969	0.7432	0.5788	9.1912	0.5681
12-12.9	0.8836	0.6260	11.0620	0.5033	0.7877	0.4068	8.2668	0.4515	0.7612	0.4934	8.2263	0.5419
13-13.9	0.8301	0.5677	10.5080	0.4782	0.7980	0.3669	5.9791	0.5819	0.7780	0.6102	9.9488	0.5461
14-14.9	0.8091	0.7732	13.2130	0.5046	0.8398	0.8345	12.4770	0.5790	0.7564	0.5985	9.7231	0.5468
15-15.9	0.8520	0.7277	13.4360	0.4849	0.8203	0.6626	10.4770	0.5502	0.7638	0.4208	6.7618	0.5792
16-16.9	0.8406	0.7145	12.5870	0.4889	0.7877	0.3787	8.0059	0.4680	0.7624	0.8270	11.9290	0.6106
ANOVA (p)	0.0427*	0.2418**	0.3221**	0.6896**	0.4565**	0.0475*	0.0913**	0.1353**	0.9452**	0.6985**	0.6892**	0.8336**

* Significant difference, but considered border line value (near p = 0.05).
** Not significant (p > 0.05).
nd = no data (no fish captured).

TABLE 3

Minimum, maximum, and mean values ($\overline{X}$), standard deviation (sd) and number (n) per site and parameter
(diam = oocyte diameter, K = condition factor, GI = gonadal index,
GSR = gonado-somatic relationship).

	Monjolo				Parobé				São Leopoldo			
	min.-max.	$\overline{X}$	sd	n	min.-max.	$\overline{X}$	sd	n	min.-max.	$\overline{X}$	sd	n
Diam (mm)	0.2740-1.0960	0.8326	0.1492	660	0.2740-1.2330	0.8185	0.1736	400	0.2740-1.2330	0.7612	0.2076	600
K	0.4054-0.5747	0.4891	0.0400	33	0.3867-0.6969	0.5618	0.0758	19	0.4478-0.6440	0.5545	0.0531	29
GI	0.2508-1.0731	0.6821	0.2098	33	0.2296-1.2673	0.6324	0.3017	19	0.2790-1.0901	0.5611	0.2046	29
GSR	4.8095-19.4240	12.2240	3.3895	33	3.2567-16.4560	9.9381	4.0409	19	4.5033-16.8380	9.0307	2.8957	29

Only the comparison of oocyte diameter and GI between Monjolo and Parobé indicated no significant differences.

The mean condition factor (K) displayed an inverse relationship. It increased from up to downriver. The differences found between the condition factors at Monjolo and the other sites were significant.

DISCUSSION

Reproduction-related parameters of *A. fasciatus,* such as gonadal index and gonadal-somatic relationship, decreased with the increasing pollution gradient of the Rio dos Sinos. Fish from the most contaminated site at São Leopoldo displayed significantly smaller oocyte diameter, lower gonadal

index, and gonadal-somatic relationship. Water contamination, particularly from heavy metals, is considered to be the major cause of the phenomenon.

In his review of the subject, Von Westernhagen (1988) mentions numerous studies which show that heavy metals may reduce oocytes volume, as indicated by reduction of the oocyte diameter. Exposure of fish to low levels (micrograms per liter) of zinc, cadmium, copper, mercury, or pesticides may lead to an 80% reduction of egg production. Egg deposition of fathead minnows (*Pimephales promelas*) at copper concentrations of 18-32 µg/L was completely interrupted (Mount & Stephan 1969); low concentrations of zinc (180 µg/L) reduced the number of eggs by some 83% (Brungs, 1969). A progressive decrease in spawning activity and egg numbers in this species was reported by Eaton (1973) after exposure to 3.7-31 µg/L copper and 0.6-60 µg/L cadmium. Zinc concentrations of 130-200 µg/L caused a 21% reduction in eggs spawned in minnows (*Phoxinus phoxinus*; Bengtsson, 1974). The life-bearing guppy *Poecilia reticulata* reduced the clutch size of the young about 50% when exposed to 170-360 µg zinc/L (Uviovo & Beatty, 1979). According to Dave & Xiu (1991) low concentrations of copper (0.25 µg/L), lead (30 µg/L), mercury, (0.2 µg/L) and nickel (80 µg/L) can interfere in hatching and survival of the zebra fish (*Brachydanio rerio*). Maximum values of cadmium, lead, copper, and mercury reached critical levels in São Leopoldo and to a lesser degree in Parobé (Table 1, FEPAM, 1999). Only nickel and zinc concentrations are lower at all sites.

Synergistic effects cannot be excluded. Low pH values such as those measured at São Leopoldo, might enhance the toxic effects on reproductive systems of fish (von Westernhagen, 1988). Bioaccumulation leads to elevated heavy metal concentration in fish. In an unpublished analysis of heavy metals in *Astyanax* (probably *A. fasciatus*) upstream from São Leopoldo, the Department of Environmental Protection of Novo Hamburgo found high concentrations of copper (3.26 mg/kg), lead (2.71 mg/kg), nickel (8.96 mg/kg) and zinc (51.26 mg/kg). These concentrations exceed recommended maximum values up to a factor of 100 (DMAE, 1983).

The high organic load and hydrological characteristics of Rio dos Sinos in the lower reaches cause low concentrations of dissolved oxygen in the area of São Leopoldo. Mean values of between 2 and 3 mg/L can drop to 0.2 mg/L (Table 1; FEPAM, 1999). Mitchell & Bo-Collings (1997), suggesting that decline in oogenesis and vitellogenesis may be associated with low concentrations of dissolved oxygen, which might interfere in yolk accumulation, and consequently, alter oocyte size.

TABLE 4

Results of student-test comparison between the mean ($\overline{X}$) of parameters of the reference site with the mean of the parameters of polluted sites (diam = oocyte diameter, K = condition factor, GI = gonadal index, GSR = gonado-somatic relationship).

Parameters	Site comparison	$\overline{X}$ reference site	$\overline{X}$ polluted site	p
Diam (mm)	Monjolo x Parobé	0.8326	0.8185	0.1740**
K	Monjolo x Parobé	0.4891	0.5618	0.0007*
GI	Monjolo x Parobé	0.6821	0.6324	0.5305**
GSR	Monjolo x Parobé	12.2240	9.9381	0.0338*
Diam (mm)	Monjolo x São Leopoldo	0.8326	0.7612	0.0000*
K	Monjolo x São Leopoldo	0.4891	0.5545	0.0000*
GI	Monjolo x São Leopoldo	0.6821	0.5611	0.0241*
GSR	Monjolo x São Leopoldo	12.2240	9.0307	0.0002*

* Significant difference (p < 0.05);
** Nonsignificant (p > 0.05).

Decreasing reproductive parameters were accompanied by increasing condition factors in *A. fasciatus*. Adams *et al.* (1996), working on redbreast sunfish (*Lepomis auritus*), found an increase of condition factors in sites contaminated by pulp and paper effluents and a decrease of the visceral-somatic index, which reflects lipid stores in viscera. Their explanation for these effects coincides with that of McMaster *et al.* (1991) who postulate that an increased condition factor and a decreased energetic commitment to visceral lipid storage and reproduction could be explained by a disruption in metabolic capability and altered energy allocation. Since this study did not measure the visceral somatic index, it is not known if *A. fasciatus* from contaminated Rio dos Sinos sites follow exactly the same pattern. But the decreased gonadal indices and gonadal-somatic relationships showed clearly that energy was not allocated to the gonads. Altered energy allocation may not reflect environmental stress but other environmental factors, such as habitat and food availability, which usually increase from upstream to downstream.

Comparison of our data with the studies cited strongly suggests that water contamination of Rio dos Sinos interferes with the reproductive system of *A. fasciatus*. Since this species is considered to be resistant to water pollution, interference of pollutants with other more sensitive species is very likely. The long-term decline in species abundance and an unknown amount of species richness may be attributed to the high impact from pollution.

The results of this study recommend the use of *A. fasciatus* in biomonitoring studies. It is a common, easily catchable species with a reproductive system sufficiently sensitive to contaminants.

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