ARTIGO | *ARTICLE*

Frequency of morphological alterations in the fish of Lake *Guaíba* and its application to environmental monitoring

Frequência de alterações morfológicas em peixes do Lago Guaíba e sua aplicação no monitoramento ambiental

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ABSTRACT

This paper examined the association between the frequency of morphological alterations in fish and the physical and chemical parameters often used in monitoring water quality, with the aim of determining which places show most environmental degradation and which species are most affected. Fish samplings were standardized and carried out seasonally, using seine nets, in eleven locations in the catchment area of the *Guaíba* Lake. A total of 53,408 specimens were collected. Eight species were found to be constant in the samples and were therefore considered to be potential bioindicators. The highest frequency of morphological alterations was observed in the areas where the quality of water was the worst. Deformations of the opercular bones and of fin rays and spines were the most frequent alterations found. The repeated spatial pattern of the occurrence of deformations of fin rays and spines during two periods of study, points to this alteration as a good indicator of environmental quality.

Key words: *Guaíba*. Environmental impact. Water quality.

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Resumo

Este artigo avaliou a associação entre a frequência de alterações morfológicas em peixes e os parâmetros físico-químicos frequentemente usados no monitoramento da qualidade da água, com o objetivo de determinar quais os locais que apresentam maior degradação ambiental e quais são as espécies mais afetadas. A amostragem de peixes foi padronizada e realizada sazonalmente com redes de arrasto do tipo picaré em onze pontos da bacia hidrográfica do Lago Guaíba. Foi coletado um total de 53 408 exemplares. Oito espécies foram consideradas constantes, o que as torna potencialmente bons indicadores. As maiores frequências de alterações morfológicas foram observadas nos pontos com pior qualidade da água. As anomalias mais frequentemente observadas foram deformação dos ossos do opérculo e deformação dos espinhos e raios das nadadeiras. A repetição dos padrões espaciais de ocorrência de deformação dos espinhos e raios das nadadeiras em dois períodos de estudo aponta este tipo de alteração como um bom indicador de qualidade ambiental.

Palavras-chave: *Guaíba. Impacto ambiental. Qualidade da água.*

INTRODUCTION

The classification of environmental quality in aquatic ecosystems cannot be solely based on the measurement of the level of contaminants in the water and in the sediment. In order to study the fate (bioavailability, bioaccumulation and biotransformation) of chemical substances in the aquatic environment, it is important to measure the quantity of contaminants in the biota through biological monitoring (van der Oost *et al*., 1996). New methods have been developed, especially in the last two decades, to evaluate environmental quality and monitor effluent through direct observation of chronic or sublethal effects caused by contaminants in aquatic organisms in different stages of their life cycle (Malabarba *et al*., 2004).

An increasing number of studies have reported the occurrence of external alterations to fish. These studies discuss the possible relationship between these alterations and the quality of the environment and they have consistently reported a low frequency of anomalies in non-polluted sites and high frequency in polluted areas, resulting from sewage discharge, industrial waste or both. Sanders *et al*. (1999) found the percentage of alterations to be reliable in assessing the condition of the fish community. This indicator has been very effective for a wide range of types of environmental stress, and it has been particularly useful in identifying places which were degraded by the accumulation of various stress agents.

Goettems *et al*. (1987) and Malabarba & Goettems (1987) used, with satisfactory results, the analysis of the frequency of morphological change in skull bones observed in samples of *Astyanax jacuhiensis,* in monitoring the tertiary treatment system of a petrochemical plant. Adams (1990), Sindermann (1990), Flores-Lopes *et al*. (2001), Schulz & Martins-Junior (2001), Flores-Lopes *et al*. (2002), Malabarba *et al*. (2004), Flores-Lopes *et al*. (2005), Flores-Lopes & Thomaz (2011), realized the importance of using morphological alterations as indicators of environmental quality in environmental monitoring programs. The results presented by these authors showed the importance of using morphological alterations as a tool for evaluating the quality of aquatic environments.

This paper examines the association between the frequency of morphological alterations in fish and the physical and chemical parameters often used to measure water quality according to (Brasil, 2005) resolution #357, with the aim of determining the locations which show the greatest environmental degradation and the species which are most affected.

Study area

Guaíba Lake is one of the most important water resources in the state of *Rio Grande do Sul* in Brazil, supplying 1,500,000 inhabitants in the city of Porto Alegre and neighboring municipalities. The hydrographic basin of *Guaíba* Lake has an area of 85,950 km^2 or 30% of the area of *Rio Grande do Sul* (Fundação Estadual de Proteção Ambiental, 1992), with 80% of the GDP and 70% of the state's population (Empresa de Assistência Técnica e Extensão Rural, 1991). Due to this high concentration of homes and factories, the lake receives a large amount of domestic and industrial waste from several sources, either directly or through its main tributaries (the rivers Gravataí, Sinos and Caí). According to Morandi & Bringhenti (1997), *Guaíba* Lake receives a large amount of pollutants from animal and rice-farming systems, besides domestic and industrial waste, via its tributaries. Analyzing the physical and chemical parameters in the *Gravataí* River near where it empties into *Guaíba* Lake, during 1992 and 1993, Morandi & Bringhenti (1997) found critically polluted waters with a high concentration of nitrates, indicating the low depurative capacity of this tributary, while faced with high levels of waste in its hydrological regime.

MATERIAL AND METHODS

Fish samples were taken seasonally from December 2002 until October 2004. Data was analyzed in two separate periods of one year. The first year comprised the period between December 2002 and December 2003. The second year comprised the period between January and December 2004.

The samples were collected using a seine net (15m x 1.5m; mesh size 0.5cm) (Malabarba & Reis, 1987), at eleven locations. Six were in *Guaíba* Lake (Pt.1 - *Gasômetro, Porto Alegre* municipality (30°02'06.3"S and 51°14'29.3"W); Pt.2 - *Saco da Alemoa, Eldorado do Sul* municipality (29°59'15.6"S and 51°14'24.1"W); Pt.3 - *Foz do Celupa, Guaíba* municipality (30°06'10"S and 51°18'42.4"W); Pt.4 - *Praia da Alegria, Guaíba* municipality (30°08'28.7"S and 51°18'53.4"W); Pt.5 - *Barra do Ribeiro, Barra do Ribeiro* municipality (30°17'11.4"S and 51°18'01"W), and Pt.6 - *Praia de Ipanema, Porto Alegre* municipality (30°08'03"S and 51°14'07"W)) and five locations on three tributary rivers. Of these five places, two were on the *Caí* River (Pt.7 - next the bridge and BR-386 highway (29°49'22.6"S and 51°21'00"W); and Pt.8 - locality of *Morretes*, next to the mouth of the *Jacuí* River (29°55'43.9"S and 51°17'13.8"W), both in *Nova Santa Rita* municipality), one at the *Sinos* River (Pt.9 - next the bridge and BR-386 highway (29°52'36.5"S and 51°14'35.4"W)), two on the *Gravataí* River (Pt.10 - near the RS-118 highway (29°57'43.7"S and 51°00'09"W), and Pt.11 - *Passo das Canoas* (29°57'25.7"S and 51°00'23.2"W), both in the *Gravataí* municipality) (Figure 1).

At each site, a seine net was used on 4 occasions near to the shore. The specimens collected were preserved in 10% formalin. In the laboratory, the collected material was sorted, identified to the species level, according to Malabarba (1989) and preserved in 70% alcohol. Part of the analyzed material was deposited in the scientific collection of the *Universidade Federal do Rio Grande do Sul* (UFRGS). All specimens from each sample were analyzed to determine morphological alterations which were classified and grouped according to the following categories proposed by Malabarba & Goettems (1987) and Malabarba *et al.* (2004): A) Bone dysplasia (atrophy, hypertrophy, torsion, deformation): a1 - opercular bones; a2 - maxillo-mandibular apparatus; a3 - branchiostegal bones; a4 - fin rays and spines; a5 - other bone dysplasia. B) Tumors (neoplasias): b1 - bone neoplasias; b2 - skin neoplasias. C) Vertebral column: c1- kyphosis, lordosis and scoliosis. D) Eyes: atrophy and other dysplasia.

The chi-square test for contingency tables was applied in order to determine the association between the frequency of alteration and the sampling site. Data included the absolute frequency of specimens exhibiting each kind of morphological alteration at

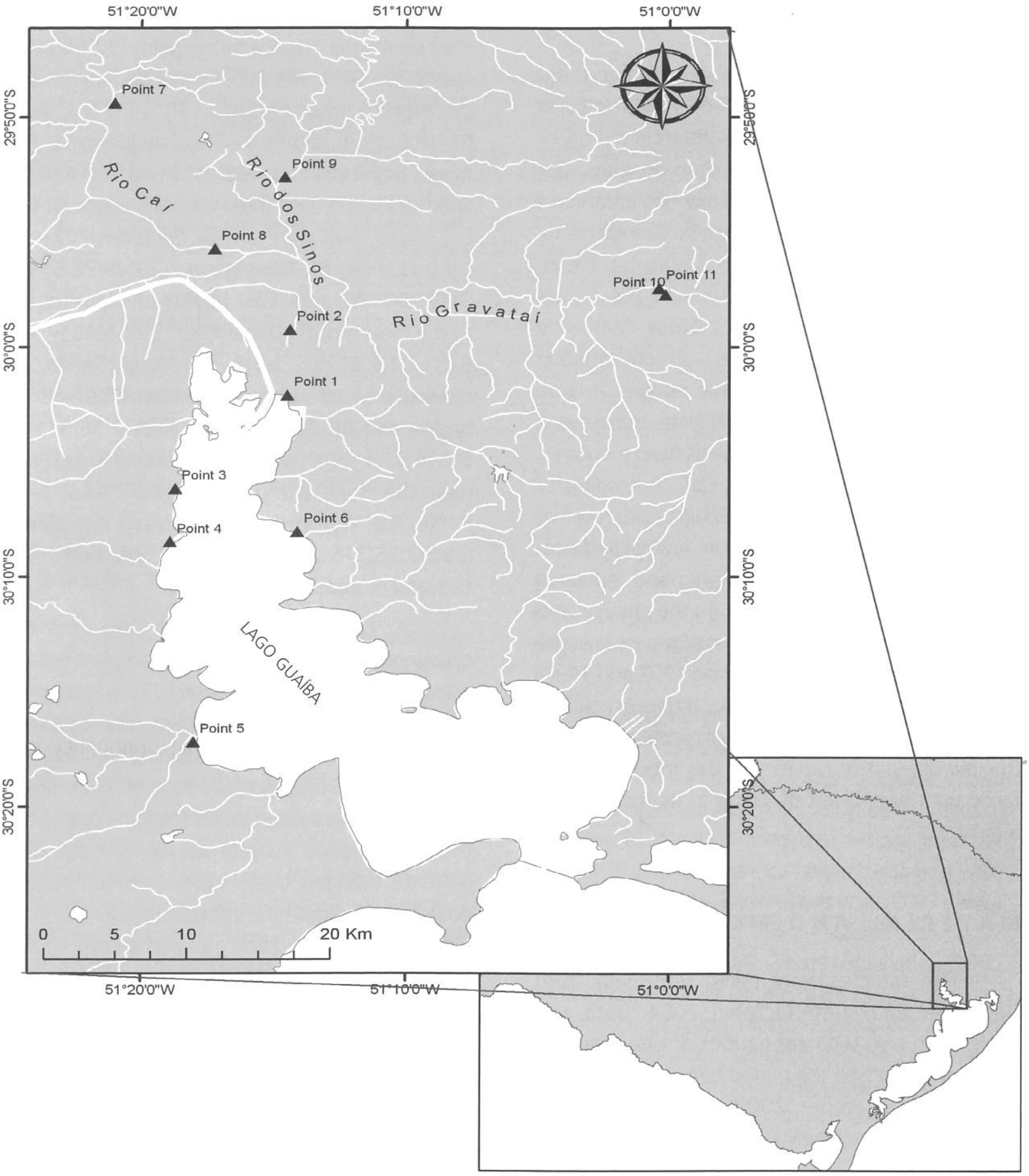

Figure 1. Map of State of *Rio Grande do Sul* (Brazil) and, in detail, the Lake *Guaíba* basin showing the flowing rivers and sampling sites.

Notes: 1) *Gasômetro*, 2) *Saco da Alemoa*, 3) *Foz do Celupa*, 4) *Praia da Alegria*, 5) *Barra do Ribeiro*, 6) *Praia de Ipanema*, 7) *Caí* River, 8) *Morretes*, 9) *Sinos* River, 10) *Gravataí* River near the RS-118 Highway and 11) *Passo das Canoas*.

each sampling site. The simple chi-square test was applied separately for each alteration and for each species in order to determine which ones demonstrated non-random frequency related to the collection locations (Malabarba *et al*., 2004). The test was applied independently for data of every one-year sampling period, as well as for data of two one-year sampling periods. In all cases, a 95% level of significance was established.

The species rate constancy was calculated according to the constancy index: C = (p x 100)/P, where C is the constancy index, p is the number of samples in which the species appears and P is the total number of samples collected. The species were grouped into the following categories, according to C values: constant species - present in more than 50% of the samples; accessory species - present in 25% to 50% of the samples; accidental species - present in less than 25% of the samples.

Temperature, pH, BOD_5, O_2 and fecal coliform data were obtained from *Departamento Municipal de Água e Esgoto* (DMAE), *Fundação Estadual de Proteção Ambiental* (FEPAM), and *Companhia Riograndense de Saneamento* (CORSAN). The physical and chemical quality of the water was determined by matching the average values obtained for each parameter in the classes established by *Conselho Nacional do Meio Ambiente* (CONAMA) resolution number 357 (Brazil, 2005), which provides for the classification of bodies of water and environmental guidelines for its regulatory framework and establishes the conditions and standards for effluent discharge. This resolution defines four categories of water quality based on its physical and chemical characteristics. A principal components analysis was performed with the purpose of identifying physical and chemical parameters that influenced water quality, using the Multivariate Statistical Package v. 3.11.

RESULTS

A total of 53,408 specimens, representing sixty-six (66) species, were caught and analyzed. The species considered constant were: *Cyanocharax alburnus*, *Astyanax fasciatus*, *Astyanax jacuhiensis*, *Rineloricaria cadeae*, *Gymnogeophagus gymnogenys*, *Cyphocharax voga*, *Hyphessobrycon luetkenii* and *Corydoras paleatus*, therefore these species could be potential bioindicators (Annex).

Of all the species examined, morphological alterations were found in nineteen of them, as follows: *Aphyocharax anisitsi*, *Astyanax fasciatus*, *Astyanax jacuhiensis*, *Cheirodon ibicuhiensis*, *Corydoras paleatus*, *Cyanocharax alburnus*, *Cyphocharax voga*, *Cyphocharax spilotus*, *Geophagus brasiliensis*, *Gymnogeophagus gymnogenys*, *Hyphessobrycon luetkenii*, *Hypostomus aspilogaster*, *Parapimelodus nigribarbis*, *Pimelodella australis*, *Pimelodus maculatus*, *Pseudocorynopoma doriae*, *Rineloricaria cadeae*, *Rineloricaria strigilata* and *Serrapinnus calliurus*.

The highest frequency of morphological alteration was observed at *B. Ribeiro, Gasômetro* and *Gravataí* RS-118. The point *Gasômetro* showed all forms of morphological alteration. The alterations a1 (opercular bones) and a4 (fin rays and spines) were observed in all locations studied.

The chi-square test for contingency tables applied to the total frequency of alterations, showed that the occurrence of alterations is not random in *Guaíba* Lake as a whole, as well as at points *Gasômetro, Gravataí* RS-118, *P. Alegria*, Passo *Canoas* and *B. Ribeiro*. The occurrences of the alterations a1 (opercular bones), a2 (maxillo--mandibular apparatus) and a4 (fin rays and spines) were also not considered to be random, showing significant results for the alterations a1 (opercular bones) at points *Gasômetro, Passo Canoas* and *B. Ribeiro*, a2 (maxillo-mandibular apparatus) at point *P. Alegria* and a4 (fin rays and spines) at point *Gravataí* RS-118 (Table 1).

The results of the chi-square test for contingency tables applied to species that showed some kind of alteration, were statistically significant for *Astyanax fasciatus* and *Cyanocharax alburnus*. When the simple chi-square test was used for each species, only *Astyanax fasciatus* and *Corydoras paleatus* gave statistically significant results in both sampling periods.

The alterations observed in *Astyanax fasciatus* (n=10955) corresponded to a1 (opercular bones) (n=119, 1.08%), a2 (maxillo-mandibular apparatus) (n=11, 0.1%), a4 (fin rays and spines) (n=12, 0.1%), c1 (kyphosis, lordosis and scoliosis) (n=3, 0.02%), and d (eye) (n=8, 0.07%). Taking into consideration the whole sampling period, the occurrence of morphological alterations in this species was not random in the *Guaíba* Lake catchment area (χ^2=102.57), nor at point *Gasômetro* (χ^2=36.25). The test result was also statistically significant for the alteration a1 (opercular bones) (χ^2=75.97), at *Gasômetro*. When considering separately two sampling periods of one year, the results were significant for point *Gasômetro* in both periods, and for the alteration a1 (opercular bones) at *Gasômetro* for the first sampling period (Table 2).

The alterations of *Corydoras paleatus* (n=5871) corresponded to a1 (opercular bones) (n=1, 0.01%), a2 (maxillo-mandibular apparatus) (n=7, 0.11%), a4 (fin rays and spines) (n=128, 2.18%), a5 (other bone dysplasia) (n=3, 0.051%), c1 (kyphosis, lordosis and scoliosis) (n=8, 0.13%), and d (eye) (n=2, 0.03%). The result of the chi-square test for contingency tables for this species was not statistically significant (χ^2=51.44), indicating randomness in the occurrence of morphological alterations in the catchment area of *Guaíba* Lake. The chi-square test results were statistically significant for points *Gasômetro* and *Morretes*, indicating a lack of randomness in the occurrence of alterations in these sites. The test result was statistically significant for the alteration a4 (fin rays and spines) (χ^2= 33.61). When the frequencies obtained were compared to the expected frequencies, the test showed the occurrence of frequencies higher than expected for the alteration a4 (fin rays and spines) at *Morretes*. The simple chi-square test result for *Corydoras paleatus* was significant for the whole catchment area in both sampling periods, and was also significant for *Morretes* in the first period and for *Gasômetro* in the second period. The test result was also significant for the alteration a4 (fin rays and spines) for both periods, at *Morretes* in the first year and *Gasômetro* in the second year (Table 3).

The alterations of *Cyanocharax alburnus* (n=3257) corresponded to a1 (opercular bones) (n=47, 1.44%), a2 (maxillo-mandibular apparatus) (n=1, 0.03%), a4 (fin rays and spines) (n=7, 0.21%), b2 (skin neoplasias) (n=4, 0.12%), c1 (kyphosis, lordosis and scoliosis) (n=1, 0.03), and d (eye) (n=10, 0.30%). The result of the chi-square test for contingency tables for this species was statistically

Table 1. Chi-square test for contingency tables of morphological alterations observed in the catchment area of Lake *Guaíba* during the sampling period (from 2002 to 2004).

Sampling point	Type of alteration									χ^2
	a1	a2	a3	a4	a5	b1	b2	c1	d	
Gasômetro	133.8	0.397	0.01	5.912	5.303	0.01	3.988	2.10	0.314	**151.859**
S. Alemoa	0.451	2.165	0.40	6.451	0.201	0.40	0.035	1.71	0.69	12.5077
F. Celupa	0.642	0.971	0.07	0.586	0.036	0	0.216	0.31	0.022	2.849974
P. Alegria	3.008	16.88	0.10	0.077	0.048	0.10	0.29	0.84	1.16	**22.50629**
Barra Ribeiro	32.58	0.883	3.63	0.592	0.092	0.18	0.551	0.78	2.205	**41.49101**
P. Ipanema	5.719	8.849	0.14	0.07	0.07	0.14	0.422	0.27	0.057	15.73918
Caí River	3.107	9E-06	0.07	0.663	0.037	0.07	0.223	1.48	0.013	5.674545
Morretes	0.716	8.781	0.30	1.918	0.15	0.30	0.012	2.34	0.706	15.2262
Sinos River	3.634	0.137	0.11	3.284	0.054	0.11	0.321	0.46	0.063	8.163309
Gravataí RS118	12.03	0.21	0.45	45.69	0.227	5.25	0.097	2.21	5.638	**71.82277**
Passo Canoas	54.6	1.514	0.43	0.788	0.633	1.27	0.167	0.07	0.521	**59.98378**
Total	**250.3**	**40.79**	5.71	**66.03**	6.851	7.835	6.323	12.58	11.39	**407.8237**

(GL= 80, χ^2 tab= 101.87, α= 0.05); simple χ^2 test for collecting spot comparison (GL= 10, χ^2 tab= 18.30, α= 0.05); simple χ^2 test for the frequencies of alterations (GL= 8, χ^2tab= 15.50, α= 0.05); Pt. 1) *Gasômetro*; Pt. 2) *S. Alemoa*; Pt. 3) *F. Celupa*; Pt. 4) *P. Alegria*; Pt. 5) *B. Ribeiro*; Pt. 6) *P. Ipanema*; Pt. 7) *Caí* River; Pt. 8) *Morretes*; Pt. 9) *Sinos* River; Pt. 10) *Gravataí* RS 118 and Pt. 11) *Passo das Canoas*.
Values in bold type: statistically significant.

Table 2. Simple chi-square test for the alteration a1 - opercular bones, for the species *Astyanax fasciatus*.

	December 2002 to December 2003					January to December 2004			
	n	f	Fi	χ^2		n	f	Fi	χ^2
Gasômetro	2,433	95	51.5	**36.7**	*Gasômetro*	2,401	1	0.88	0.02
Saco da Alemoa	645	10	13.7	1.0	*Saco da Alemoa*	563	0	0.21	0.21
Foz do arroio Celupa	335	0	7.1	7.1	*Foz do Arroio Celupa*	142	0	0.05	0.05
Praia da Alegria	356	2	7.5	4.1	*Praia da Alegria*	234	0	0.09	0.09
Barra do Ribeiro	752	1	15.9	14.0	*Barra do Ribeiro*	277	0	0.10	0.10
Praia de Ipanema	313	0	6.6	6.6	*Praia de Ipanema*	571	0	0.21	0.21
River *Caí*	8	0	0.2	0.2	River *Caí*	2	0	0.00	0.00
River *Caí-Jacuí*	295	9	6.2	1.2	River *Caí-Jacuí*	382	0	0.14	0.14
River *Sinos*	162	0	3.4	3.4	River *Sinos*	121	0	0.04	0.04
River *Gravataí* RS118	73	0	1.5	1.5	River *Gravataí* RS118	68	0	0.03	0.03
River *Gravataí* PC	154	0	3.3	3.3	River *Gravataí* PC	668	1	0.25	2.31
Total	5,526	117		**79.1**	**Total**	5,429	2		3.19

(GL = 10, χ^2tab= 18.307 for α= 0.05; n: number of individuals; f: observed frequency; Fi: expected frequency for the sample); Pt. 1) *Gasômetro* Pt. 2) *S. Alemoa*; Pt. 3) *F. Celupa*; Pt. 4) *P. Alegria*; Pt. 5) *B. Ribeiro*; Pt. 6) *P. Ipanema*; Pt. 7) *Caí* River; Pt. 8) *Morretes*; Pt. 9) *Sinos* River; Pt. 10) *Gravataí* RS 118 and Pt. 11) *Passo das Canoas*.
Values in bold type: statistically significant.

Table 3. Simple chi-square test for the alteration a4 - fin rays and spines, for the species *Corydoras paleatus*.

Sampling point	December 2002 to December 2003				January to December 2004			
	n	*f*	*Fi*	χ^2	n	*f*	*Fi*	χ^2
Gasômetro	1,553	6	20.422	10.185	15	5	0.4671	**43.986**
S. Alemoa	15	0	0.1972	0.1972	0	0	0	0
F. Celupa	12	0	0.1578	0.1578	4	0	0.1246	0.1246
P. Alegria	0	0	0	0	0	0	0	0
B. Ribeiro	0	0	0	0	0	0	0	0
P. Ipanema	4	0	0.0526	0.0526	0	0	0	0
Caí River	64	1	0.8416	0.0298	4	1	0.1246	6.1523
Morretes	413	21	5.4309	**44.633**	1	0	0.0311	0.0311
Sinos River	58	0	0.7627	0.7627	11	0	0.3426	0.3426
Gravataí RS 118	1,030	14	13.544	0.0153	1,051	28	32.73	0.6836
Passo Canoas	121	1	1.5911	0.2196	1,515	47	47.18	0.0007
Total	3,270	43		**56.253**	2,601	81		**51.321**

(GL = 10, χ^2tab= 18.307 for α= 0.05; n: number of individuals; f: observed frequency; Fi: expected frequency for the sample); Pt. 1) *Gasômetro* Pt. 2) *S. Alemoa*; Pt. 3) *F. Celupa*; Pt. 4) *P. Alegria*; Pt. 5) *B. Ribeiro*; Pt. 6) *P. Ipanema*; Pt. 7) *Caí* River; Pt. 8) *Morretes*; Pt. 9) *Sinos* River; Pt. 10) *Gravataí* RS 118 and Pt. 11) *Passo das Canoas*.
Values in bold type: statistically significant.

significant (χ^2=178.81), indicating no randomness in the occurrence of morphological alterations in the catchment area of *Guaíba* Lake. The chi-square test result was statistically significant for point *Caí* River, indicating no randomness in the occurrence of alterations at this site. The test results were statistically significant for the alterations a1 (opercular bones) (χ^2=115.5), a4 (fin rays and spines) (χ^2=34.59) and d(eye) (χ^2=16.12). When the frequencies obtained were compared to expected frequency, the test showed the frequency occurrence higher than expected for the alterations a1 (opercular bones), a4 (fin rays and spines) and c1 (kyphosis, lordosis and scoliosis) at *Caí* River. The simple chi-square test for *Cyanocharax alburnus* yielded a significant result, taking into consideration the whole catchment area only in the first sampling period and for *Gasômetro* in the first period. The test results were also significant for the alteration a1 (opercular bones) at *Gasômetro* in the first year and for the alterations b2 (skin neoplasias) at *Gasômetro* and d(eye) at *Caí* River in the second sampling period.

Analysis of the physical, chemical and biological parameters

The main component analysis of the physical and chemical parameters produced two significant axes that showed a cumulative percentage of 67.21% of the variation among the sampling sites. The point *B. Ribeiro* was the one with the best water quality of the whole catchment area due to the highest average O_2 and the lowest average for fecal coliforms and BOD_5. This site was classified as class I, per the CONAMA resolution, for all the parameters studied. The localities *S. Alemoa*, *F. Celupa*, *P. Alegria* and *P. Ipanema* showed lower water quality than did *B. Ribeiro*, varying from good to reasonable during the sampling period. Both sites were classified as class I for pH, O_2 and BOD_5. With regard to the presence of fecal coliforms, *S. Alemoa* was classified as class IV, *F. Celupa* as class II and *P. Alegria* as class III.

The highest variations in temperature were observed in points *F. Celupa* and *B. Ribeiro*. The site *P. Ipanema* showed a reasonable water quality, which varied from good to reasonable during the sampling period. The points *Gasômetro*, *Caí* River, *Morretes* and *Sinos* River showed the worst quality, which was due to the lowest averages of O_2 and the highest averages of fecal coliforms and BOD_5. A small variation in temperature and a high variation in pH were also observed at these sites. The *Gasômetro* may be considered to have the worst quality due to a high average of fecal coliforms and BOD_5 and a low average of O_2 during the whole sampling period. The sites *Gravataí* RS-118 and *P. Canoas* showed a reasonable quality. The variation in temperature observed at these points was very small.

DISCUSSION

Analysis of the frequency of morphological alterations was used with satisfactory results for Goettems *et al*. (1987) in monitoring the efficiency of waste stabilization ponds in the tertiary treatment of effluent end petrochemicals.

The analysis of the fish in the catchment area of *Guaíba* Lake showed that there was no randomness in the occurrence of morphological alterations, and that the frequency of alterations in fish as a whole, as well as for some species, indicates low environmental quality. The analysis also demonstrated that there was no randomness in the point *Gasômetro*, *P. Alegria*, *B. Ribeiro*, *Gravataí* RS-118 and *P. Canoas*.

Malabarba *et al*. (2004) observed a frequency occurrence higher than expected for alterations in the fins (a4 - fin rays and spines) of *Corydoras paleatus* in their first period of sampling (1992) in *S. Alemoa*. These authors also showed that the occurrence of this anomaly was considered casual for the entire basin. Unlike these authors, the result observed in this study for *Corydoras paleatus* was the same for both sampling periods, showing no randomness in the occurrence of this alteration for this species and higher frequency at points *Gasômetro* and *Morretes* which also showed higher environmental degradation according to the physical and chemical parameters established in the CONAMA resolution. The difference between the results obtained by Malabarba *et al*. (2004) and those obtained in this study are probably due to different, natural or environmental factors present in this environment. *Guaiba* Lake receives a variety of substances, many of which are not detectable in traditional physical and chemical properties. These authors pointed out that each species demonstrated a different kind of alteration, which was probably associated with different origins, such as the high frequency of alterations in *C. alburnus* caused by parasites and *A. fasciatus*, probably caused by chemicals. Furthermore, in this basin, there is a large flow of water and a large dilution of the substances that are released into this environment, so that there is a higher concentration of them at different times.

Similarly, the chi-square test for contingency tables, considering all species and all alterations, showed an association between the higher frequency of alterations and the degree of contaminant impact (CONAMA resolution for points *Gasômetro*, *P. Alegria*, *Gravataí* RS-118 and P. *Canoas*). This association was demonstrated again through the simple chi-square test which associated point

Gasômetro with the frequency of alteration a1 (opercular bones) in *Astyanax fasciatus* and *Cyanocharax alburnus*, a4 (fin rays and spines) in *Astyanax jacuhiensis*, *Cheirodon ibicuhiensis* and *Corydoras paleatus*, b1 (bone neoplasias) in *Gymnogeophagus gymnogenys,* and b2 (skin neoplasias) in *Cyanocharax alburnus*. Flores-Lopes & Thomaz (2011), in a study of the same area, observed that the species *A. fasciatus* displayed mean values for the intensity of histopathological alterations that were at their highest at *Praia das Pombas* and the sites *Gasômetro*, *S. Alemoa*, *F. Celupa* and *P. Ipanema*. These authors demonstrated that the highest mean values of HAI (Histopathological Alteration Index) were also observed in *Guaiba* Lake and that *F. Celupa* differed significantly from the other sampling sites for species *A. fasciatus* and *Cyanocharax alburnus*, as it had higher HAI values and mean values (over 100 for *C. alburnus*), showing itself to be associated with environments with major degradation of environmental quality, mainly chemical contamination. These results demonstrate that, based on this method, *F. Celupa* can be considered as having the worst environmental quality in the hydrographic basin studied.

Sanders *et al.* (1999) showed that high alteration percentages are associated with fish assemblages of poor or very poor quality, and that low levels of alterations are related to assemblages of very good or exceptional quality. Among all the sampling points, *Gasômetro* can be considered the one with the worst environmental quality, both by the frequency of morphological alterations in the fish as well as by CONAMA resolution criteria, being considered as class IV. The parameters that most influenced the analysis were fecal coliforms and BOD_5, and the one that had least influence was O_2. These results are very similar to those observed by Bendati *et al.* (2003) who ranked *Guaíba* Lake as class IV or higher, and the quality of the water at *Gasômetro* from average to poor. These authors observed that this region receives a large amount of discharge from the sewers of Porto Alegre's central neighborhoods in the *Ponta da Cadeia* (Gasômetro) and from the *Dilúvio* and *Cavalhada* creeks. According to Faria & Lersch (2001), the contamination of this region is predominantly organic in origin, also due to the direct influence of the *Gravataí* River which receives waste from the largest number of sewer pipes.

In this study, the chi-square test result demonstrated that the point *Gravataí* RS-118 showed no randomness in the occurrence of morphological alterations a1 (opercular bones) and the point *P. Canoas* showed no randomness in occurrence of a4 (fin rays and spines). These results agree with Bendati *et al.* (1998) and Bendati *et al.* (2003) who reported that the mouth of the *Gravataí* river is the most contaminated area, indicated by the absence of macro-invertebrates, the amount of waste in the water, the high values of biochemical oxygen demand, conductivity and reduced dissolved oxygen, refuting the reasonable quality of the water, this study being in agreement with the CONAMA resolution. Bendati *et al.* (2003), also report that the left bank, where our samples were taken, is influenced by local, geographic conditions with the formation of bays, where the water shows greater stagnation and poor renewal, so that contaminants remain there for longer periods. The fact that point *Gasômetro* has the biggest problem with water quality explains why it shows the highest variety in types of morphological alteration.

Although the point *B. Ribeiro* has shown no randomness in the occurrence of morphological alterations a1 (opercular bones), it was classified as a good-quality spot according to the CONAMA resolution, being considered as class I. At this site, however, *Cyphocharax voga* and *Gymnogeophagus gymnogenys* have exhibited a higher frequency than expected for the alteration a4 (fin rays and spines) in the second sampling period, which was not observed in the first sampling period. Malabarba *et al.* (2004) observed, during the years 1992 – 1996, a high occurrence of vertebral deformities c1 (kyphosis, lordosis and scoliosis) in *Cyanocharax alburnus* in *Guaíba* Lake and Flores-Lopes *et al.* (2002) associated this alteration infection with by metacercarians of digenetic trematodes in the vertebral column.

Malabarba *et al.* (2004) also noted high alteration frequency in the eyes (d) of *Astyanax* *fasciatus* and a high alteration frequency in the fins (a4) and tumors (b1) in *Rineloricaria strigilata* in samples taken between the years 1992 and 1996, and they and Flores-Lopes *et al.* (2001) related this to environmental factors, especially chemicals. The high frequency of these kinds of alterations was not observed in *Cyanocharax alburnus* and *Astyanax fasciatus* in this period of study (2002-2004), and samples of *Rineloricaria strigilata* were too small to compare to data obtained previously. This contrast with the classification as classes shows the importance of using other methods for the evaluation of water quality, not just relying on physical and chemical parameters. These results indicate the possible presence of other stressing agents in this area that are not detected by traditional analysis methods, a reduction of chemicals discharged into the environment or a greater dilution of these compounds. The results demonstrated the enhanced sensitivity of individual to various substances or environmental factors.

The use of methodologies that assess the condition of the bodies of water together with physical and chemical parameters, permits a greater understanding of the complexity of aquatic environments, since these tests provide information on abiotic factors, biotic parameters of the fish assemblage and ecosystems. According to Graney *et al.* (1995), depending on the variety of environmental factors, such as the dynamic aspects of biological cycles in the short and long terms, the critical importance of the organisms' interaction to determine the response of ecosystems to a stressor, can create considerable uncertainty, the level of responses that result from tests with a single species and can be extrapolated to field situations.

Based on the results obtained, we may draw the conclusion that the analysis of morphological alterations for some species of fish can be used as a tool in the evaluation of water quality in environmental monitoring programs, indicating which areas, periods and species are being affected most by the degradation of the environment. Through this study, it was possible to observe that the alterations of type a1 (opercular bones) and a4 (fin rays and spines) were the most frequent and this is probably linked to the presence of chemical environmental factors not found in traditional physical and chemical properties. The results of this study, as well as those obtained previously by Malabarba *et al.* (2004), demonstrate the need to study all the fish populations of the *Guaíba* Lake basin, as well as its sensitivity to the presence of various stressors that are discharged into it. These studies reinforce the need to decrease the discharge of waste from various sources, such as pesticides, organic waste, detergents and heavy metals into the basin, in order to preserve the species in these places.

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ANNEX

A LIST OF THE SPECIES CAPTURED IN THE CATCHMENT AREA OF LAKE *GUAÍBA* DURING FIELDWORK, ACCORDING TO REIS *et al.* (2003)

Order/Family	Species	n	C%
Clupeiformes			
Clupeidae	*Platanichthys platana* (Regan, 1917)	57	17.0
Engraulidae	*Lycengraulis grossidens* (Spix & Agassiz, 1829)	384	24.8
Characiformes			
Curimatidae	*Cyphocharax spilotus* (Vari, 1987)	487	27.2
	Cyphocharax voga (Hensel, 1870)	950	59.0
Prochilodontidae	*Prochilodus lineatus* (Valenciennes, 1836)	1	1.1
Anostomidae	*Leporinus obtusidens* (Valenciennes, 1836)	2	2.2
	Schizodon jacuiensis (Bergman, 1988)	3	2.2
Crenuchidae	*Characidium rachovii* (Regan, 1913)	1	1.1
	Characidium tenue (Cope, 1894)	346	30.6
	Characidium zebra (Eigenmann, 1909)	40	6.8
Characidae	*Aphyocharax anisitsi* (Eigenmann & Kennedy, 1903)	54	18.1
	Astyanax eigenmanniorum (Cope, 1894)	37	4.5
	Astyanax fasciatus (Cuvier, 1819)	10,955	90.9
	Astyanax jacuhiensis (Cope, 1894)	3,226	85.2
	Astyanax sp. 1	25	2.2
	Astyanax sp. 2	4	1.1
	Bryconamericus iheringii (Boulenger, 1887)	185	23.8
	Charax stenopterus (Cope, 1894)	6	2.2
	Cheirodon ibicuhiensis (Eigenmann, 1915)	11,601	39.7
	Cheirodon interruptus (Jenyns, 1842)	25	4.5
	Cyanocharax alburnus (Hensel, 1870)	3,257	95.4
	Diapoma speculiferum (Cope, 1894)	1	1.1
	Hyphessobrycon bifasciatus (Ellis, 1911)	11	5.6
	Hyphessobrycon luetkenii (Boulenger, 1887)	8,297	59.0
	Hyphessobrycon meridionalis (Ringuelet, Miquelarena & Menni, 1978)	1	1.1
	Hyphessobrycon sp.	1	1.1
	Oligosarcus jenynsii (Günther, 1864)	41	12.5
	Oligosarcus robustus (Menezes, 1969)	157	40.9
	Pseudocorynopoma doriae (Perugia, 1891)	654	35.2
	Serrapinnus calliurus (Boulenger, 1900)	926	40.9
Erythrinidae	*Hoplias malabaricus* (Bloch, 1794)	43	20.4
Siluriformes			
Aspredinidae	*Ancistrus brevipinnis* (Regan, 1904)	3	1.1
	Bunocephalus doriae (Boulenger, 1902)	13	1.1
	Bunocephalus iheringii (Boulenger, 1891)	28	15.9
Trichomycteridae	*Homodiaetus anisitisi* (Eigenmann & Ward, 1907)	28	10.2
Callichthyidae	*Corydoras paleatus* (Jenyns, 1842)	5,871	50.0
	Corydoras undulates (Regan, 1912)	1	1.1
Loricariidae	*Ancistrus brevipinnis* (Regan, 1904)	3	1.1
	Hisonotus sp.	88	19.3
	Hypostomus aspilogaster (Cope, 1894)	92	10.2
	Hypostomus commersoni (Valenciennes, 1836)	7	3.4
	Loricariichthys anus (Valenciennes, 1836)	35	17.0

ANNEX

A LIST OF THE SPECIES CAPTURED IN THE CATCHMENT AREA OF LAKE *GUAÍBA* DURING FIELDWORK, ACCORDING TO REIS *et al.* (2003)

Conclusion

Order/Family	Species	n	C%
	Otocinclus flexilis (Cope, 1894)	848	22.7
	Rineloricaria cadeae (Hensel, 1868)	1,239	62.5
	Rineloricaria sp. 1	1	1.1
	Rineloricaria strigilata (Hensel, 1868)	230	40.9
Pseudopimelodidae	*Microglanis cottoides* (Boulenger, 1891)	2	1.1
Heptapteridae	*Pimelodella australis* (Eigenmann, 1917)	527	37.5
	Rhamdia aff. *quelen* (Quoy & Gaimard, 1824)	7	3.4
Pimelodidae	*Parapimelodus nigribarbis* (Boulenger, 1889)	624	10.2
	Pimelodus maculatus (La Cepède, 1803)	15	11.3
Gymnotiformes			
Gymnotidae	*Gymnotus carapo* (Linnaeus, 1758)	1	1.1
Atheriniformes			
Atherinopsidae	*Odontesthes aff. perugiae* (Evermann & Kendall, 1906)	35	18.1
Cyprinodontiformes			
Rivulidae	*Cynopoecilus nigrovittatus* (Costa, 2002)	1	1.1
	Cynopoecilus melanotaenia (Regan, 1912)	1	1.1
Poeciliidae	*Phalloceros caudimaculatus* (Hensel, 1868)	6	4.5
Anablepidae	*Jenynsia multidentata* (Jenyns, 1842)	105	18.1
Perciformes			
Sciaenidae	*Pachyurus bonariensis* (Steindachner, 1879)	162	27.2
Cichlidae	*Cichlasoma portalegrense* (Hensel, 1870)	4	4.5
	Crenicichla lepidota (Heckel, 1840)	21	13.6
	Crenicichla punctata (Hensel, 1870)	53	22.7
	Geophagus brasiliensis (Quoy & Gaimard, 1824)	430	36.3
	Gymnogeophagus gymnogenys (Hensel, 1870)	911	60.2
	Gymnogeophagus labiatus (Hensel, 1870)	124	25
	Gymnogeophagus rhabdotus (Hensel, 1870)	115	11.3
	Tilapia rendalli (Boulenger, 1897)	1	1.1
Total		53,408	

n=number of individuals; C= Constancy index.