HISTOPATHOLOGY AS AN APPROACH TO EVALUATE THE EFFECT OF AN OIL SPILL ON FISHES OF THE ARROIO SALDANHA AND RIO IGUAÇU (BRAZIL)

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ABSTRACT: *In July of 2000, an accident occurred at the Presidente Getúlio Vargas Refinery (REPAR), located in the Municipality of Araucaria, Southern Brazil which involved the spilling of about 4 million liters (25,160 barrels) of crude oil. This was considered the largest oil spill in the history of Brazil. Despite of the use of containment barriers, the oil slick reached a small creek (Arroio Saldanha) and, further downstream, two important rivers in the region (Rio Barigüi and Rio Iguaçu). In this stretch, the Rio Iguaçu and the Rio Barigui are highly polluted, having crossed Curitiba, a city that has 2+ million inhabitants, and several industrial areas. The histopathological study of the gills and liver of 2 species of fishes was used to evaluate the impact of the oil on the fish fauna in the affected rivers. The choice of fish species reflects their year-around availability and their predicted roles in the ecosystem.* Astyanax sp. *is mainly pelagic and* Corydoras paleatus *is demersal; these fish species should, therefore, indicate the environmental health of these two compartments of the streams. The spatial and temporal distribution of histological alterations was analyzed from up to 10 specimens of each species of fish from collections made in 5 sites. A relative impact index (Ri) was determined by assigning a weight to the extent of each pathology observed in sections of each organ/fish (i_i = 1 or 2, for moderate or severe pathologies, respectively). The Ri of each organ and fish specimen was calculated as the average impact of the observed histological injuries using the formula:* $Ri = \Sigma i_i/n$. *Variations of the gill and liver Ri suggest a relative regeneration of the environmental health of the impacted streams. A better understanding of the impact caused to the aquatic system by the oil spill is greatly hindered by the presence of other persistent or sporadic events of environmental aggression to which these streams are submitted.*

Introduction

An accident in the Presidente Getúlio Vargas Refinery (REPAR), in Araucaria, State of Paraná, Brazil, in July of 2000, caused an oil leak that reached the Arroio Saldanha (a small creek in the area of the refinery) and, further downstream, the Rio Barigüi and Rio Iguaçu. Contention barriers were placed in the affected streams and the petroleum was stopped in the proximity of the city of Balsa Nova, about 45 km downstream on the Rio Iguaçu.

In order to evaluate the initial impact and monitor the health of the fish communities (using them as a marker of the overall environmental health) of the impacted rivers, the histopathology of 2 organs, gill arches and liver, of representative species in two assemblages of the fish communities of this river system was studied from October, 2000 to May 2002. This paper presents the results of this monitoring.

Material and methods

In order to evaluate both temporally and spatially the extent of the impact on the fish fauna of the affected rivers caused by the oil spill of the REPAR, 5 collection sites were established (Fig. 1), at variable distances from the point of the oil spill. Since no data were available on the histopathological status of fishes from the affected rivers prior to the accident, two of these points, one located upstream of the locale of entry of the oil in the Rio Iguaçu (site 5) and another located in the headwaters of the Arroio GLP (site 0) – a small creek very similar and parallel to the impacted Arroio Saldanha but never exposed to the oil from the spill - were tentatively considered to represent the state of the aquatic system previous to the accident in comparisons. Other sites represent affected areas: site 3, within the Arroio Saldanha was heavily impacted by the oil spill and subjected to relatively persistent aggressions associated to the cleanup activities; site 6, at the municipality of Guajuvira, distant about 60 Km downstream from the locale of the entry of the oil in the Rio Iguaçu; and site 8, in Água Azul, located further downstream from site 6 in the Rio Iguaçu. Numbering of these sites refers to diagnostic and monitoring studies of the fish fauna (see Ostrensky et al.). Collections were made in October and November of 2000 (about 3 months after the accident), and monthly from January 2001 to May 2002.

Representative species of two fish assemblages were utilized as indicators for the study. *Corydoras paleatus (*Jenyns, 1842) (Siluriformes, Callichthyidae) is a demersal species that feeds on organisms of the zoobenthos, algae, and members of the infauna, such as annelids and some crustaceans (Froese et al., 2002). *Astyanax spp.* (Characiformes, Characidae) are benthopelagic species and feed mainly on zoobenthos (especially insects), aquatic plants, and fishes (Froese et al., 2002). These fish species were utilized with the objective of indirectly evaluating the health of distinct compartments of the aquatic system affected by the oil-spill accident.

Fishes were captured with several fishing methods (gill net, cast net, traps and seine). From each fish, the 2º left branchial arch and the liver were removed, fixed in ALFAC (Beçak and Paulete, 1976) for 24 hours, and preserved in 70% ethanol. The samples were submitted to the routine histological processing (Beçak and Paulete, 1976). At least 6 random sections of each organ were made at 5 µm and stained with Hematoxilin of Harris and Eosin (Horobin and Bancroft, 1998) for the morphological study under light microscope.

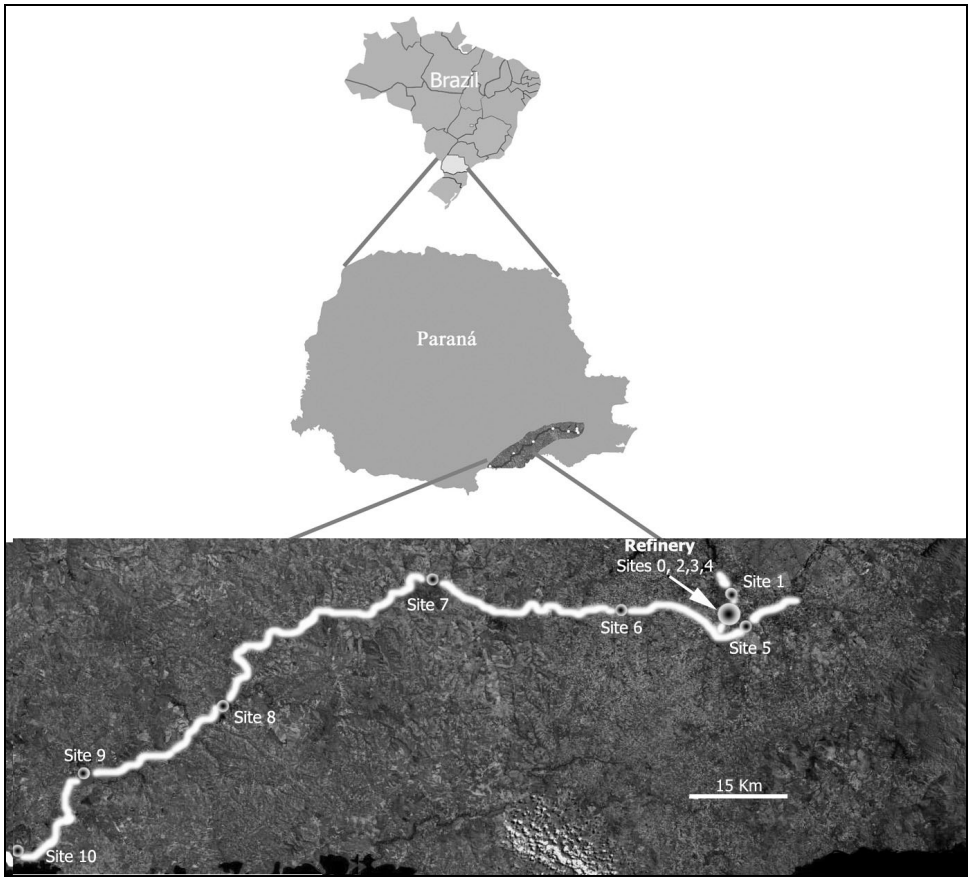


Figure 1. Location of the sampling sites utilized in the present study.

Table 1. Histopatologies utilized in the calculation of the relative impact index (Ri) from the gills and liver (terminology according to Ribelin and Migaki, 1975; Mallat, 1985; and/or Walter et al., 2000).

Gill filaments
• Epithelial hyperplasia
• Fusion of secondary lamellae
• Hypertrophy of gill epithelium
• Edematous lamellae
• Cytoplasmic vacuolization
• Hemorrhage
• Aneurism
• Increase in mucus cells
Liver
• Hepatocytes intensely eosinophylic with hyperchromatic nuclei
• Inflammatory infiltration
• Cellular necrosis
• Hydropic vacuolization

A list of the histopathologies (which follows terminology of Ribelin and Migaki, 1975; Mallat, 1985; and/or Walter et al., 2000) considered for each organ in this study is presented in Table 1. For each sample (fish), these pathologies were classified as absent, moderate, and severe and the information was later converted into the values 0, 1 and 2, respectively, to allow a semi-quantitative analysis. A pathology is considered moderate if it has a focal distribution in the sections examined. Intense pathologies were denser and more widespread in the examined material. A relative index of impact (Ri) for each sample (fish and organ) was determined by the following formula: $Ri = \Sigma i_i/n$ (where n=number of samples).

Relative indexes of impact (Ri) were compared for the gills and liver of the fishes from all collection sites and dates of collection using non-parametric statistical tests; the Mann-Whitney U Test was used to compare two independent samples. Kruskal-Wallis ANOVA was used to compare distribution and median of more than two samples (followed by visual inspection of a Box & whisker plot to identify graphic similarities between samples). The significant level considered in this paper is $p < 0.05$. All tests were performed with the software Statistica 6.0 (StatSoft, Inc. 98).

Results

The consistency of capture of specimens of the fish groups varied greatly within the sampling period. *Corydoras paleatus* was most consistently captured at sites 5, 6, and 8, while species of *Astyanax* were more often captured at sites 0, 3, and 8. This difference of distribution apparently reflects relative abundance of the fish species and the natural conditions of each site. The number of captured specimens per site and date varied from 0 to 10.

Corydoras paleatus and species of *Astyanax* were sympatric only in site 8. Since the Ri for the gills and liver of these two fish groups are significantly different in this site, the results of these two species were analyzed separately. In this site, the impact, as quantified by the relative impact indexes (Ri), was significantly greater for *C. paleatus* than for *Astyanax spp.*

Corydoras paleatus. From October 2000 to June 2002, 358 specimens of *C. paleatus* were captured and processed for the study of histopathologies of gills and liver. The number of specimens of *C. paleatus* captured in each site and date varied from 1 to 10 (mean=6).

The most prevalent pathologies observed in the gills of *C. paleatus* in the period of this study is edematous lamellae (n=305), followed by hypertrophy of the gill epithelium (n=222) and epithelial hyperplasia (n=218). The $Ri_{(gills)}$ of all dates combined of *C. paleatus* for fish collected at the site 8 is significantly different from both sites 5 and 6 (Fig. 2). No significant difference was observed among sites for the combined $Ri_{(liver)}$.

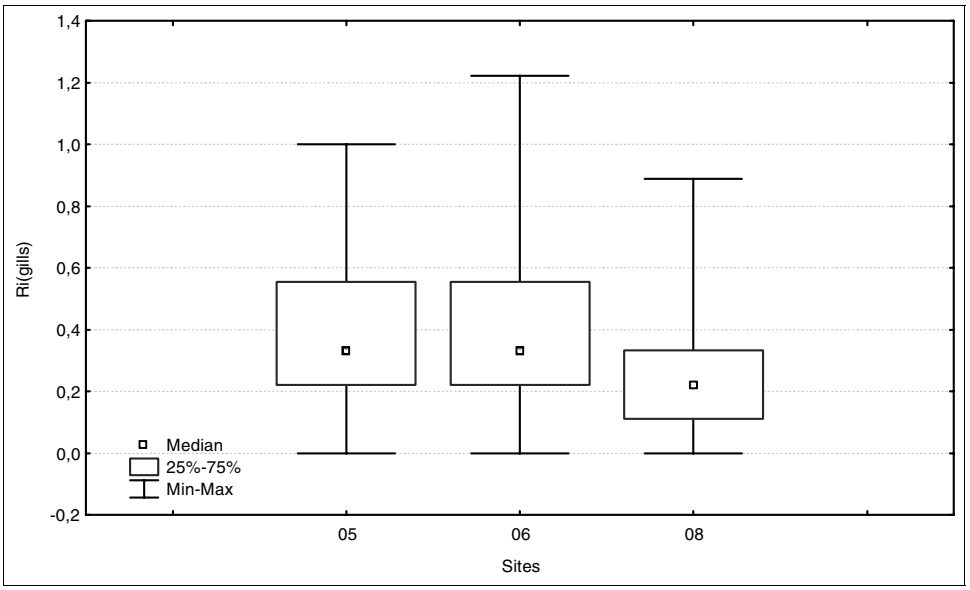
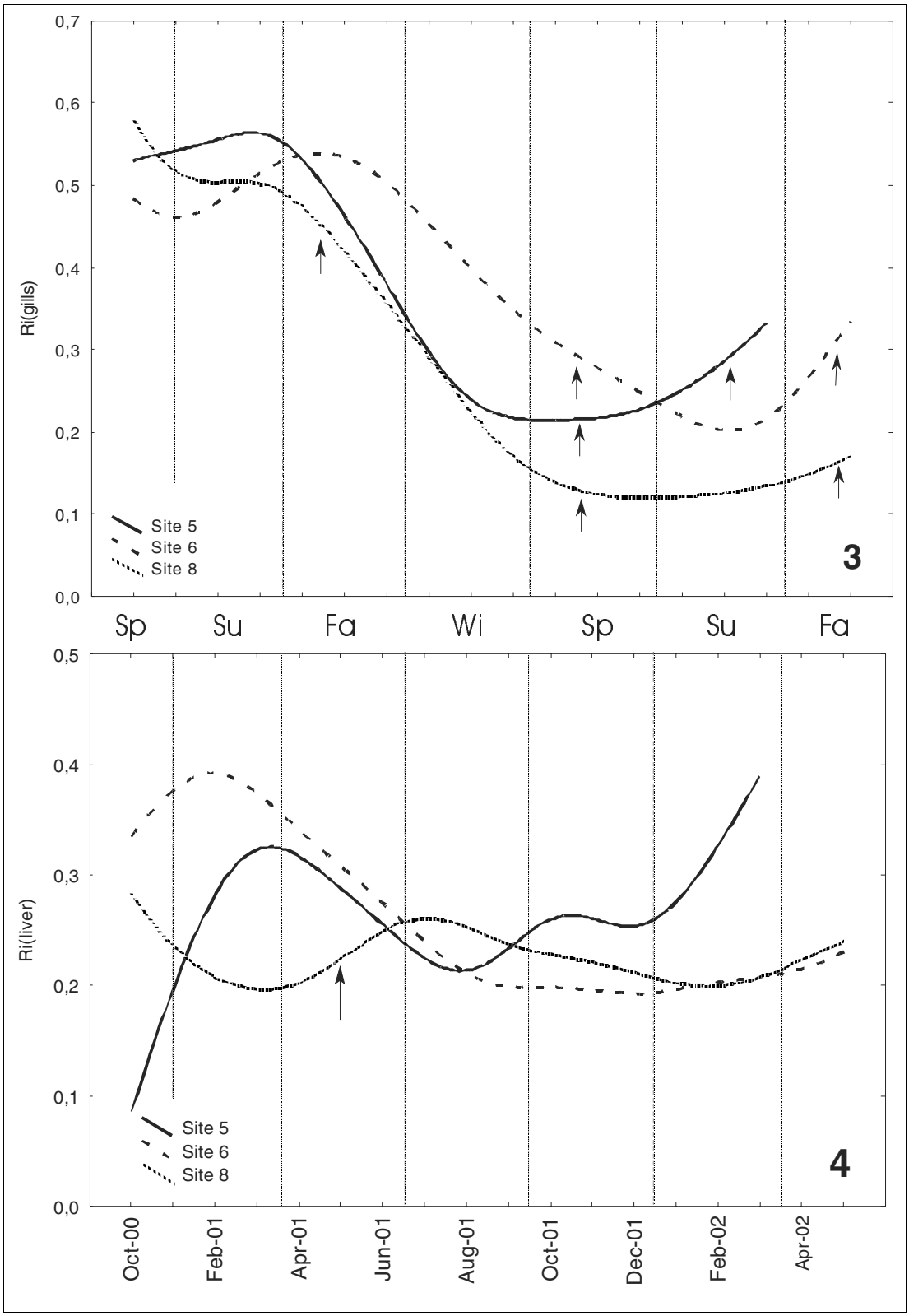


Figure 2. Box & whisker plot of the $Ri_{(gills)}$ (all collection dates combined) for the sampling sites for *Corydoras paleatus*.



Figures 3-4. Curves of the temporal variation of Ri fitted by distance weighted least squares of *Corydoras paleatus* collected from 3 study sites. Fig. 3. Gills. Fig. 4. Liver. Arrows depict significantly different values of $Ri_{(gills)}$ for that specific season (between vertical lines). Sp= spring; Su=summer; Fa=fall; Wi=winter.

A comparison of the temporal variation of the mean relative impact index (Ri) for the gills between the 3 sites from which *C. paleatus* were collected during this study, site 5 (control), site 6 (Guajuvira) and Site 8 (Água Azul), is presented in Figure 3. Significant statistical differences (arrows in Fig. 3) between sites were observed only in the Fall (site 8) and Spring (all sites significantly different) of 2001, and Summer (site 5) and Fall (sites 6 and 8) of 2002. The median $Ri_{(gills)}$ from fish collected at the site 5, located upstream from the entry of the oil in the Rio Iguaçu, is not significantly different from those observed from fishes at sites 6 and 8 until the Spring of 2001. There is however, a remarkably similar pattern of temporal variation of the $Ri_{(gills)}$ of fish collected at these locations during the entire study period. The observed temporal variation for each site is highly significant ($p<0.0004$). All sites initially depict relatively high $Ri_{(gills)}$ values that decrease strongly at the beginning of 2001; both sites 5 and 6 shows a trend of increase in the first months of 2002 (Fig. 3).

The most common injuries observed in the histological sections of the liver of *C. paleatus* were cellular necrosis (n=203) followed by inflammatory infiltration (n=165) and hepatocytes intensely eosinophylic with hyperchromatic nuclei (n=122). Except for $Ri_{(liver)}$ for fishes captured in site 8 in the Fall of 2001, the values observed in each season do not depict any significant difference between sites (Fig. 4). Indeed, no difference was detected when the $Ri_{(liver)}$ from all dates combined were compared among sites using the Kruskal-Wallis ANOVA. Temporal variation of $Ri_{(liver)}$ was only slightly significant ($p=0.042$) for site 6.

***Astyanax* spp.** A total of 226 specimens were captured and processed during this study. *Astyanax* spp. were available only from 2 stations located in the Arroio Saldanha (sites 0 and 3) and in a single site at the Rio Iguaçu itself (site 8). The number of specimens of each sampling unit varied from 1 to 10 (mean=6). Taxonomy of this genus is complicated and probably about 6 undescribed species of *Astyanax* exist in this part of the Rio Iguaçu and tributaries (L. F. Duboc, personal communication). Thus, they were treated as *Astyanax* spp. in the present study.

The most common gill injury observed in the specimens examined was edematous lamellae (n=111), followed by epithelial hyperplasia (n=99) and hypertrophy of the gill epithelium (n=87). When all dates are combined, the $Ri_{(gills)}$ of *Astyanax* spp. from site 0 and 8 are undistinguishable but both are significantly different from those observed for fishes collected in site 3 (Fig. 5).

The temporal variation of the mean $Ri_{(gills)}$ for the species of *Astyanax* spp. observed during this study is presented in Figure 6 but only the variation of the the median of site 8 is statistically significant among seasons. The $Ri_{(gills)}$ of site 0 and 8 are not significantly different for most of the seasons while values observed for site 3 are statistically higher for most of the sampling period (Fig 6).

The most common liver pathology observed in *Astyanax* spp. was inflammatory infiltration (n=114), followed distantly by cellular necrosis (n=40). Not much can be said about the variation and relationship of $Ri_{(liver)}$ for this fish group considering the sampled sites and period. The observed $Ri_{(liver)}$ values are not significantly different between sites (Fig. 7) and except for site 0, no significant variation was observed along the study period.

Discussion

This study attempts to monitor the variation of the environmental health of the streams exposed to the oil spill from the accident of July 2000 at the Presidente Getúlio Vargas Refinery in Araucária, Paraná, Brazil, using histopathological parameters of two fish groups. There are some practical limitations that hinder a more comprehensive interpretation of the results. Sampling did not start immediately after the accident. There are no data on the histopathologies of the two fish groups studied in the streams involved prior to the accident. This problem has been tentatively circumvented by utilizing the sites located upstream from the point of introduction of the oil from the accident (site 0, in the Arroio GLP, and site 5, in the Rio

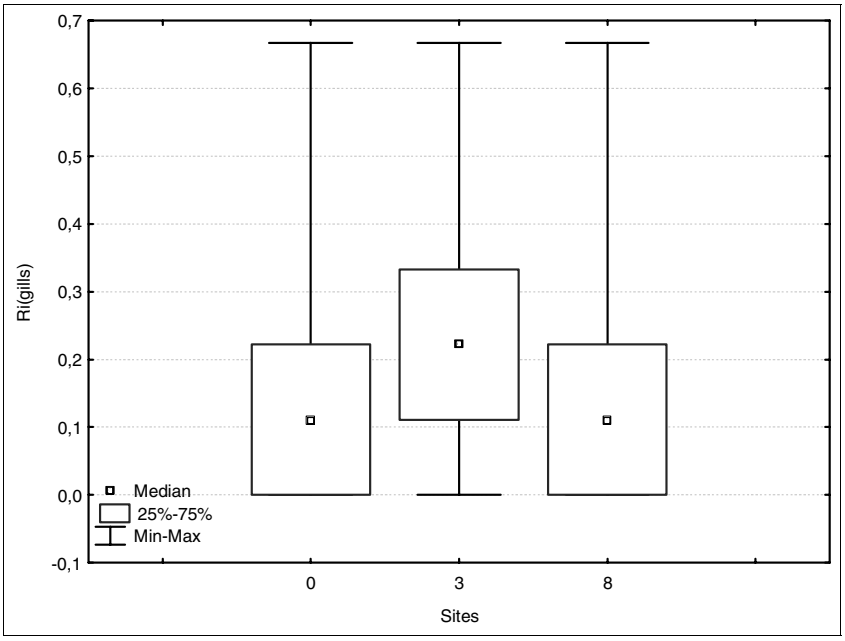
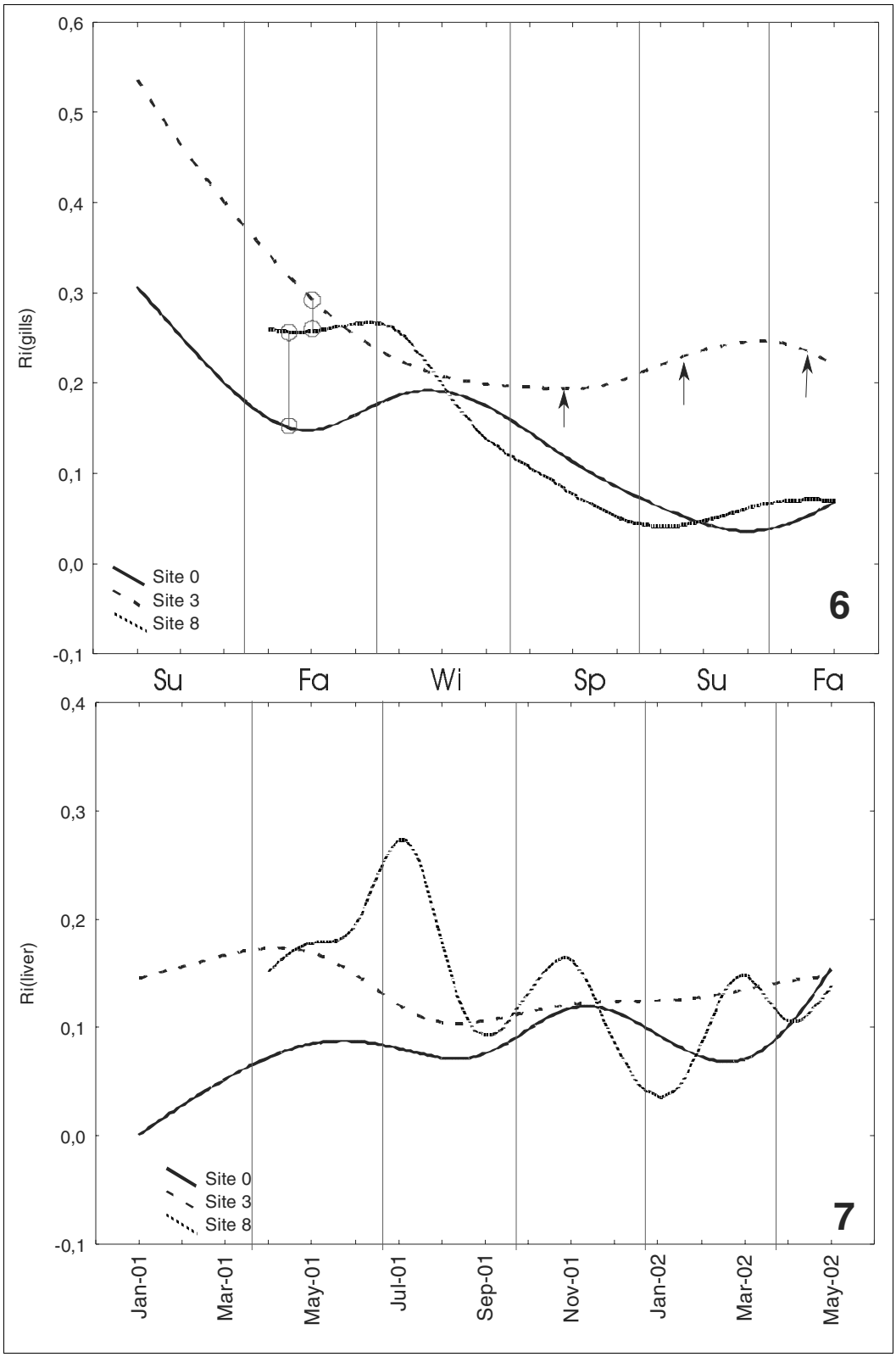


Figure 5. Box & whiskers plot of the $Ri_{(gills)}$ (all collection dates combined) for the sampling sites for *Astyanax* spp.



Figures 6-7. Curves of the temporal variation of Ri fitted by distance weighted least squares of *Astyanax* spp. collected from 3 study sites. Fig. 6. Gills. Fig. 7. Liver. Arrows depict significantly different values of $Ri_{(gills)}$ for that specific season (between vertical lines). Lines connected by vertical lines with circles in the extremities in Fig. 6 depict statistically similar Ri values. Sp= spring; Su=summer; Fa=fall; Wi=winter.

Iguaçu). Unfortunately, these "control" sites are located within the refinery (site 0) or in the metropolitan area of Curitiba (site 5) and, thus, are far from being pristine environments. Thus, interpretations of the results are relative to these sites, which were never directly exposed to the oil of that specific accident, and to the compared temporal variation of the relative impact indexes (Ri).

Differences observed between the Ri of the liver and gills of *C. paleatus* and *Astyanax* spp. when in sympatry (site 8) most likely reflects differences in habitat occupation, as suggested in the Material and Methods. *Corydoras paleatus* is a demersal species and feeds greatly on the bottom which probably exposes it more frequently to eventual pollutants accumulated in the sediment. Hence, the greater values of Ri observed for this species.

For both species of fish studied, however, the data from the histopathology of the gills are far more informative than those produced for the liver (compare Figs 4 and 5; Figs. 6 and 7). Gills tissue apparently respond more rapidly to the presence of aggressive agents in the environment and in the food (Eller, 1975; Mallat, 1985) while the liver tissue appear to be related to a more chronic response.

Gill Ri values provide evidence to evaluate variations of the environmental health of the aquatic habitats exposed to the crude oil spilled during the accident in July 2000. The results obtained from the liver did not show significant variation and will not be considered in the discussion.

Data from the gills of both fish groups suggest a general improvement in time of the environmental conditions at all study sites (Figs. 3, 6), except for site 3 (see below). It is reasonable to associate the usually higher $Ri_{(gills)}$ values observed in the study period from species of *Astyanax* collected at site 3 to impacts associated to the accident of oil spill. Site 3 is located in the Arroio Saldanha, a small stream, directly exposed to the oil spill and frequently impacted by the activities associated with the cleanup of the area. In fact, the $Ri_{(gills)}$ for this fish group did not show any significant temporal variation during the the study period, suggesting the chronic contact to aggressive factors in the area. For *Astyanax spp.*, the mean $Ri_{(gills)}$ of site 8, which is located farther downstream within the oil-impacted system, is both statistically and graphically indistinct from the values observed in the site 0 (located in another stream, never impacted by oil) for this fish group, which suggests similar and better environmental health of these aquatic habitats.

The results of this study do not associate the changes in the $Ri_{(gills)}$ of *C. paleatus* with exposure to the crude oil originating from the accident at the REPAR. The $Ri_{(gills)}$ values obtained for sites 6 and 8 are not significantly different to those calculated for site 5, located upstream to the entry of oil in the Rio Iguaçu (never directly exposed to the crude oil from the accident) (Fig. 4). Further, the statistically similar curves of variation of the $Ri_{(gills)}$ of the 3 sites where *C. paleatus* was collected (which includes the upstream site 5) points to influence of factors that have their origin upstream from the point of entry of the oil from the accident (Fig. 4). This conclusion is further supported by the similar shape of the curve of $Ri_{(gills)}$ observed for *Astyanax* spp. collected from site 8 (Fig. 6). If regional factors, of wider geographical influence, were responsible for these similarities, the same pattern should be expected to occur also for *Astyanax* spp. captured in site 0.

The headwaters of the Rio Iguaçu, upstream from site 5, crosses metropolitan and industrial areas and is, thus, exposed to a great variety of both organic and inorganic pollutants, and other environmentally harmful activities. The upper Rio Iguaçu, for example, was intensively dredged during 2000-2001. Upstream activities in the proximities of the metropolitan area of Curitiba are, apparently, also responsible by the significant increase in $Ri_{(gills)}$ values observed for *C. paleatus* in site 6 in the Fall of 2002, following, and probably responding to, a correspondent increase in the same values for site 5 observed during Summer, 2002.

The available histopathological data, thus, support the idea that the impact of the exposure to the oil and related cleanup activities has negatively affected the environmental health of the small stream Arroio Saldanha. However, the results of this study did not detect any lasting influence of the exposure to the spilled oil on the environmental conditions of the wider downstream portions of the Rio Iguaçu. The factors influencing the aquatic health of the river, at least up to site 8, appear more related to aggressive events occurring upstream, towards the region of the headwaters of this river. These apparently chronic environmental aggressions buffer any evidence of damage caused by the exposure to the oil from the accident at the REPAR. The river system, however, is apparently capable of regenerating both spatially and temporally, but the extent of this ability is unknown at the moment. Further monitoring of the histopathological parameters of the fishes examined, especially those related to the gills, can certainly provide additional evidence of the process of regeneration of this aquatic habitat.

Biography

Walter A. Boeger, Ph.D. is a Full Professor at the Universidade Federal do Paraná. Along with other members of the Grupo Integrado de Aqüicultura e Estudos Ambientais, he has developed several projects associated with the evaluation and mitigation of environmental impacts in both marine and freshwater environments.

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