



Physiological Changes in Largemouth Bass Exposed to Paper Mill Effluents Under Laboratory and Field Conditions

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Accepted 19 March 2003

Abstract. We report here on studies designed to assess the effects of paper mill effluents on non-reproductive functions of free-ranging and captive Florida largemouth bass (*Micropterus salmoides floridanus*). This was accomplished by conducting an outdoor tank study, in which fish were exposed to well water or to 10%, 20%, 40%, and 80% full strength effluent for 28 or 56 days, and by sampling largemouth bass from sites within the St. Johns River, Florida, upstream and downstream from a paper mill plant. Blood and plasma samples from fish from the tank study and from fish sampled from the ambient sites were analyzed for over 20 variables. We also determined liver and spleen weights and examined them histologically. The most significant finding from the tank study was an increase in the concentration of albumin and hepatosomatic index for bass exposed to $\geq 20\%$ effluents for 56 days. Splenosomatic index and number of melanomacrophage centers were decreased in bass from effluent-dominated sites (Palatka and Rice Creek), whereas concentrations of calcium, phosphorous, glucose, and creatinine were elevated in fish from these sites, compared to fish from reference streams. Fish from Rice Creek also had fewer red blood cells, and male bass from Palatka had lower concentrations of cholesterol. Plasma concentrations of albumin and hepatic concentrations of glutathione were elevated in males from Palatka, and both females and males from Rice Creek had higher concentrations of globulin. These results indicate a complex pattern of effects of paper mill effluents on several physiological functions. However, despite the myriad of treatment and site-related effects, most physiological parameters fell within normal ranges when compared to reports on largemouth bass and other freshwater species.

Keywords: paper mill effluents; largemouth bass; hematology; liver; histology

Introduction

Exposure of fish to sublethal concentrations of contaminants may impose considerable physiological stress, resulting in a number of manifestations such as reduced growth, impaired

reproduction, predisposition to disease, reduced locomotory and predatory performance, or reduced capacity to tolerate subsequent stress (Adams et al., 1989). Accordingly, when evaluating the sublethal effects of contaminants on fish, it is necessary to assess a variety of responses at several levels of biological organization in order to achieve results that have biological and ecological meaning. Indicators that reflect conditions at

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lower organizational levels (e.g., biochemistry) usually respond relatively rapid to stress and have mechanistic significance, whereas indicators that reflect conditions at higher organizational levels (e.g., organism, population) respond more slowly but may have more ecological relevance (Adams et al., 1989).

Biochemical responses of fish to chemical stimuli have been studied extensively. The increase in mono-oxygenase enzyme activity (measured as ethoxyresorufin-*O*-deethylase or EROD activity) in fish livers sampled downstream of bleached kraft mills effluents (BKME) is one example (Lehtinen, 1990; Huuskonen and Lindström-Seppä, 1995). Although an induction in EROD activity has been recognized as an indicator of exposure to *Ah*-receptor agonists present in BKME, a major disadvantage of this approach is the lack of understanding on the physiological significance of these changes (Thomas, 1990). In addition, factors such as temperature, age, sex, and nutritional and reproductive status of fish can modify the expression and activity of these detoxification enzymes, which could complicate the interpretation of induction responses (Jimenez and Stegeman, 1990). Alternatively, measurements of physiological indices for assessing the effects of different stressors on fish are extremely valuable because they incorporate several levels of biological organization. Indeed, laboratory and field studies have demonstrated that exposure of fish to BKME can negatively affect many physiological functions. Some of these changes include alterations in hepatic metabolism of carbohydrates leading to altered growth, and negative effects on hematological, immunological, and osmoregulatory functions (Swanson et al., 1996). In addition, exposure of fish to BKME can lead to altered reproductive function. In this regard, results from our laboratory have indicated altered reproductive biomarkers for Florida largemouth bass (*Micropterus salmoides floridanus*) sampled in the St. Johns River, downstream from a paper mill plant (Palatka, Florida) (Sepúlveda et al., 2002a). Fish inhabiting effluent-dominated streams had lower circulating levels of vitellogenin; decreased concentrations of plasma 11-ketotestosterone and 17 β -estradiol; and elevated EROD activity. Similar reproductive changes have also been

documented in bass exposed to BKME under controlled laboratory conditions (Sepúlveda et al., 2001, 2003).

Because of the reproductive alterations observed in largemouth exposed to paper mill effluents, we were interested in evaluating the potential effects of these effluents on other physiological functions. Thus the objective of the current study was to evaluate the effects of paper mill effluents on a suite of hematological, osmoregulatory, and hepatic functions of largemouth bass. Since parameters were measured under both laboratory and field conditions, an additional objective was to compare these responses among captive and free-ranging bass.

Materials and methods

Description of paper mill plant

The kraft mill studied (Georgia-Pacific Operation at Palatka, Florida) produces a 50/50 mix of bleached/unbleached kraft market pulp, so the effluents are referred to hereafter as bleached/unbleached kraft mill effluents (B/UKME). When this study was conducted, the mill released 36 million gallons of effluent/day. Bleaching sequences were C₉₀d₁₀E_{op}HD_p and CEHD for the softwoods and hardwoods, respectively, where Cd is the mixture of chlorine (C) and chlorine dioxide (d) in proportions designated by subscripts; E_{op} the extraction with alkali and the addition of elemental oxygen (o) and hydrogen peroxide (p); H the hypochlorite; and Dp the 100% d substitution with the addition of p. Effluents received secondary treatment, which consisted of both anaerobic (200-ha basin) followed by aerobic (200-ha basin) biological degradation during a retention period of 40 days.

Laboratory study: Exposure conditions

We exposed Florida largemouth bass to four concentrations of B/UKME (10%, 20%, 40%, and 80% of full-strength) for 28 or 56 days to test the effects of these effluents on several physiological parameters. A negative control (i.e., tank containing well water only) was included in this experiment. The tanks that were used as test

chambers were 1500 l in volume and were used in a flow-through mode. We used two replicate tanks for each treatment concentration (10 tanks total). The tanks were positioned about 100 m from the B/UKME discharge point. To create the different concentrations of B/UKME for testing, we used in-line digital flow meters (ECOSOL[®], Ontario, Canada). Tanks were aerated with a single, high volume, low-pressure air pump. The average flow-through rate was 15 l/min.

We obtained fish for the experiment during March and April 1998, from the Richloam Fish hatchery (Terry Town, FL). These fish were ~1.5 years of age, on average and were reproductively active. Forty fish were added to each tank on March 4. The fish were fed *ad libitum* once a week with commercially available fish pellets ("Floating Fish Nuggets", Zeigler, Gardners, PA). Tanks were also checked every other day for the presence of dead fish, and for measurements of water quality. A total of 28 fish died during the course of the experiment (8, 3, 6, 7, and 4 from the 0%, 10%, 20%, 40%, and 80%, respectively, for the 28 and 56 days combined). Every other day (between 10 and 12 AM), we measured dissolved oxygen, temperature, and pH in all tanks using portable instruments (dissolved oxygen and temperature with a YSI Inc., model 55 (Yellow Springs, OH, USA) and pH with a hand-held pH meter, (Hanna Instruments, model H19025C, Bedfordshire, UK). The range of dissolved oxygen, temperature, and pH for the tank system was 4.5–10 mg/l, 16.2–27.6 °C, and 6.7–8.7, respectively.

At the end of each experimental exposure, we collected ~10 females and 10 males from each tank. These fish were weighed individually; we also collected a blood sample from each fish, before they were euthanized and necropsied as described below.

Field study: sampling sites and fish collections

During March 1998, approximately 10 largemouth bass of each sex (total of 61 females and 53 males) were collected by electroshocking from six sites within the St. Johns River (mainstream) and tributaries (small creeks) (Fig. 1). Areas sampled included two tributary reference sites: Cedar Creek (located approximately 30 km downstream from the mill) and Etonia Creek (primary water source for the mill, located 100–200 m upstream from the

effluent discharge), and one effluent-dominated site (Rice Creek, a small tributary stream receiving direct discharge from the mill). Fish were also sampled from three mainstream sites: reference sites Welaka and Dunn's Creek (approximately located 40 and 20 km upstream from effluent discharge, respectively), and contaminated site Palatka (mainstream receiving the discharge from tributary Rice Creek). The average estimated paper mill effluent concentrations in the Rice Creek and Palatka sites are 60% and less than 10%, respectively (Georgia-Pacific, personal communication and also see Quinn et al., 2003). However, water flow in Rice Creek is tidally influenced, so that during periods of low flow, mill effluents can account for up to 90% of the total flow (Schell et al., 1993). Reference sites were matched to contaminated sites in most physico-chemical characteristics, with the exception of presence of effluent. In order to minimize variation in parameters measured in relation to timing of reproductive season, all fish within each site were collected within an average of 4 h and all sites were sampled within a 1-week period. Rice Creek was the only exception to this strict sampling protocol, where it was necessary to collect largemouth bass on three different occasions over a 2-week period to achieve adequate numbers. The scarcity of bass in this stream would indicate absence of adequate prey and/or nesting substrate, thus making this area unsuitable for long-term residency.

Blood collection, necropsies, and age determination

Fish were measured (body weight and total length), bled, euthanized, and necropsied as described in Sepúlveda et al. (2001). Livers and spleens were excised and weighed to the nearest 0.01 g, and hepato (HSI) and spleenosomatic indices (SSI) calculated by dividing the weight of the organ by the weight of the fish \times 100. We also removed sagittal otoliths from each of the fish; these were used to determine fish age (Crawford et al., 1989).

Histopathology

Spleen and liver sections were fixed in Notox[®] and representative samples were cut transversally,

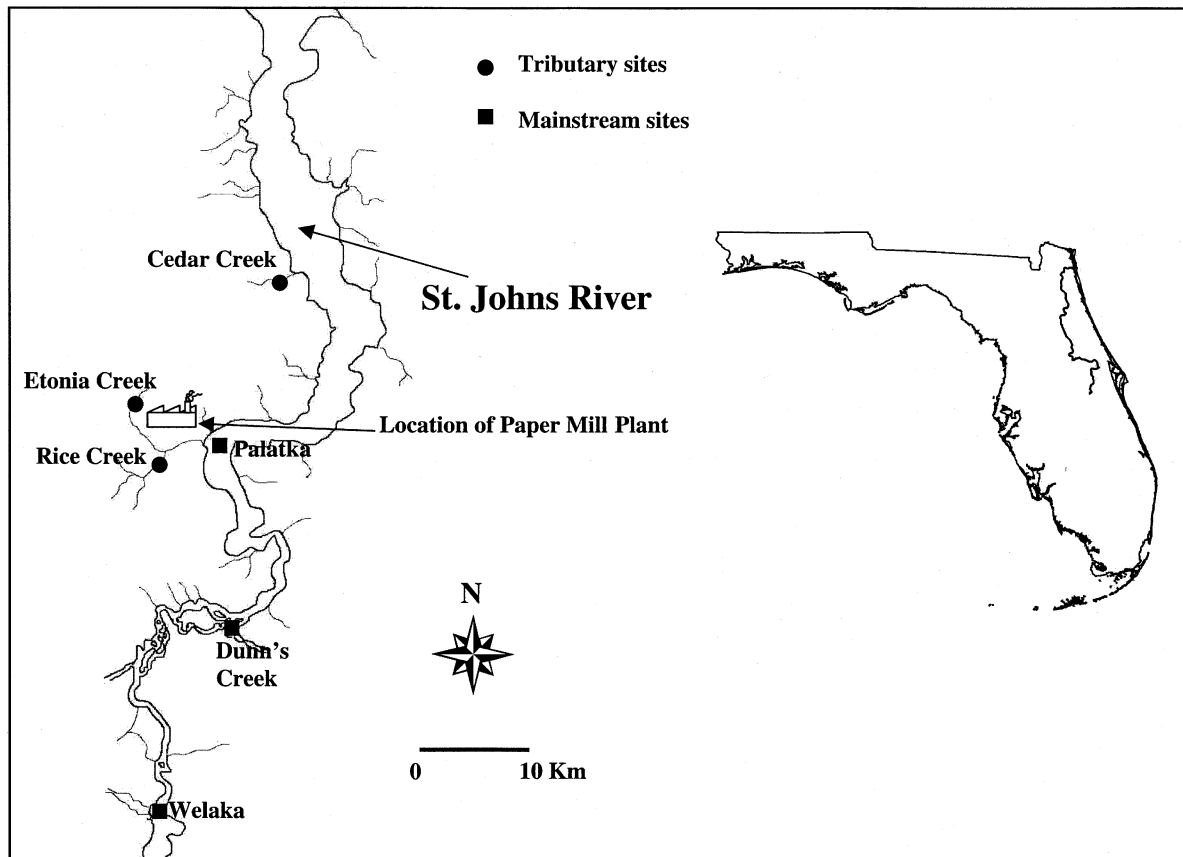


Figure 1. Map of the St. Johns River (mainstream) and tributaries in North-Central Florida from which largemouth bass were sampled during March 1998. The direction of River flow is north.

embedded in paraffin, and sectioned to a thickness of 5- μ m. The sections were then mounted on glass slides, air dried and stained with Mayer's hematoxylin and eosin (H&E). Some of the sections were also stained with Perl's Prussian Blue, which allows for the differentiation of three pigments within melanomacrophage centers (MMCs): hemosiderin (ferric ion) stains bright blue, melanin appears as black to brown granules, and lipofuscin/ceroid pigments stain yellow brown (Blazer et al., 1987). By microscopic examination at 40 \times , we counted the number of MMCs and parasites (mainly immature forms of trematodes and nematodes) in each liver and spleen section. In addition, liver glycogen content and perivascular/pericanalicular inflammation were graded in the wild bass using a scale of 1 to 3 (low, moderate, and abundant). The presence of glycogen was verified in a

subset of slides through the use of periodic acid-Schiff stain. To reduce bias, codes for sites of collection were covered until the histopathologic examination had been completed. Histological evaluations of spleens were only conducted in wild bass, and livers from captive bass were not graded for amounts of glycogen and perivascular inflammation.

Clinical chemistry and concentrations of hepatic glutathione

The blood and plasma values were analyzed <6 h after they had been collected. We measured several hematological parameters (packed cell volume, PCV; hemoglobin, Hb; and number of red blood cells, RBCs) as described in Sepúlveda et al. (2002b). Osmolality was determined using a vapor

pressure osmometer (Wescor, Model 5500, Logan, UT, USA). For this technique, two plasma samples of 5 μ l each were used. A 290 mosM/l standard was run after every two fish plasma samples, and corrections to the concentrations obtained were made accordingly for deviations that exceeded expectations for the 290 value. Chemistry panels were run with \sim 350 μ l of fish plasma and included: glucose; proteins (albumin and globulin); electrolytes (sodium, chloride, potassium, calcium, phosphorous); total bilirubin; creatinine; uric acid; blood urea nitrogen; and the enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (AKP). A Corning Clinical Chemistry Analyzer was used to measure electrolytes (Model 664) and Model 550 Express (Norwood, MA, USA) was used for the remaining measurements. The determination of plasma electrolytes was given second priority when there was not enough plasma to run all measurements. Parameters were measured in 10 bass (5 males and 5 females) from each site/treatment. Total hepatic glutathione (GSH) concentrations were determined using the methods described by Gallagher et al. (2000) using an enzymatic recycling assay adapted for a 96-well microplate reader. We were interested in determining if GSH concentrations were affected by exposure to B/UKME, and tested this possibility by analyzing plasma samples of five males collected at each site. Levels of GSH were not analyzed in females because of the potential of these being modulated by estrogens.

Statistical analyses: Laboratory study

Pairwise comparisons were conducted using a two-way analysis of covariance (ANCOVA) (SAS Institute, Inc. 1988) to test for differences in the dependent variables between treatments. We used weight of the fish as a covariate because fish exposed to the B/UKME mixture for 56 days were significantly heavier than fish exposed for 28 days, ($F = 62$, $p = 0.001$, and $F = 64$, $p = 0.001$ for females and males, respectively). Data sets from females and males were pooled for those variables that were not affected by gender, and were log or arcsin-transformed if they did not meet the criteria for normality and homogeneity of variance (PROC UNIVARIATE). If the ANCOVA showed

a significant treatment effect, a Dunnett's multiple comparison test was used to determine which contaminated site(s) differed from the reference/control. Statistical significance was assessed at $p \leq 0.05$.

Statistical analyses: Field study

Pairwise comparisons were conducted using a two-way analysis of covariance (ANCOVA) (SAS Institute, Inc. 1988) to test for differences in the dependent variables between sites. Type of stream (tributary or mainstream) was used as the second cofactor and age was used as the covariate. Data sets from females and males were pooled for those variables that were not affected by gender, and were log or arcsin-transformed if they did not meet the criteria for normality and homogeneity of variance (PROC UNIVARIATE). If the ANCOVA showed a significant site effect, a Dunnett's multiple comparison test was used to examine which contaminated site(s) differed from the reference. The degree of glycogen storage and of perivascular/pericanalicular inflammation in livers was compared between sites using a χ^2 Test (PROC FREQ). Statistical significance was assessed at $p \leq 0.05$.

Results

Laboratory study

The effects of different concentrations of B/UKME on physiological parameters of bass exposed to effluents for 28 and 56 days are summarized in Fig. 2. The only significant changes observed were: a decline in the number of RBCs in bass exposed to 40% effluent for 28 days (Fig. 2A); an increase in the concentration of albumin in females and males exposed to $\geq 20\%$ effluent for 56 days (Fig. 2B); and an increase in HSI for males exposed to $\geq 20\%$ effluent for either 28 or 56 days (Fig. 2C); and an increase in the concentration of AKP in females exposed to 20% and 40% effluent for 28 days and to 40% effluent for 56 days (Fig. 2D). The remaining physiological parameters (body weight and length; SSI; PCV; Hb; glucose; creatinine; osmolality; cholesterol; sodium;

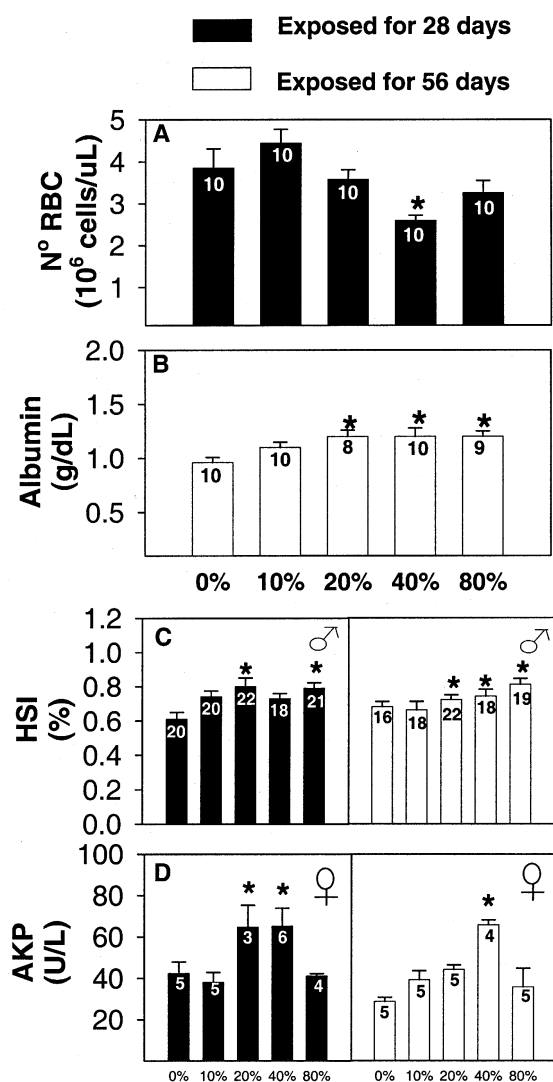


Figure 2. Summary of significant physiological parameters for largemouth bass exposed to four B/UKME concentrations (10%, 20%, 40%, and 80%), plus a negative control, for 28 or 56 days (ANCOVA, Dunnett's multiple comparison test; $\alpha = 0.05$). Values reported are means \pm SE, both sexes combined unless otherwise specified. Numbers inside bars indicate sample size. Asterisks indicate differences relative to controls.

chloride; calcium; phosphorous; globulin; blood urea nitrogen; uric acid; total bilirubin; ALT; and AST) were not affected by treatment.

An unexpected finding was that bass livers showed evidence of different degrees of chronic injury, regardless of treatment. The livers of captive bass had accumulation of brown pigment in

their hepatocytes; fatty change ranging from mild cytoplasmic vacuolation to complete replacement of hepatocellular cytoplasm; loss of normal tissue architecture and tissue degeneration with the formation of regenerative nodules; and mild to moderate inflammation. These changes did not appear to be related to B/UKME exposure, because the frequency distribution of lesions did not differ across treatments.

Field study

Site-related effects on the measured parameters of bass are summarized in Fig. 3. SSI (Fig. 3A) and number of spleen MCCs (Fig. 3B) were significantly less common in bass from B/UKME-mainstream and tributary sites (Palatka and Rice Creek), whereas plasma concentrations of calcium, phosphorous, glucose, and creatinine were increased in fish from these sites in relation to reference streams (Fig. 3C–F, respectively). Fish from Rice Creek also had lower numbers of RBCs when compared to bass from Cedar Creek and Etonia (Fig. 3G), and females from this contaminated tributary site were approximately 1 year younger when compared to females from reference tributary sites (Fig. 3H). For mainstream sites, males from Palatka had lower concentrations of cholesterol in relation to males from Dunn's Creek and Welaka (Fig. 3I). Plasma concentrations of albumin (Fig. 3J) and hepatic concentrations of GSH (Fig. 3K) were greater in males from Palatka, and both females and males from Rice Creek had greater concentrations of globulin, compared to bass from the reference streams (Fig. 3L). The remaining physiological parameters (body weight and length; HSI; number of spleen and liver parasites; number of liver MMCs; PCV; Hb; creatinine; osmolality; cholesterol; sodium; chloride; blood urea nitrogen; uric acid; total bilirubin; ALT; AST; and AKP) were not affected by site.

Histological examination of the liver revealed comparable amounts of hepatic glycogen and perivascular inflammation in bass from reference and contaminated sites. For amount of hepatic glycogen, 70%, 18% and 12% of the females and 40%, 16% and 44% of the males examined had livers that contained low, moderate, and abundant

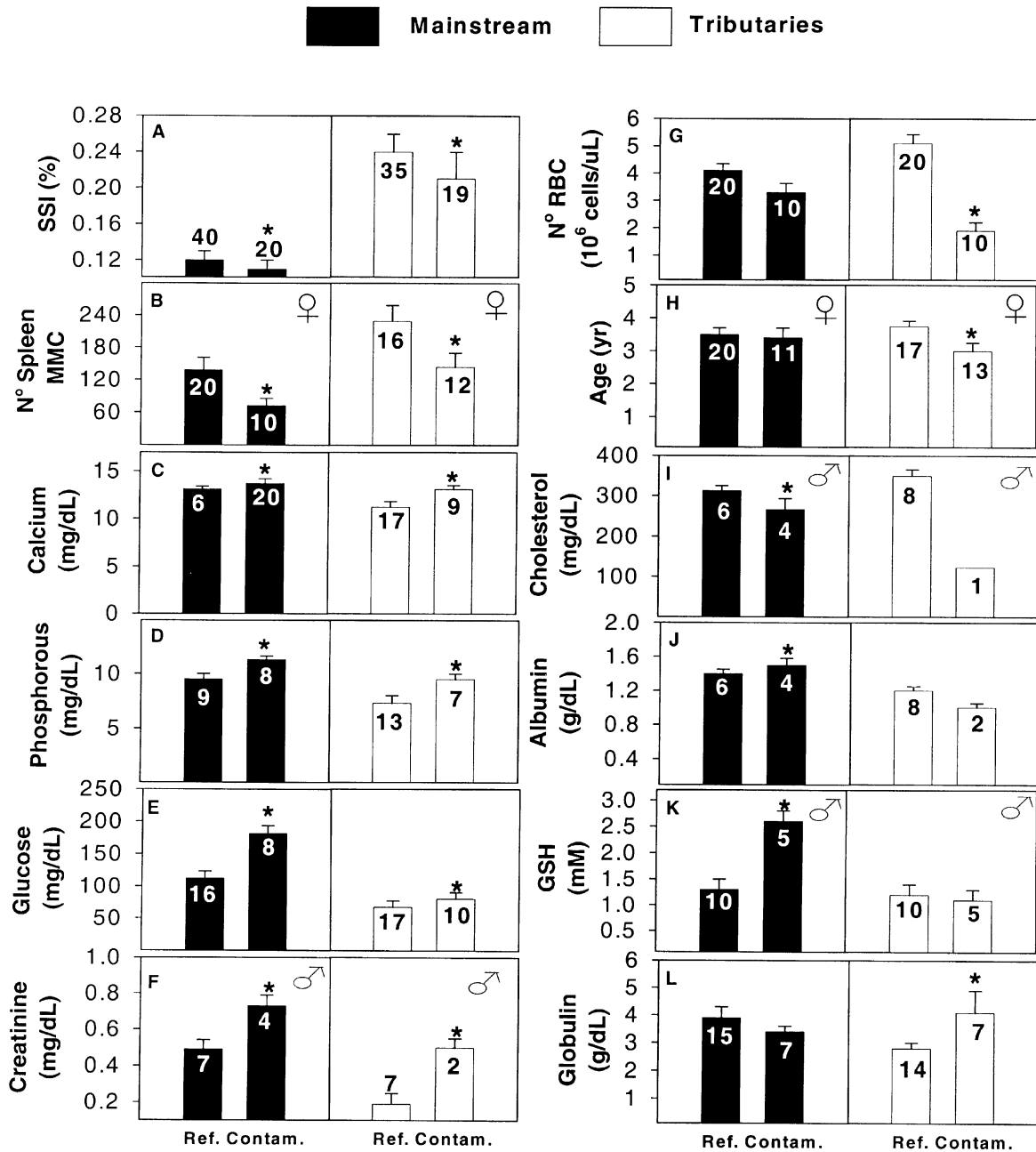


Figure 3. Summary of significant physiological parameters for largemouth bass from the St. Johns River (March 1998); ANCOVA, with Dunnett's multiple comparison test, $\alpha = 0.05$. Fish were collected from tributaries or mainstream sites. Values reported are means \pm SE, both sexes combined unless otherwise specified. Numbers inside bars indicate sample size. Asterisks indicate differences between tributary (Rice Creek) and mainstream (Palatka) sites in relation to reference streams (Cedar and Etonia Creeks for tributaries, or Welaka and Dunn's Creek for mainstream sites).

glycogen, respectively ($\chi^2 < 2.6, p > 0.3$). The respective values for perivascular inflammation were 68%, 20%, and 12% and 57%, 31%, and

12% for females and males having low, moderate, and severe inflammation, respectively ($\chi^2 < 5.8, p > 0.06$).

Discussion

In the study reported here, bass exposed to B/UKME responded with hematological changes, such as reductions in the number of RBCs and decline in the weight of the spleen. Other blood measurements (Hb and PCV), however, remained unaltered. Several field and laboratory studies have reported anemia in fish due to a decline in the number of RBCs and/or Hb concentrations after exposure to BKME (Everall et al., 1991; Swanson et al., 1992; Khan et al., 1996; Soimasuo et al., 1998). Others have postulated that declines in the number of RBCs and Hb may result from increased breakdown of RBCs (hemolysis), since this phenomena has been induced *in vitro* after exposure of RBCs to resin acids (Bushnell et al., 1985). The mechanism by which resin acids cause hemolysis is not clear, but these compounds are associated with a reduction in cellular ATP and diminished oxygen consumption. Declines in Hb concentration caused by hemolysis usually result in jaundice and elevated concentrations of bilirubin in plasma (Nikinmaa and Oikari, 1982; Everall et al., 1991). In our study, the lack of changes in Hb and total bilirubin concentrations, suggests that the decline in number of RBCs was caused by alterations in the hematopoietic capacity of the spleen and/or head kidney. This possibility is supported by the fact that exposed fish also had lower values for SSI. The fact that electrolytes and proteins tended to increase in concentration rather than decrease in B/UKME-exposed bass, also suggests that the decrease in RBCs was not due to impaired osmoregulation.

Fish that are exposed to paper mill effluents do not always respond with declines in hematological parameters. For example, Servizi et al. (1992) reported no differences in PCV of Chinook salmon (*Oncorhynchus tshawytscha*) exposed to up to 4% biotreated BKME for 210 days, and Soimasuo et al. (1998) found no changes in Hb and PCV in whitefish (*Coregonus lavaretus*) exposed to up to 7% BKME for a month. Similarly, Borton et al. (1996) exposed several species of freshwater fish species, including largemouth bass, to up to 8% of effluent (produced by a paper mill plant that used chlorine dioxide as a bleaching agent) for 263 days and found no effects on SSI or PCV.

In the present study, bass from contaminated sites had greater concentrations of plasma proteins (albumin and globulin), cholesterol, creatinine, calcium, and phosphorous, compared to bass from reference streams. Similarly, bass exposed to $\geq 20\%$ effluent for 56 days had higher concentration of albumin compared to controls. These changes might suggest an osmoregulatory dysfunction generally seen as a result of an adaptive stress response. The hyperglycemia observed in bass sampled from the Rice Creek and Palatka sites, is also considered a typical stress response probably associated with effluent exposure.

Concentrations of hepatic GSH were greater in fish from the Palatka site (where exposure to B/UKME was likely) than in fish from the reference mainstream sites. Electrophilic compounds in B/UKME, such as chlorinated aromatics, have been reported to trigger GSH synthesis in fish (Gallagher et al., 1992). The lack of GSH induction at the site immediately downstream from the mill (Rice Creek) may simply reflect inadequate levels of GSH-inducing compounds, or possibly dietary factors (Gallagher et al., 1992). In previous studies, whitefish harvested near a pulp mill outfall in Lake Saimaa, Finland, had higher levels of hepatic GSH (Oikari et al., 1991). Similarly, we have found that channel catfish (*Ictalurus punctatus*) exposed to sediments contaminated with industrial hydrocarbons respond with an increase in GSH synthesis by the liver (Di Giulio et al., 1993).

Carbohydrate metabolism can be affected by exposure to BKME. Coho salmon (*Oncorhynchus kisutch*) exposed to an effluent at a concentration equivalent to 80% of the 96-h LC50 caused immediate hyperglycemia, and a 48-h exposure reduced liver glycogen concentration almost to zero (McLeay and Brown, 1975). In another study, Oikari and Nakari (1982) exposed trout to components of paper mill effluent for 11 d and observed an exhaustion of liver glycogen. Other studies have failed to detect changes in liver glycogen and/or blood glucose concentrations in fish that have been exposed to BKME (Oikari et al., 1988; Swanson et al., 1992; Soimasuo et al., 1998). Although we did observe an increase in blood glucose in bass sampled from effluent-contaminated streams, we did not detect changes in hepatic glycogen levels in these fish. We evaluated glycogen stores in the present by histological grading.

This method might lack the sensitivity needed present in more conventional, quantitative methods (e.g., analysis of glucose equivalents from digested liver samples).

Circulating levels of corticosteroids may increase after exposure of fish to BKME which could lead to immunological disruptions, such as reductions in leucocrit and in immunoglobulins (Soimasuo et al., 1995; Khan et al., 1996). These changes can result in an increased susceptibility to pathogens such as bacteria and parasites. Kennedy et al. (1995) exposed juvenile trout to sublethal concentrations of chlorinated resin acids for 24 h and observed a reduced resistance to infection by *Aeromonas salmonicida*. Several studies have also reported an increase in the prevalence and intensity of infection with ecto and endoparasites in fish exposed to pulp and paper effluents (Axelsson and Norrgren, 1991; Khan et al., 1992, 1994b). In the present study, histological evaluation of liver and spleen sections did not reveal differences in the number of immature nematode and trematode parasitic cysts.

Fish exposed to BKME can respond with changes at the cellular level. Hepatic lesions associated with BKME exposure include: biliary hyperplasia; carcinomas; necrosis; fibrosis; focal vacuolation; lysosomal alterations; and loss of cellular compartmentalization (Lehtinen, 1990; Axelsson and Norrgren, 1991; Bucher et al., 1992; Khan et al., 1994a). In addition, both spleens and livers of BKME-exposed fish have been reported to contain increased numbers of MMCs (Khan et al., 1992, 1994a). MMCs have been proposed as useful indicators of contaminant exposure in fish, since they collect different pigments (including hemosiderin, a breakdown product of red blood cells, lipofuscin, and melanin) indicative of pathological processes and tissue destruction (Blazer et al., 1987). In goldfish (*Carassius carassius*) these centers have also been implicated in the processing and trapping of antigens (Herraez and Zapata, 1986). The number and size of these aggregates, however, can vary in relation to fish age, starvation, presence of infectious diseases, and season (Blazer et al., 1987). In this respect, we observed a significant positive correlation between the number of MMCs in liver and spleen of bass and the number of parasites in these organs (data not shown). In addition, bass with larger spleens and

livers tended to have more MMCs. Since bass from contaminated mainstream sites had decreased spleen weights, it is not surprising that fish from these sites also had a decrease number of spleen MCCs. Except for the presence of parasites and some perivascular inflammation, no other pathological lesions were observed in spleens and livers of wild bass.

In summary, the results from this study support three main conclusions. First, physiological responses were most evident in free-ranging largemouth bass, with few significant trends observed in captive fish exposed to different effluent concentrations. Second, although many of the physiological parameters measured were statistically different from control or reference fish, they fell within normal physiological ranges when compared to reports on largemouth bass and other freshwater species (Denyes and Joseph, 1956; Wedemeyer and Yasutake, 1977; Burns and Lantz, 1978; Hazen et al., 1978; Clark et al., 1979; Borton et al., 1996; Sepúlveda et al., 2002b). This suggests that, under the exposure conditions reported in this study, this B/UKME would cause few or no deleterious physiological effects in bass. This is in contrast to previous field and laboratory studies from our laboratory, that have shown that largemouth bass exposed to these effluents can respond with decreased reproductive output (Sepúlveda et al., 2002a, 2003). And third, since many of the parameters measured in this study are likely to be affected by a suite of environmental conditions other than chemical exposure (e.g., water temperature, dissolved oxygen, diet, etc.) it is essential that these factors not be dismissed when evaluating impacts of contaminants like paper mill effluents on populations of wild fish.

Acknowledgements

The authors express their gratitude to Georgia-Pacific Corporation (Atlanta, GA) for funding. Special thanks to Stewart Holm for his help in designing and overseeing this project. David Spraley and Myra Carpenter (Georgia-Pacific, Palatka Operation, FL) were responsible for the construction and maintenance of the treatment tank system. Shane Ruessler, Carla Wieser, Jon Wiebe, and Nicola Kernaghan (USGS-BRD, Center for Aquatic

Resource Studies, Gainesville, FL) assisted in the collection, treatment, and processing of fish. Trenton Schoeb (Department of Genomics and Pathobiology, University of Alabama, Birmingham, AL) helped in the histological evaluation of tissues, and Karen Pastos (Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL) assisted with the hepatic glutathione analyses. William Johnson and Dave Douglas (Florida Fish and Wildlife Conservation Commission, Eustis, FL) helped collect and determine the age of largemouth bass. Steve Walsh (USGS, Center for Aquatic Resource Studies, Gainesville, FL) provided the use of equipment, and finally, Galin Jones (Department of Statistics, University of Florida, Gainesville, FL) helped with the statistical analyses.

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