PANAMJAS
Pan-American Journal of Aquatic Sciences

Histopathological alterations in gills of juvenile Florida pompano *Trachinotus carolinus* (Perciformes, Carangidae) following sublethal acute and chronic exposure to naphthalene

THAÍS C. A. SANTOS[1*]; VICENTE GOMES[1]; MARIA JOSÉ A. C. R. PASSOS[1]; ARTHUR J. S. ROCHA[1]; RENATO B. SALAROLI[2] & PHAN VAN NGAN[1]

[1] *Universidade de São Paulo, Instituto Oceanográfico, Laboratório de Ecofisiologia de Animais Marinhos. Praça do Oceanográfico 191, 05508-900, São Paulo, SP, Brazil. *Corresponding author: thaiss@usp.br*
[2] *Universidade de São Paulo, Departamento de Fitopatologia, Avenida Pádua Dias 11, 13418-900, Piracicaba, SP, Brazil.*

Abstract. Juvenile Florida pompanos (*Trachinotus carolinus)* were exposed to sublethal concentrations of naphthalene (0.15 ppm and 0.30 ppm) for 24 hours (acute) and 12 days (chronic). Control fish were maintained for the same periods in clean seawater and seawater with ethanol, which is a carrier to dissolve naphthalene. Gill samples from 56 fish were prepared for histological analysis and examined under optical and scanning electron microscopy. Alterations in the gills of fish exposed to naphthalene were semi-quantitatively ranked based on the severity of tissue lesions and comparisons were made with fish kept in clean water and water with ethanol. Fish of the control groups exhibited functionally normal gills, apart from sparse, slight alterations, such as the lifting of epithelial cells. Acute exposure to naphthalene caused slight to moderate alterations in the gills, whereas chronic exposure led to significant, progressively irreparable damage, especially at the greatest concentration. Chronic exposure resulted in a greater number and diversity of alterations. Hypertrophied epithelial cells, epithelial lifting, telangiectasia, fusion of secondary lamellae or their tips, rupture of lamellar epithelium, stasis and necrosis were the most common lesions. Naphthalene caused severe damage to the gills of the Florida pompano which was related to concentration and exposure time.

Key words: Fish, marine pollution, PAH

Resumo. Alterações histopatológicas em brânquias de juvenis de pampos *Trachinotus carolinus* (Perciformes, Carangidae) após exposições subletais aguda e crônica ao naftaleno. Pampos juvenis (*Trachinotus carolinus*) foram expostos a concentrações subletais de naftaleno (0,15 ppm e 0,30 ppm) por 24 horas (aguda) e 12 dias (crônica). Peixes do grupo controle foram mantidos pelo mesmo período em água do mar limpa e água do mar com etanol, usado como solvente do naftaleno. Amostras de brânquias de 56 peixes foram preparadas para análises histológicas e examinadas aos microscópios óptico e eletrônico. Alterações nas brânquias de peixes expostos ao naftaleno foram semi-quantitativamente classificadas segundo o grau de severidade das lesões dos tecidos e comparadas com os tecidos de peixes mantidos em água do mar e água do mar contendo etanol. Peixes dos grupos controle apresentaram brânquias funcionalmente normais, exceto por pequenas e esparsas alterações tais como descolamento de células epiteliais. Exposições agudas ao naftaleno causaram alterações branquiais leves a moderadas enquanto que exposições crônicas ocasionaram danos significativos, progressivamente irreparáveis, especialmente na maior concentração. Exposições crônicas resultaram em um maior número e maior diversidade de alterações. As lesões mais observadas foram hipertrofia das células epiteliais, descolamento epitelial, telangiectasia, fusão das lamelas secundárias ou das suas extremidades, ruptura do epitélio lamelar, aneurisma e necrose. O naftaleno causou danos severos às brânquias de pampos, relacionados à concentração e ao tempo de exposição.

Palavras chave: Peixes, poluição marinha, PAH

Introduction

The Florida pompano, *Trachinotus carolinus* (Linnaeus 1766), is a member of the family Carangidae and is found in abundance from the coast of Massachusetts in the United States to the coasts of Central and South America (Hoese & Moore 1998). This species is usually found in the surf zone of sandy beaches with strong wave action and intense mixture process.

Fish play an important role in the food chain of marine ecosystems (Du Preez *et al.* 1990) and are a valuable source of proteins. Fish are also considered good indicators of environmental quality and are therefore receiving special attention in ecotoxicological studies. These organisms can absorb contaminants in the water. Those that inhabit waters in the vicinities of urban areas may frequently be exposed to sublethal concentrations of pollutants.

Polycyclic aromatic hydrocarbons (PAHs) are important constituents of petroleum, and naphthalene has been one of the most intensively studied PAHs because of its high toxicity, lower sensitivity to photo-oxidation, high persistence in water and low molecular weight (Vijayavel *et al*. 2004). PAHs accumulate rapidly in aquatic animals, reaching greater concentrations than in the surrounding environment, which affects normal vital functions (Nagabhushanam *et al*. 1991, Elumalai & Balasubramanian 1999). Laboratory studies have shown that the presence of PAH metabolites in the bile of organisms is correlated with the degree of exposure (Collier & Varnasi 1991, Britvic *et al*. 1993, Upshall *et al.* 1993, Yu *et al.* 1995, Silva *et al.* 2006). Moreover, PAHs of lower molecular weight are generally found in greater concentrations in fish tissues (Swapan *et al*. 2000, Silva *et al.* 2006).

PAHs in fish organs are not directly responsible for the death of the organism, but sublethal concentrations may affect its functionality and normal physiology by damaging biological structures (Vargas *et al.* 1991, Santos *et al.* 2006). PAHs can exert toxic effects at tissue concentrations of only a few $\mu g.g^{-1}$ (Pitot III & Dragan 1996). The main mechanism behind the toxicity of polyaromatic hydrocarbons is their direct binding to hydrophobic sites of macromolecules, thereby disturbing their normal function (Molven & Gooksoyr 1993, Santos *et al*. 2006) and resulting in toxic effects to the fish (Payne *et al*. 2003, Albers 2003). It has been suggested that the concentration of PAHs frequently found in many aquatic environments is a significant risk factor with regard to various aspects of fish health (Payne *et al*. 2003). PAHs have been reported to cause structural damage to the respiratory lamellae of the gills (DiMichele & Taylor 1978, Correa & Garcia 1990, Prasad 1991, Nero *et al*. 2006). PAHs are also reported to have narcotic action (Correa & Garcia 1990, Alkindi *et al*. 1996).

Naphthalene is one of the most intensively studied PAHs due to its high toxicity, low sensitivity to photo-oxidation, long persistence in water and low molecular weight (Vijayavel *et al*. 2004). It is a two-ring PAH and ubiquitous pollutant introduced into the aquatic environment mainly as a result of discharge from coal tar production and distillation processes (ATSDR 1995) as well as from petroleum products and by-product spillages (Irwin *et al.* 1997, Pacheco & Santos 2001).

Gills are the major organ for osmotic regulation, excretion and respiration in fish. The gills of fish are located on each side of the head beneath a gill-covering operculum and are composed of finger-like filaments attached to a cartilaginous gill bar. Numerous, delicate, leaf-like structures, the lamellae, project from each filament and these consist of minute capillaries covered by a single layer of thin epithelial cells. The epithelium forms a barrier between the fish's blood and the surrounding water.

Gills are generally considered a good tissue indicator of the water quality and are appropriate for the assessment of environmental impact (Mallatt 1985, Winkaler *et al.* 2001, Fanta *et al.* 2003). There are few articles available on gill histology of fish exposed to naphthalene (DiMichele & Taylor 1978, Black *et al.* 1991, Spies *et al.* 1996, Schirmer *et al.* 1998, Ahmad *et al*. 2003). Moreover, studies on the histopathology of different fish organs exposed to contaminants are often carried out with freshwater or brackish-water species (El-Sayed *et al*. 1995, Spies *et al.* 1996, Dwivedi *et al.* 1997, Simonato *et al.* 2008). Histopathological studies are performed to evaluate the direct effects of contaminants on fish in laboratory bioassays (Schwaiger *et al*. 1992, 1997, Ortiz-Delgado *et al.* 2007). However, despite its broad range of distribution on coasts throughout the Americas and its suitability for aquaculture, few studies have reported the effects of pollutants on the species *T. carolinus* (Hymel *et al.* 2002, Santos *et al.* 2006).

The aim of the present study was to describe the effects of naphthalene on the gills of *T. carolinus*, assessing alterations in the tissues and gill function in relation to concentration and exposure time. To our knowledge, this is the first investigation on alterations of gill morphology in *T. carolinus* exposed to acute and chronic sublethal concentrations of naphthalene.

Materials and methods

Field sampling

Juvenile Florida pompanos (body weight: 1.6 ± 1 g; body length: 6 ± 2 cm) were collected from the Enseada beach in the city of Ubatuba, Brazil (23°30'S; 45°07'W). This area was selected due to the low concentrations of petroleum aromatic hydrocarbons detected in water samples (Zanardi 1996). The fish were kept in 500-liter tanks filled with filtered water (1μm) at a constant temperature (24°C) and salinity (35) for at least 10 days to minimize the stress of capture prior to exposure to the pollutant. The water was 75% replaced daily. The fish were fed with a dry commercial feed containing 45% protein, as recommended for the genus (Heilman & Spieler 1999). Food was withdrawn 48 hours before beginning the experiments. Throughout the experiment, the water was filtered, salinity was maintained at 35 ± 1, the ammonium level was kept lower than $0.01x10^{-3}$ $mM.NH_4/L$ and temperature was maintained at 24 ± 1 °C.

Exposure to naphthalene

Naphthalene (99% pure) was purchased from Sigma Chemicals Co. (São Paulo, Brazil). A total of 56 fish were randomly divided into two batches. The fish in one batch were submitted to acute 24-hour exposure and those in the other batch were used for chronic 12-day exposure. Each batch consisted of four groups of seven fish: two groups as controls and two for exposure to two different concentrations of naphthalene (0.15 ppm and 0.30 ppm). The concentrations of naphthalene were one-fifth and one-tenth the lethal concentration (LC50-96h), determined for this species in a previous study (Santos *et al*. 2006). There were no mortalities throughout the exposure period. One control group was maintained in clean seawater (water control) and the other in water with ethanol (solvent control), which is the solvent used for naphthalene dilution. Ethanol was added to the aquaria at the same concentration of 0.05% for both the solvent control and the fish exposed to naphthalene. DiMichele & Taylor (1978) reported that ethanol is a good solvent for naphthalene because it has no effect on fish.

The experiments were carried out in 40-liter aquaria under controlled laboratory conditions. The water in all the aquaria was partially changed (¾) every twelve hours and the pollutant or the ethanol was replaced. Naphthalene concentration was monitored in some aquaria every 12 hours to determine its diffusion into the air or biodegradation, using the UV spectrophometric method described by Neff & Anderson (1975). The monitoring of naphthalene concentration in the experiments revealed a maximal reduction of 22% in the water at the end of twelve hours. These data [previously published by Santos *et al.* (2006)] agree with those described by Wakeham *et al.* (1983), who established 19 hours as the half-life of naphthalene in seawater. At the end of the exposure period, the oxygen consumption and ammonium excretion of the fish was determined to analyze the physiological alterations caused by exposure to the pollutant. These results were also published elsewhere (Santos *et al*. 2006). Subsequently, the same fish were promptly sacrificed by rupturing their central nervous system without anesthesia.

Anesthesia for fish is usually delivered in the water and the anesthetic agent is absorbed through the gills. Under anesthesia, breathing is reduced and fish may go through an excitement phase as inhibitory neurons are depressed. Under the effects of anesthesia, fish may become hypoxic, with low blood oxygen levels (Bowser 2001). Fish that become highly stressed may experience hemorrhaging of the gills, which could damage the tissue and incur undesirable lesions. Therefore, the fish were quickly killed by rupturing their central nervous system.

Body length and weight were measured before the gills were dissected.

Histological analysis

Gill samples were immediately fixed in Dietrich (10% paraformaldehyde, 30% absolute alcohol, 2% acetic acid in distilled water), dehydrated in graded ethanol and embedded in historesin. Sections of 4 μm were stained with hematoxylin and eosin (H&E). Alterations in the structure of a central section of the two first gill arches were semi-quantitatively evaluated by the degree of tissue change (DTC), which is based on the severity of the lesions according to the methodology described by Poleksic & Mitrovic-Tutundzik (1994) and Simonato *et al.* (2008). For the calculation of DTC, the alterations in each gill were classified in progressive stages of tissue damage. First-stage lesions (I) are slight and would be reversible with an improvement in the environmental conditions; second-stage lesions (II) are more severe, leading to effects on tissue function; and third-stage lesions (III) are very severe, with irreparable damage. The sum of the number of lesion types within each of the three stages multiplied by the stage coefficient represents the numerical value of the DTC, based on the formula $DTC = (10^0 \Sigma I) + (10^1 \Sigma II) + (10^2 \Sigma III)$, in which I, II and III correspond to the sum of the number of alterations found in stages I, II and III, respectively. The DTC was obtained for the fish of

all the experimental groups and used in the statistical analysis to compare the mean degree of tissue damage between groups.

DTC values between 0 and 10 indicate normal gill function; values between 11 and 20 indicate slight damage; values between 21 and 50 indicate moderate changes; values between 50 and 100 indicate severe lesions; and values above 100 indicate irreversible damage to the organ (Poleksic & Mitrovic-Tutundzik 1994, Simonato *et al*. 2008).

Scanning electron microscopy

Gill samples from each group fixed in Diethich's solution were dehydrated and critical point dried using liquid CO_2. Dried specimens were mounted on aluminum stubs and sputter coated with gold. Specimens were examined and photographed using scanning electron microscopy (SEM) (Zeiss LEO 435VPA).

Statistical analysis

Histological alterations in the gills of the 56 fish, quantified as DTC values, were analyzed using the non-parametric Kruskall-Wallis test to determine differences between groups. The Mann-Whitney U-Test for independent samples was used to determine differences between the control and exposed groups. The significance level was $P < 0.05$.

Results

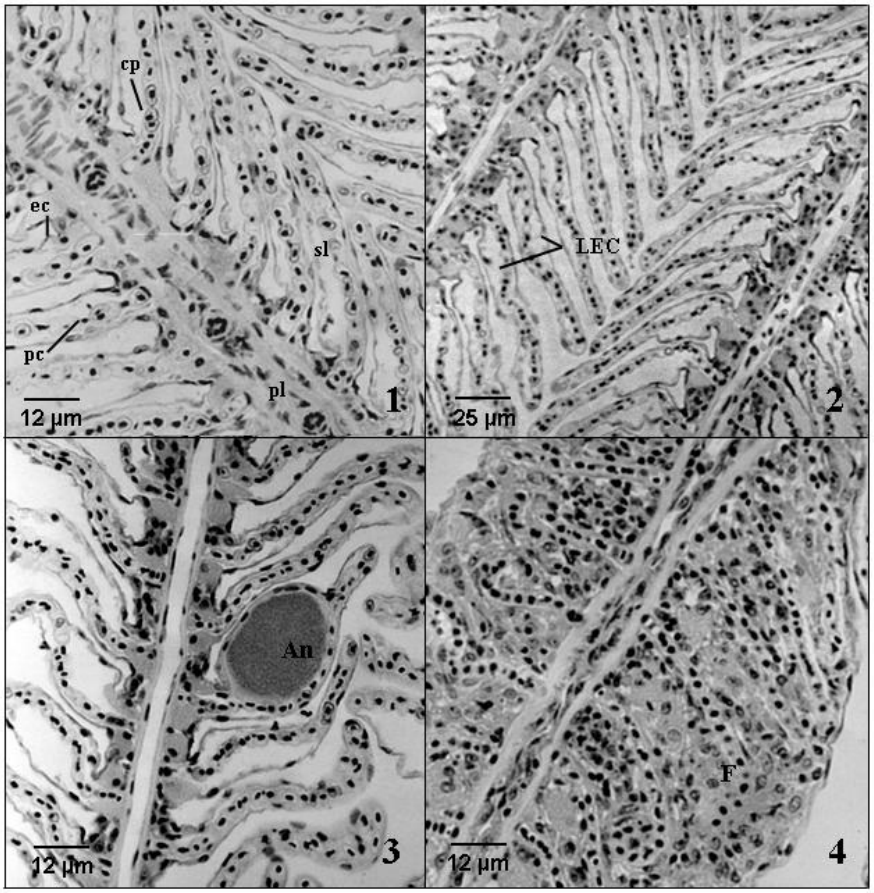
General remarks

The gill filaments of fish are straight and secondary lamellae line both sides. The surface of the gill lamellae in the control groups was covered with epithelial cells running parallel (Fig. 1).

Using the criteria described by Poleksic & Mitrovic-Tutundsic (1994) as reference, 21 different types of lesions were identified in the gills of *T. carolinus* (Table I), 14 of which were first-stage lesions, five were second-stage lesions and two were third-stage lesions. Hypertrophied epithelial cells, epithelial lifting, telangiectasia, focal proliferation of primary and secondary lamellar epithelial cells, fusion of adjacent secondary lamellae or their tips, rupture of the lamellar epithelium cells, stasis and the presence of microscopic parasites were the most common alterations in the fish exposed to naphthalene (Figs 2, 4 and 5-8). A moderate presence of aneurysm (Fig. 3) and necrosis of pillar cells were also detected in fish exposed to naphthalene, but at a lower percentage. Telangiectasia (Fig. 6) consisted of dilatation of the terminal blood vessel in secondary lamellae, in which erythrocytes were easily recognized. Stasis or aneurysm was determined by the congestion of blood cells, thereby becoming a compact homogenous mass (Fig. 3).

Table I. List of severity of gill lesions in *Trachinotus carolinus.*

(a) Hypertrophy and hyperplasia of gill epithelia	Stage
Hypertrophy of respiratory epithelium	I
Lifting of respiratory epithelial cells	I
Leukocyte infiltration of gill epithelium	I
Thinning of respiratory epithelium	I
Rupture and peeling of lamellar epithelium	II
Focal hyperplasia of epithelial cells	I
Hyperplasia from base to approximately half the length of secondary lamellae	I
Irregular ("chaotic") hyperplasia of epithelial cells	I
Fusion of tips of secondary lamellae	I
Fusion of primary lamellae tips	I
Uncontrolled thickening of proliferated tissue	II
Fusion of several secondary lamellae	I
Shortening of secondary lamellae	I
Complete fusion of all the secondary lamellae	II
(b) Blood vessel changes	Stage
Lamellar telangiectasia	I
Filament blood vessel enlargement	I
Hemorrhages with rupture of epithelium	II
Stasis	II
(c) Gill parasites	Stage
Presence of parasites	I
(d) Terminal stages	Stage
Scar tissue – fibrosis	III
Necrosis	III



Figures 1-4. Longitudinal sections of primary lamellae of *Trachinotus carolinus*. 1. Control with unaltered primary and secondary lamellae. 2. Gill exhibiting moderate lifting of epithelial cells after acute exposure to naphthalene at 0.15 ppm (LEC). 3. Gill exhibiting aneurysm (An) following chronic exposure to naphthalene at 0.30 ppm. 4. Gill exhibiting fusion of secondary lamellae following acute exposure to naphthalene at 0.15 ppm (F). pl. primary lamellae sl. secondary lamellae, cp. capillaries, pc. pillar cells, ec. epithelial cells.

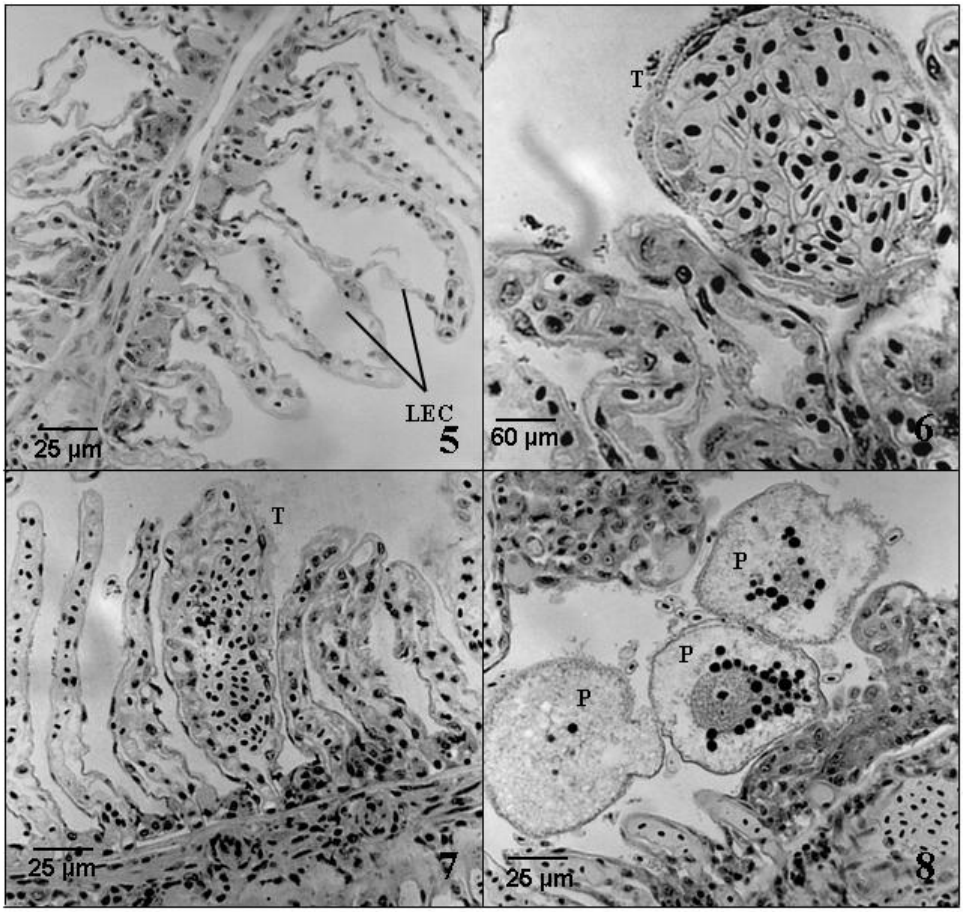
To a lesser degree, some of these alterations were also observed in control fish. Considering the scarce, reparable alterations, there were no statistically significant differences in the control or ethanol groups between the 24-hour and 12-day exposure, as mean DTC values were low (Fig. 9).

Third-stage lesions and necrosis occurred only in fish exposed to the highest concentration of naphthalene after the longest period of exposure. There was no fibrosis or scar tissue in any of the gills analyzed.

Acute exposure – 24-hours

Twenty-four hour exposure to naphthalene at 0.15 ppm and 0.30 ppm resulted in maximal DTC values of 17, indicating slight to moderate gill damage in fish (Fig. 9). Mean DTC value in the control groups was 2. There were no irreparable lesions in tissues in this period. Second-stage alterations were rare and, when observed, limited to one alteration per individual. The most frequent alterations were the presence of parasites, fusion of secondary lamellae, lifting of respiratory epithelial cells and hypertrophy of epithelial cells (Table II). Gill stasis occurred in some fish in both groups exposed to naphthalene.

Histopathological lesions in the gills of fish exposed to naphthalene at 0.15 ppm and 0.30 ppm for twenty four-hours were distinguished from the slight tissue lesions found in the controls. Differences between control and ethanol groups were non-significant, whereas both groups differed significantly from the naphthalene groups. Despite the greater DTC value for gills exposed to the higher concentration of the pollutant, differences between the naphthalene groups were non-significant during acute exposure.



Figures 5-8. Frequent lesions found in Florida pompanos. 5. Lifting of epithelial cells (LEC) following acute exposure to naphthalene at 0.30 ppm. 6 & 7. Gill exhibiting subepithelial hemorrhage (telangiectasia) following chronic exposure to naphthalene at 0.15 ppm (T). 8. Parasite infestation following chronic exposure to naphthalene at 0.15 ppm (P).

Chronic exposure – 12-days

After chronic exposure to naphthalene for twelve days, the survival rate was 85%. The lowest DTC value was 2 in both the control and ethanol groups and the highest value was 134 in fish exposed to naphthalene at 0.30 ppm (Fig. 9). After chronic 12-day exposure to the pollutant, the animals had higher mean DTC values. Only at the highest concentration of naphthalene did the animals exhibit irreparable third-stage lesions – necrosis of the gill tissue. There was a greater quantity and diversity of alterations in the chronic experiment, the most common of which were lamellar epithelium hypertrophy, fusion of lamellae and presence of microscopic parasites, which were even found in the control groups (Table II).

There were no significant differences in DTC values between control and ethanol groups in chronic exposure (Fig. 9). The naphthalene groups differed significantly from control and ethanol groups with regard to DTC. Moreover, there were significant differences in DTC values between the two naphthalene groups. The gills of fish submitted to long-term exposure to naphthalene exhibited extensive epithelial lifting and blood congestion (aneurysm) in many areas of the secondary lamellae, with the breakdown of the pillar cell system. Twelve days of exposure to naphthalene resulted in a mean DTC of 26 ± 7 at a concentration of 0.15 ppm and 125 ± 5 at 0.30 ppm.

Comparing the 24-hour and 12-day periods, the longer the period of exposure to naphthalene led to more severe and diversified damage to the gills.

Table II. Most frequently alterations observed at the gills of *Trachinotus carolinus* according to the exposure time to naphthalene.

Exposure time	Alterations most commonly observed	Stage of lesion severity
24 hours	presence of parasites	I
(acute exposure)	fusion of secondary lamellae	I
	lifting of respiratory epithelial cells	I
	hypertrophy of epithelial cells	I
	stasis	II
12 days	lamellar epithelium hypertrophy	I
(chronic exposure)	fusion of lamellae	I
	presence of microscopic parasites	I
	extensive lifting of respiratory epithelial cells	I
	stasis (aneurysm)	II
	necrosis	III

Scanning electron microscopy

The SEM examination confirmed the partial fusion of many secondary lamellae in the fish exposed to naphthalene (Figs 10-12). SEM of the control fish revealed normal gill surfaces, with no alterations (Fig. 13). Although not considered for the DTC value, SEM analysis confirmed the disarrangements in gill filaments with damage to the structure of the secondary lamellae, clearly observed in the fish exposed to naphthalene.

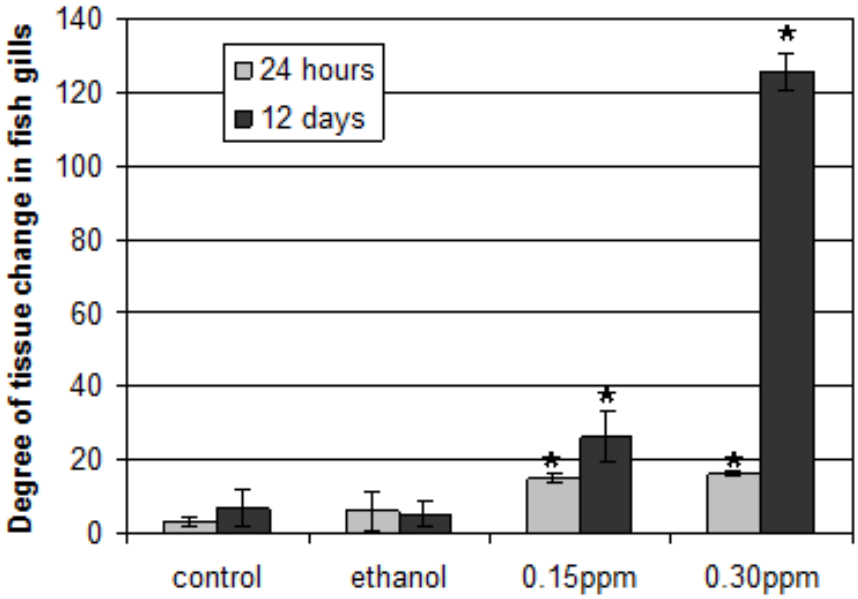


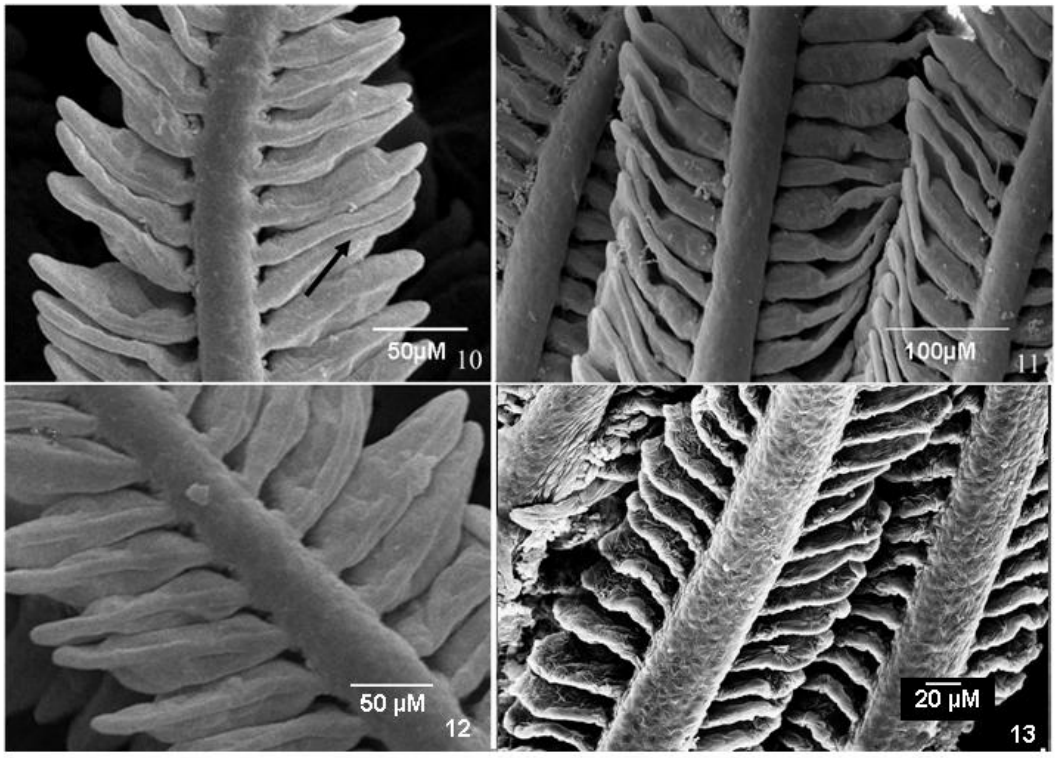
Figure 9. Degree of tissue change in *Trachinotus carolinus* used as controls and exposed to different sublethal concentrations of naphthalene. Grey bars correspond to 24-hour acute exposure and dark bars correspond to 12-day chronic exposure. Asterisks denote significant differences ($P < 0.05$) from control groups. Control refers to clean-water group; ethanol refers to control group exposed to solvent of naphthalene; 0.15 ppm and 0.30 ppm are naphthalene concentrations.

Discussion

Histopathological studies are recommended for the evaluation of fish health. Such studies allow reliable assessments of biochemical responses in animals exposed to a variety of environmental stressors. Alterations in fish gills are among the most commonly recognized responses to environmental pollutants (Mallatt 1985, Laurent & Perry 1991, Au 2004).

The method applied to the Florida pompano, to calculate the degree of tissue change, enabled the comparison of alterations in fish exposed to naphthalene and control groups, and to correlate the effects of exposure to gill functionality. Epithelial

lifting was the first alteration detected. The epithelium covering the secondary lamellae was lifted away from the pillar cell system as a continuous sheet, thereby increasing the diffusion distance between water and blood. This process affects respiration and ionic regulation by inhibiting key transport processes (Mallatt 1985, Wood 2001, Van Heerden *et al*. 2004).



Figures 10-13. Gill lamella with extensive lifting of epithelial cell layer observed under scanning electron microscope. 10. Lamellae of fish exposed to 0.30 ppm of naphthalene after acute exposure. 11. Slight lifting of epithelial cell layer in fish exposed to naphthalene at 0.15 ppm following exposure for 24 hours. 12. Extensive lifting of epithelial cell layer in fish exposed to 0.30 ppm of naphthalene following chronic exposure. 13. Lamellae of control fish after 24 hours of experiment.

The histopathological lesions in both control groups should be considered carefully. Background knowledge on the control conditions is essential to interpreting pathological responses (Poleksic & Mitrovic-Tutundsic 1994). Despite a few sparse lesions, the mean DTC values in the control groups remained within the range expected for gills with normal function. The slight lesions in both control groups may be due to the stress of confinement, as *T. carolinus* normally exhibits intense swimming activity, which characterizes it as a species with a high energy demand (Santos *et al*. 2006).

Although ethanol causes physiological alterations in oxygen consumption and ammonia excretion in fish (Vargas *et al.* 1991, Santos *et al*. 2006), there were no significant histological differences between ethanol and clean-water controls. The concentration of ethanol used in this study, was not considered harmful to gill tissues.

As observed in the pompanos exposed to naphthalene, a number of studies have demonstrated that interstitial edema, or epithelial lifting, is one of the most frequent lesions found in the gill epithelium of fish exposed to pollutants (Mallatt 1985, Sola *et al.* 1995, Bury *et al.* 1998, Figueiredo-Fernandes *et al.* 2007). Epithelial lifting is one of the first responses of gills even with exposure to low concentrations of contaminants (Segers *et al*. 1984). In studies on *Lates calcarifer*, exposed to acute and subchronic cadmium, Thophon *et al.* (2003) also described epithelial lifting in the fish gills.

The fusion of secondary lamellae, hyperplasia and the lifting of epithelial cells, with the presence of edema and an increase in mucus secretion, are the

main lesions described in fish exposed to petroleum hydrocarbons (DiMichele & Taylor 1978, Engelhardt *et al.* 1981, Correa & Garcia 1990, Prasad 1991). All of these alterations affect gas exchange. The complete fusion of secondary lamellae is usually induced by high doses of chemical compounds or is a final result of hyperplasia in sublethal poisoning following chronic exposure (Temmink *et al*. 1989). The non-specificity of the histological gill alterations suggests stereotyped physiological reactions of the gills to stress and many alterations may plausibly be considered defensive reactions (Mallatt 1985).

After 24 hours of exposure, the fish in the control and ethanol groups exhibited normal gills whereas those exposed to naphthalene exhibited slight to moderate alterations affecting the normal functioning of the respiratory organ. When fish are exposed to contaminants, alterations in their normal functions make them more susceptible to parasites and illness (Ahmad *et al*. 2003). As in the present study, Ahmad *et al.* (2003) also found that naphthalene exposure had a harmful effect on cell membranes in *Anguilla anguilla*, impairing cell viability. The significant results of tissue change impairing alteration on the normal function of gills of fish exposed, even to low concentrations of naphthalene as that of 0.15 ppm, indicates that even low doses of pollutants can alter the tissue function and, in spite of being reversible, alterations at the cellular structure can result even in organism death.

Stasis, which restricts blood flow through the lamellae, was evident in both periods in the groups exposed to the greatest concentration of naphthalene. This condition reduces both gas and ion exchange, which is extremely harmful to the organism and may lead to death.

After chronic exposure to naphthalene at a concentration of 0.30 ppm, the fish exhibited the greatest amount of tissue damage, mainly due to the presence of focal necrosis in pillar cells. Necrosis in fish gills is believed to reflect direct harmful effects of irritants (Temmink *et al.* 1983) and cell death is characteristic of irreparable damage. Thus, chronic exposure to high doses of naphthalene affected the vital organs of the fish and the longer the period of exposure led to a higher degree of tissue change in the gills. Rodrigues & Fanta (1998) also report increasing tissue damage associated to exposure time and pollutant concentration. Degenerative lesions are frequently found in fish due to exposure to high concentrations of pollutants and after chronic exposure periods (Pacheco & Santos 2002, Rodrigues & Fanta 1998).

Mallatt (1985) reviewed studies on the main alterations induced by toxic substances in the structure of fish gills and the most common of which were necrosis, hyperplasia, hypertrophy, rupture of gill tissue, lamellar fusion, hyper secretion and proliferation of mucous cells, alteration in chloride cells and vascularization. The author reports that even lamella disorganization and first-stage lesions indicate that the environment is not completely favorable to the development and survival of fish.

The alterations found in the present study proved to be a semi-quantitative response to the action of naphthalene on the gills of *T. carolinus*. The analysis methodology employed can be used in laboratory to analyze the effects of exposure time and dose of toxic substances on fish. Despite the non-specificity of the lesions, histological analysis on fish proved an efficient tool for the assessment of environmental quality, as individual effects of pollutants in nature - thousands of different compounds - are relatively rare. Nonethelless, the assessment of the effect of a single component is very useful for toxicological studies as well as the establishment of monitoring programs and environmental laws.

Acknowledgements

The authors are grateful to the Oceanographic Institute of the Universidade de São Paulo for the facilities offered and the Brazilian fostering agency Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP – 02/10566-7) for the scholarship granted to the first author. We are also grateful for the use of the Nap/Mepa (Esalq) scanning electron microscope.

References

Ahmad, I., Pacheco, M. & Santos, M. A. 2003. Naphthalene-induced differential tissue damage association with circulating fish phagocyte induction. **Ecotoxicology and Environmental Safety,** 54(1): 7-15.

Albers, P. H. 2003. **Petroleum and individual polycyclic aromatic hydrocarbons.** In: Hoffman, D. J., Rattner, B.A., Burton Jr., G. A. & Cairns Jr., J. (Eds.). Handbook of Ecotoxicology. 2nd ed. Lewis Publishers, Boca Raton, Florida, pp. 341-371.

Alkindi, A. Y. A., Brown, J. A., Waring, C. P. & Collins, J. E. 1996. Endocrine, osmoregulatory, respiratory and haematological parameters in flounder exposed to the water soluble fraction of crude oil. **Journal of Fish Biology,** 49(6): 1291–1305.

ATSDR (Agency for Toxic Substances and Disease Registry). 1995. **Toxicological profile for naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene.** Report of the U.S. Department of Health and Human Services, Public Health Service.

Au, D. W. T. 2004. The application of histo-cytopathological biomarkers in marine pollution monitoring: a review. **Marine Pollution Bulletin,** 48(9-10): 817-834.

Black, M. C., Millsap, D. S. & McCarthy, J. F. 1991. Effects of acute temperature-change on respiration and toxicant uptake by rainbow-trout, *Salmo gairdneri* (Richardson). **Physiological Zoology,** 64(1): 145-168.

Bowser, P. 2001. **Anesthetic option for fish.** In: Gleed, R. D. & Ludders, J. W. (Eds.). Recent advances in veterinary anesthesia and analgesia: Companion animals (available at http://www.ivis.org, Document No. A1412.0701).

Britvic, S., Lucic, D. & Kurelec, B. 1993. Bile fluorescence and some early biological effects in fish as indicators of pollution by xenobiotics. **Environmental Toxicology and Chemistry,** 12(4): 765-773.

Bury, N. R.; Li, J., Flik, G., Lock, R.A.C. & Wendelaar-Bonga, S.E. 1998. Cortisol protects against copper induced necrosis and promotes apoptosis in fish gill chloride cells in vitro. **Aquatic Toxicology,** 40(2): 193-202.

Collier, T. K. & Varnasi, U. 1991. Hepatic activities of xenobiotic metabolizing enzymes and biliary levels of xenobiotics in English sole (*Parophyrs vetulus*) exposed to environmental contaminants. **Archives of Environmental Contamination and Toxicology,** 20(4): 462-473.

Correa, M. & Garcia, H. I. 1990. Physiological responses of juvenile white mullet, *Mugil curema*, exposed to benzene. **Bulletin of Environmental Contamination and Toxicology,** 44(3): 428-434.

DiMichele, L. & Taylor, M. H. 1978. Histopathological and physiological responses of *Fundulus heteroclitus* to naphthalene exposure. **Journal of the Fisheries Research Board of Canada,** 35(8): 1060-1066.

Du Preez, H. H., McLachlan, A., Marais, J. F. K. & Cockcroft, A. C. 1990. Bioenergetics of fishes in a high-energy surf-zone. **Marine Biology,** 106(1): 1-12.

Dwivedi, H., Sari, R. & Wate, S. 1997. Long term effects of DI-aromatic hydrocarbon in catfish *Heteropneustes fossilis.* **Journal of Ecobiology,** 9: 247-254.

El-Sayed, N. K., Salem, S. A., Moursy, A. & Ibrahim, B. M. 1995. Acute and chronic toxicity of some aromatic hydrocarbons on *Tilapia zillii*. **Bulletin of the National Institute of Oceanography and Fisheries,** 21: 613-630.

Elumalai, M. & Balasubramanian, M. P. 1999. Influence of naphthalene on esterase activity during vitellogenesis of marine edible crab, *Scylla serrata*. **Bulletin of Environmental Contamination and Toxicology,** 62(6): 743–748.

Engelhardt, F. R., Wong, M. P. & Duey, M. E. 1981. Hydromineral balance and gill morphology in rainbow trout *Salmo gairdneri*, acclimated to fresh and sea water, as affected by petroleum exposure. **Aquatic Toxicology,** 1(3-4): 175-186.

Fanta, E., Rios, F. S., Romão, S., Vianna, A. C. C. & Freiberger, S. 2003. Histopathology of the fish *Corydoras paleatus* contaminated with sublethal levels of organophosphorus in water and food. **Ecotoxicology and Environmental Safety,** 54(2): 119-130.

Figueiredo-Fernandes, A., Ferreira-Cardoso, J. V., Garcia-Santos, S., Monteiro, S. M., Carrola, J., Matos, P. & Fontaínhas-Fernandes, A. 2007. Histopathological changes in liver and gill epithelium of Nile tilapia, *Oreochromis niloticus*, exposed to waterborne copper. **Pesquisa Veterinária Brasileira,** 27(3): 103-109.

Heilman, M. J. & Spieler, R. E. 1999. The daily feeding rhythm to demand feeders and the effects of timed meal feeding on the growth of juvenile Florida pompano, *Trachinotus carolinus*. **Aquaculture,** 180(1-2): 53–64.

Hoese, H. D. & Moore, R. H. 1998. **Fishes of the Gulf of Mexico: Texas, Louisiana and adjacent waters.** 2nd ed. Texas A&M University Press, Texas, 422 pp.

Hymel, M. K., Baltz, D. M., Chesney, E. J., Tarr, M. A. & Kolok, A. S. 2002. Swimming performance of juvenile Florida pompano exposed to ethylene glycol. **Transactions of the American Fisheries Society,** 131(6): 1152-1163.

Irwin, R. J., VanMouwerik, M., Stevens, L., Seese, M. D. & Basham, W. 1997. **Environmental contaminants encyclopedia.** National Park Service, Water Resources Division, Colorado, 114 pp.

Laurent, P. & Perry, S. F. 1991. Environmental effects on fish gill morphology. **Physiological Zoology,** 53(1): 4-25.

Linnaeus, C. 1766. Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Laurentii Salvii, Holmiae. **Systema Naturae,** 12: 1-532.

Mallatt, J. 1985. Fish gill structural changes induced by toxicants and other irritants: a statistical review. **Canadian Journal of Fisheries and Aquatic Sciences,** 42(4): 630-648.

Molven, A. & Gooksoyr, A. 1993. **Biological effects and biomonitoring of organochlorines and PAH in the marine environment.** In: Mervyn, R. (Ed.). Ecotoxicology Monitoring. VCH, New York, pp 137-162.

Nagabhushanam, R., Machale, P. R., Kkatyayani, R. V., Reddy, P. S. & Sarojini, R. 1991. Erythrophoretic responses induced by naphthalene in freshwater prawn, *Caridina rajadhari*. **Journal of Ecotoxicology and Environmental Monitoring,** 1: 185-191.

Neff, J. M. & Anderson, J. W. 1975. An ultraviolet spectrophometric method for the determination of naphthalene and alkyl naphthalenes in the tissues of oil-contaminated marine animals. **Bulletin of Environmental Contamination and Toxicology,** 14(1): 122-128.

Nero, V., Farwell, A., Listen, A., Van Der Kraak, G., Lee, L. E. J., T. Van Meerd, Mackinnon, M. D. & Dixon, D. G. 2006. Gill and liver histopathological changes in yellow perch (*Perca flavescens*) and goldfish (*Carassius auratus*) exposed to oil sands process-affected water. **Ecotoxicology and Environmental Safety,** 63(3): 365-377.

Ortiz-Delgado, J. B., Segner, H., Arellano, J. M. & Sarasquete, C. 2007. Histopathological alterations, EROD activity, CYP1A protein and biliary metabolites in gilthead seabream *Sparus aurata* exposed to Benzo(a)pyrene. **Histology and Histopathology,** 22(4): 417-432.

Pacheco, M. & Santos, M. A. 2001. Biotransformation, endocrine and genetic responses of *Anguilla anguilla* L. to petroleum distillate products and environmental contaminated waters. **Ecotoxicology and Environmental Safety,** 49(1): 64–75.

Pacheco, M. & Santos, M. A. 2002. Biotransformation, genotoxic and histopathological effects of environmental contaminants in European eel (*Anguilla anguilla* L.). **Ecotoxicology and Environmental Safety,** 53(3): 331-347.

Payne, J. F., Mathieu, A. & Collier, T. K. 2003. **Ecotoxicological studies focusing on marine and freshwater fish.** In: Douben, P. E. T. (Ed.). PAHs: An Ecotoxicological Perspective. Wiley, UK, pp. 191-224.

Pitot III, H. C. & Dragan, Y. P. 1996. **Chemical carcinogenesis.** In: Klaasen, C. D. (Ed.). Casarett and Doull's Toxicology: The basic science of poisons. McGraw Hill Publishing, New York, pp. 241-320.

Poleksic, V. & Mitrovic-Tutundzic, V. 1994. **Fish gills as monitor of sublethal and chronic effects of pollution.** In: Müller, R. & Lloyd, R. (Eds.). Sublethal and chronic effects of pollutants on freshwater fish. Fishing News Books, Oxford, pp 339-352.

Prasad, M. S. 1991. SEM study on the effects of crude oil on the gills and airbreathing organs of climbing perch *Anabas testudineus*. **Bulletin of Environmental Contamination Toxicology,** 47(6): 882-889.

Rodrigues, E. L. & Fanta, E. 1998. Liver histopathology of the fish *Brachydanio rerio* Hamilton-Buchman after acute exposure to sublethal levels of the organophosphate dimethoate 500. **Revista Brasileira de Zoologia,** 15(2): 441-450.

Santos, T. C. A., Phan, V. N., Passos, M. J. A. C. R. & Gomes, V. 2006. Effects of naphthalene on metabolic rate and ammonia excretion of juvenile Florida Pompano, *Trachinotus carolinus*. **Journal of Experimental Marine Biology and Ecology,** 335(1): 82-90.

Schirmer, K., Dixon, D. G., Greenberg, B. M. & Bols, N. C. 1998. Ability of 16 priority PAHs to be directly cytotoxic to a cell line from the rainbow trout gill. **Toxicology,** 127(1-3): 129-141.

Schwaiger, J., Bucher, F., Ferling, H., Kalbfus, W. & Negele, R.D. 1992. A prolonged toxicity study on the effects of sublethal concentrations of bis(tri-n-butyltin)oxide (TBTO): histopathological and histochemical findings in rainbow trout (*Oncorhynchus mykiss*). **Aquatic Toxicology,** 23(1): 31-48.

Schwaiger, J., Wanke, R., Adam, S., Pawert, M., Honnen, W. & Triebskorn, R. 1997. The use of histopathological indicators to evaluate contaminant-related stress in fish. **Journal of Aquatic Ecosystem Stress and Recovery,** 6(1): 75-86.

Segers, J.H.L., Temmink, J.H.M., Van den Berg,

J.H.J. & Wegman, R.C.C. 1984. Morphological changes in gill of carp (*Cyprinus carpio* L.) exposed to acutely toxic concentrations of methyl bromide. **Water Research,** 18(11): 1437-1441.

Silva, D. A. M., Buzitis, J., Krahn, M. M., Bícego, M. C. & Pires-Vanin, A. M. S. 2006. Metabolites in bile of fish from São Sebastião Channel, São Paulo, Brazil as biomarkers of exposure to petrogenic polycyclic aromatic compounds. **Marine Pollution Bulletin,** 52(2): 175-183.

Simonato, J. D., Guedes, C. L. B. & Martinez, C. B. R. 2008. Biochemical, physiological, and histological changes in the neotropical fish *Prochilodus lineatus* exposed to diesel oil. **Ecotoxicology and Environmental Safety,** 69(1): 112-120.

Sola, F., Isaia, J. & Masoni, A. 1995. Effects of copper on gill structure and transport function in the rainbow trout, *Oncorhynchus mykiss*. **Journal of Applied Toxicology,** 15(5): 391-398.

Spies, R. B., Stegeman, J. J., Hinton, D. E., Woodin, B., Smolowitz, R., Okihiro, M. & Shea, D. 1996. Biomarkers of hydrocarbon exposure and sublethal effects in embiotocid fishes from a natural petroleum seep in the Santa Barbara Channel. **Aquatic Toxicology,** 34(3): 195-219.

Swapan, C. D., Araki, T. & Fukushima, T. 2000. Polycyclic aromatic hydrocarbons in fish organs. **Marine Pollution Bulletin,** 40(10): 882–885.

Temmink, J. H. M., Bouwmeister, P., de Jong, P. & Van Den Berg, J. 1983. An ultrastructural study of chromate-induced hyperplasia in the gill of rainbow trout (*Salmo gairdneri*). **Aquatic Toxicology,** 4(2): 165-179.

Temmink, J. H. M., Field, J. A., Van Haastrecht, J. C. & Merkelbach, R. C. M. 1989. Acute and subacute toxicity of bark tannings in carp (*Cyprinus carpio*). **Water Research,** 23(3): 341-344.

Thophon, S., Kruatrachue, M., Upatham, E. S., Pokethitiyook, P., Sahaphong, S. & Jaritkhuan, S. 2003. Histopathological alterations of white seabass, *Lates calcarifer*, in acute and subchronic cadmium exposure. **Environmental Pollution,** 121(3): 307-320.

Upshall, C., Payne, J. F. & Hellou, J. 1993. Induction of MFO enzymes and production of bile metabolites in rainbow trout (Oncorhynchus mykiss) exposed to waste crankcase oil. **Environmental Toxicology and Chemistry,** 12(11): 2105-2112.

Van Heerden, D., Vosloo, A. & Nikinmaa, M. 2004. Effects of short-term copper exposure on gill structure, methallothionein and hypoxia-inducible factor-1α (HIF-1α) levels in rainbow trout (*Oncorhynchus mykiss*). **Aquatic Toxicology,** 69(3): 271-280.

Vargas, M., Correa, M. & Chung, K. S. 1991. Indicadores fisiológicos en la evaluación de la toxicidad de hidrocarburos aromaticos. **Boletin del Instituto Oceanográfico de Venezuela,** 30(1): 57-64.

Vijayavel, K., Gomathi, R. D., Durgabhavani, K. & Balasubramanian, M. P. 2004. Sublethal effect of naphthalene on lipid peroxidation and antioxidation status in the edible marine crab *Scylla serrata*. **Marine Pollution Bulletin,** 48(5-6): 429-433.

Wakeham, S. G., Davis, A. C., Karas, I. L. 1983. Mesocosm experiments to determine the fate and persistence of volatile organic compounds in coastal waters. **Environmental Science & Technology,** 17(10): 611-617.

Winkaler, E. U., Silva, A. G., Galindo, H. C. & Martinez, C. B. R. 2001. Biomarcadores histológicos e fisiológicos para o monitoramento da saúde de peixes de ribeirões de Londrina, Estado do Paraná. **Acta Scientiarum,** 23(2): 507-514.

Wood, C. M. 2001. **Toxic responses of the gill.** In: Schlenck, D. & Benson, W. H. (Eds.). Target organ toxicity in marine and freshwater teleosts. Taylor & Francis, London, pp. 1-89.

Yu, Y., Wade, T. L., Frang, J., McDonald, S. & Brooks, J. M. 1995. Gas chromatographic-mass spectrometric analysis of polycyclic aromatic hydrocarbon metabolites in Antarctic fish (*Notothenia gibberiforns*) injectes with diesel fuel arctic. **Archives of Environmental Contamination and Toxicology,** 29(2): 241-246.

Zanardi, E. 1996. Hidrocarbonetos no canal de São Sebastião e na plataforma interna adjacente – influência do derrame de maio de 1994. **Master's Thesis.** Universidade de São Paulo, São Paulo, Brazil, 112 pp.

Received December 2010
Accepted April 2011
Published online November 2011