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Toxic *Prorocentrum lima* induces abnormal behaviour in juvenile sea bass

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Abstract Juveniles of the European sea bass *Dicentrarchus labrax* were exposed to both cell-free medium and whole cell cultures of the dinoflagellate *Prorocentrum lima* strain PL2V. Fish were also fed a commercial fish diet in tanks containing live *P. lima*, and *Artemia* that had ingested the alga. Fish exposed to the cell-free medium and to whole cell cultures were stressed and behaved abnormally when compared to the behaviour of control fish, fish in normal seawater. Stress-related behaviours included hyperactivities (jumps, fast let-right turns, surface swims, etc), poor feeding reflexes and abstinence from feeding. Fish that directly ingested the alga or that ingested *Artemia* containing the alga died. Histological studies revealed that gills and liver of treated fish were impacted, as opposed to the normal conditions of same tissues in control fish. The diseased organs could have been responsible for the abnormal behaviours and death of treated fish. The aquaculture and ecological implications of the results are discussed.

Keywords Behaviour · *Dicentrarchus labrax* · Dinoflagellate · Fish mortality · Gill and liver damages · Histological examination · Stress

Introduction

Microalgal species are especially attractive in aquaculture operations for rearing fish juveniles and shellfish larvae. A mixture of algal species (Phycopure) is employed to raise shrimps (Heerbrand and Lin 2006), while a good number of other microalgal species that include *Isochrysis galbana* and *Pavlova lutheri* are acceptable starter food for winged pearl oyster *Pteria sterna* (Martínez-Fernández et al. 2004). Similarly, juveniles of the tilapia *Oreochromis niloticus* raised on *Spirulina platensis* demonstrated enhanced growth (Lu et al. 2004). In natural waters fish also readily graze on microalgal species. For example, gizzard shad *Dorsoma cepadianum* suppresses *Ceratium* spp populations by feeding massively on them (Drenner et al. 1984), while *Tilapia galilea* suppresses populations of *Peridinium* spp by also extensively feeding on them (Drenner et al. 1987). Not all algae are beneficial to fisheries or aquaculture. Some are harmful. Prince et al. (2006) observed that microalgae exhibit a diversity of morphologies, nutritional values, and potential chemical defences (the harmful species) that could affect the feeding and fitness of predators.

Blooms of harmful microalgal species constitute a serious threat to fisheries and aquaculture in many ways, which include phycotoxin-induced fish diseases and mortalities. Cell concentration and cell toxicity play important roles in determining the level of impacts associated with phycotoxins (Runge et al. 1992; Kim et al. 2000a,b). When phycotoxins appear, fish behaviour is first impacted, leading to a cascade of events that result in fish kills. Fish may stop feeding or show signs of suffocation. Fish behaviour, then, is used as an indicator that give fish farmers early warnings on changes in environmental or fish health conditions (Linden and Al Houari 1993; Juell 1995; Ajuzie 1998).

Phycotoxins kill fish by altering tissue structure of organs and, by so doing impose some physiological disturbances on the fish. Gill, liver and intestinal tissues are the most attacked (Jones et al. 1982; Takayama and Adachi 1984; Phillips et al. 1985; Okaichi 1989; Toyoshima et al. 1985; Arzul et al. 1998; Lush et al. 1998; Ajuzie and

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Houvenaghel 2003). Herbivorous, zooplanktivorous and detritivorous fish can directly or indirectly ingest phycotoxin-producing algae and/or phycotoxins in the aquatic food webs (e.g. White 1981a,b; Kelly et al. 1992).

In the wild or in fish farm units several microalgal species have been identified as fish killers. These include phytoplanktonic *Prorocentrum* species like *Prorocentrum balticum*, *P. concavum* and *P. dentatum, P. micans, P. minimum, P. sigmoides* and *P. triestinum* (Rabbani et al. 1990; Ho and Hodgkiss 1993; Steidinger 1993; Grzebyk et al. 1997; Noga 1998; Rangel 2002; Lu and Hodgkiss 2004; Kudela et al. 2005). However, the benthic and epiphytic *P. lima* has not been associated with wild or cultured fish kills (Ajuzie 2002). The animal impact is unknown (see http://waves.marine.usf.edu/redtide_menu/dinos.html). *P. lima* is a producer of diarrheic shellfish poisoning (DSP) toxins, which include okadaic acid (OA) and dinophysistoxins (DTXs) (Lee et al. 1989; Bravo et al. 2001). These toxins readily accumulate in shellfish and provoke DSP in humans eating such tainted seafood. *P. lima* has been shown to be toxic to *P. micans* (Ajuzie and Houvenaghel 2001) and to the brine shrimp *Artemia* (Ajuzie 2007). OA also accumulates in fish tissues after appearing in the food chain (Edebo et al. 1992). Therefore, a hypothesis that *P. lima* can negatively impact the behaviour of juvenile fish and cause their death was investigated using juveniles of the European sea bass *Dicentrarchus labrax*. The hypothesis that fish behaviour alters because toxic algae impact on body organs was investigated histologically. Lastly, the hypothesis that feeding trapped or farmed fish during harmful algal bloom (HAB) events would facilitate their death was also investigated in *P. lima's* cell-free medium and in whole cell cultures.

Materials and methods

One hundred day old juveniles of the European sea bass (*Dicentrarchus labrax*) (hereafter referred-to as fish) were acquired from a commercial fish hatchery in France. They were transported to Université Libre de Bruxelles, Belgium in an oxygenated enclosure with water salinity at 12‰. During transportation and acclimatization in the laboratory, no fish died. The fish were acclimatized at 18±2°C room temperature and in natural seawater diluted with dechlorinated tap water to 24‰. They were fed the same commercial fish diet that was employed at the hatchery in France, 24 hours after arriving the laboratory, by which time they had recovered from the handling stress.

Prorocentrum lima and seawater

The PL2V strain of *Prorocentrum lima* used for this study was obtained from Instituto Español de Oceanografia, Vigo, Spain. Various workers that include Pillet et al. (1995), Barbier et al. (1999) and Bravo et al. (2001) have determined the toxin profile of this strain and reported that it consists mainly of okadaic acid (OA) and dinophysistoxin-1 (DTX-1). They, however, reported the quantity of OA to be higher than that of DTX-1. Rausch de Traubenberg and Morlaix (1995), in addition, reported that for the PL2V strain 19 to 29% of its toxin is present in the cell-free medium. The acquired inoculum was cultured in bacteria-free K-medium enriched seawater (Keller et al. 1987), at 24±1°C and at 60.19 μmol photons s^{-1} m^{-2} at a distance of ca. 10 cm from the light source, on a 12:12 h light and dark cycle. Seawater was collected from the English Channel, filtered under low vacuum on Whatman GF/C filters, and autoclaved for one hour before use.

Experiments with *P. lima* cell-free medium and live cells in culture

Prorocentrum lima cell-free medium was obtained through the gravity filtration method on cultures with about 9×10^3 cells mL^{-1}. This was achieved by gently filtering the cultures on Whatman GF/C filters to avoid rupturing of the cells (Lush and Hallegraeff 1996; Tang and Dam 2001; Ajuzie 2007). Two litres of the obtained cell-free medium was poured separately into two 4 L glass tanks, after which 10 fish were introduced into each of the tanks. In another set of experiments, 2 L of *P. lima* culture (cell concentration: ca. 9×10^3 cells mL^{-1}) were introduced into two 4 L glass tanks. Ten fish were introduced into each of the tanks after stirring the medium. The medium was stirred once a day throughout the duration of the investigation. In both sets of experiments, the two study tanks for each set were separated by a piece of plywood, thus preventing fish from one tank from seeing fish in the other tank. The observer who recorded the behaviours of the treated fish could see the two tanks at the same time. To avoid disturbing the fish and any possible interference on the fish behaviour, fish used in these experiments were not fed during the study period, which lasted one week. Air pumps supplied air to all tanks. Behaviours of treated fish were compared with those of the control fish (fish in natural seawater).

Experiment with clumps of harvested dried *P. lima* cells

Ten fish were introduced into two 4 L glass tanks containing natural seawater. *P. lima* cultures were gently filtered on Whatman GF/C filters. The cells were scraped off the filters and set aside to dry. Clumps of the dried mass were then presented to the fish.

Feeding fish with fish diet amidst live *P. lima* cells

Ten fish were held separately in two 6 L tanks, each containing 2 L of natural seawater and 2 L of *P. lima*

culture (with ca. 4.5×10^3 cells mL^{-1}). The fish were fed on a commercial fish diet during 6 weeks. Before the diet was introduced into the tanks, the tank water was moderately stirred to bring the *P. lima* cells into suspension. Fish behaviours were monitored and catalogued during the study period. Any dead fish was removed and recorded. The medium in which the fish were held was changed once, at the end of the third week, with about the same concentration of *P. lima* cells.

Feeding fish with *P. lima*-contained *Artemia*

Artemia nauplii, hereinafter referred-to as brine shrimp, were obtained after *Artemia* cysts were treated for hatching. The cysts were incubated at 27 to 29°C in a mixture of filtered seawater and distilled-water, with salinity at 20‰. Hatching occurred within 24 hours. Only metanauplii were employed in these experiments since they fed readily on the alga (Ajuzie 2002, 2007). *Prorocentrum lima* was fed to the brine shrimps. After one hour the brine shrimps were harvested on a sieve and fed to fish in two study tanks, each with 10 fish. Optimally fed brine shrimps ingested *P. lima* cells within the first hour of their initial contacts with the cells (Ajuzie 2002, 2007). These experiments lasted six weeks.

Histological examinations of fish tissues

At the end of the six weeks study period, two fish were collected each from among those fed on: (i) *P. lima*-contaminated brine shrimp, and (ii) a commercial fish diet amidst cells of *P. lima*. Another group of four fish were captured from the control tanks. All captured fish were treated for histological examinations of the gill, liver, kidney, stomach and intestinal tissues, using standard methods. The fish were killed by a quick partial cut at the junction between the head and the trunk, and preserved rapidly so as to prevent the occurrence of any post-mortem artefacts (Roberts 1978; Speare and Ferguson 1989).

Results

Fish behaviour

Fish that were placed in *P. lima* cell-free culture medium and in cultures with living *P. lima* suffered stress. They became frenzied during their initial contacts with the media, behaving quite differently from the control fish that did not show any sign of stress. Immediately after the fish were brought into contact with the cell-free medium, they repeatedly jumped out of the medium into the air, and exhibited pronounced opercular movements, window creepings and window pushings near the surface, surface swims with opened mouth, and fast left-right turns. These behaviours were highly pronounced during the first two hours in the cell-free medium. The hyperactivity subsided only after about this time. During initial contacts, fish that were in tanks with live cells of *P. lima* also exhibited jumps, pronounced opercular heaves, and surface swims with opened mouth. However, the hyperactivities subsided after the cells had settled to the bottom and the water became "clear".

Fish that were offered dried clumps of *P. lima*, rejected them. They approached the clumps, sometimes touching them with their snout, but never ingested them. Fish that hastily picked a descending clump of *P. lima* immediately spat it out. Fish in tanks with cells of *P. lima* fed on the commercial diet presented to them. Similarly, those that were fed contaminated brine shrimp accepted the prey. However, fish in the latter groups stopped feeding during the third week, and progressively became less active from then on. The loss of activity persisted until fish death occurred. They also exhibited surface swims with open mouth, which gradually changed into surface swims with permanently opened mouth. The latter situation signalled the commencement of non-feeding (total abstinence from food) by the fish. But before these stages of surface swims with permanently opened mouth and non-feeding, test fish were not able to aim properly at food items. Attempts to capture food particles ended with misses. All treated fish exhibited bursts of uncoordinated swims, head-standing, loss of righting reflex, rolling over, spiral swims and loss of feeding coordination before dying.

Mortalities

No fish that was exposed to the cell-free culture medium died, even after staying a full week in the medium without feeding. Similarly, no dead fish was seen among those brought into contacts with live cells of *P. lima*, but which were not fed during the week-long investigation. Mortalities were, however, recorded among fish that were fed *P. lima*-contaminated brine shrimp and among fish that were kept in tanks with living *P. lima* cells and fed a commercial diet (Table 1). Seventy five percent of fish fed *P. lima*-contaminated brine shrimp died by the end of the six weeks study. They started dying during the fourth week. The majority of them died during the fifth week. Fish in this treatment steadily emaciated as they refrained from feeding, particularly from the third week. Fish that were fed the commercial diet in tanks with live cells of *P. lima*, ingested *P. lima* along with the diet (Plate 1). Ninety percent mortality was recorded for this group during the six week experimental period. Fish started dying during the third week. At the end of the experimental period, only two

Table 1 Percent (%) mortalities of treated vs. control fish

Week	Contaminated *Artemia* (% mortality)	Live *P. lima* + diet (% mortality)	Control (% mortality)
1	0	0	0
2	0	0	5
3	0	35	0
4	5	55	0
5	60	0	0
6	10	0	0

fish were remaining. In the control tanks, only 5% mortality was recorded during the six weeks study period.

Histological examinations

The alimentary tract of fish fed in tanks with live *P. lima* showed they ingested the alga as well as the fish diet (Plate 1). Kidney, stomach and intestinal tissues of fish directly or indirectly exposed to *P. lima* (treated fish) showed no histopathological alteration(s). However, gill and liver tissues of treated fish were highly impacted. The gill and liver tissues of the control fish were normal (Table 2; Plates 2, 3, and 4).

Discussion

Fish behaviour

The general behaviour of treated fish was different from that of control fish. Both the cell-free medium and medium with living *P. lima* caused fish brought into contact with them to be stressed and to behave abnormally. Several workers attest to fact that toxic dinoflagellates induce abnormal behaviours in fish brought into contact with them. For example, juveniles of the red sea bream, *Pagrus major*, and the Japanese anchovy, *Engraulis japonica,* fed on *Gonyaulax excavata* (=*Alexandrium tamarense*)-contaminated-zooplankton lost their equilibrium and swam on their sides, upside down or in circles (White et al. 1989). When pure ciguatoxins-1 & 2 and brevetoxin-1 were added to water containing the mosquito fish *Gambusia affinis* they exhibited pronounced opercular movement and uncoordinated swimming (Lewis 1992). When young tilapine cichlids were fed brine shrimp containing *Gambierdiscus toxicus* cells, they displayed behavioural abnormalities that ranged from spiral swimming to loss of equilibrium Kelly et al. 1992). Similarly, juvenile green back flounder, *Rhombosolea taparina,* exhibited rapid bursts of uncontrolled swimming, heaving of the operculum/mouth, small/rapid convulsive movements of the whole body and loss of orientation when exposed to the dinoflagellate *Alexandrium minutum* (Lush et al. 1998). The present work corroborates these findings and suggest that *P. lima* is toxic to juveniles of the European sea bass. It has even been suggested that OA is genotoxic and induces DNA-adduct formation in fish embryos (Huynh et al. 1998). Torigoe et al. (1988) suggested that *P. lima* produces neurotoxins. The neurotoxins may have been responsible for the witnessed neurological disorders among the treated fish, and which involved exaggerated reflexes, loss of feeding coordination and signs of asphyxiation.

Fish that were in contact with live cells of *P. lima* accepted the commercial fish diet presented to them during the first two weeks, but during the third week they became non-feeders. Fish ingested *P. lima* cells along with the commercial fish diet. The effects of the ingested *P. lima* appeared not to have been immediate, so the fish continued to feed on the introduced fish diet. But from the third week, when the toxins of *P. lima* must have started working in the fish, they stopped feeding and progressively became less active until they died. It is, therefore, suggested that a chronic exposure of juvenile fish to *P. lima* would impact their feeding behaviour.

It was observed that *P. lima* exudes a strong smell. This strong smell, it is believed, influenced the initial reactions of fish to the flakes of *P. lima* cells presented to them. Fish do have a strong sense of smell and are attracted to feed with agreeable aroma (Ajuzie and Appelbaum 1993). Apart from its repelling smell, there is also the possible that its taste is unattractive to fish, for fish spat out clumps of *P. lima*. Behavioural or gustatory rejection of toxic dinoflagellates has also been observed with fish larvae fed on a paralytic shellfish toxin species (see Yamamori et al. 1988; Robineau et al. 1991).

The disagreeable odour and taste of *P. lima* might be of some significance to its autecology. Grazers heeding to the apparent warning messages associated with its smell and taste will refrain from preying on it. Occurrence of blooms

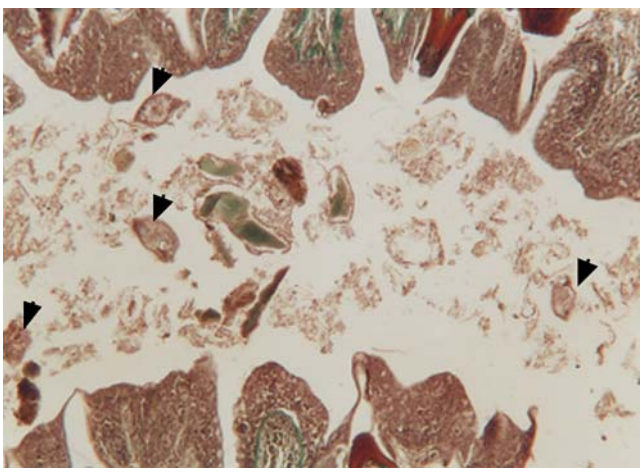

Plate 1 *Prorocentrum lima* in the digestive tract of treated fish (arrowed)

Table 2 Gill and liver pathologies: treated vs. control fish

Organ	Treated fish	Reference	Control fish	Reference
Gill	Swollen and lifted epithelium	Plate 2b	Norrmal gill lamellae	Plate 2a
	Fused and congested epithelium	Plate 2c		
	Mucous and blurred tissues	Plate 3a		
	Vacuolated tips	Plate 3b		
	Ruptured lamellae	Plate 3c		
Liver	Swollen and congested parenchyma	Plate 4b&c	Normal parenchyma	Plate 4a
	Necrotic and eroded parenchyma	Plate 4d		

of the toxic dinoflagellate *Karenia brevis* is due to grazer-avoidance (Prince et al. 2006). However, all grazers may not heed to these seemingly warning features of *P. lima*, but might consume it to their detriment. For example, *Artemia* grazes continuously on *P. lima* until it is killed by the ingested cells (Ajuzie 2007).

An interesting behaviour of fish that were in contact with the cell-free medium and cells of *P. lima* was jumping. Fish repeatedly jumped out of the different media into the air. It may have been that enough oxygen was not passing over the gills of fish due to the high viscosity of the cell-free medium, even though the media holding the fish were aerated. Jenkinson and Arzul (1998) observed that *Gymnodinium mikimotoi* (=*Karenia mikimotoi*) could thicken the water with mucus, causing fish to need more oxygen to fuel water pumping over their gills, than can be extracted from the same water. In the current work, the culture medium of *P. lima* was observed to be viscous and gummy. It could have also been that OA produced by *P. lima* (Rausch de Traubenberg and Morlaix 1995; Bravo et al. 2001) caused tissue irritation in the treated fish. The viscous and gummy culture medium was also observed to cause human skin irritation. Sueoka and Fujiki (1998) even reported that OA is a potent skin-tumour promoter.

Sea bass juveniles display a high level of social interactions (Ajuzie 1998). Fish of the same age do not grow at the same rate (Houvenaghel and Huet 1987, 1989; Ajuzie 1998). Similarly, fish used in these experiments, though of same age, were not of same size. Under normal conditions, as in the control tanks, bigger intracohort siblings exhibit various dominant traits against the smaller subordinates (Ajuzie 1998). But the treated fish were somewhat passive and exhibited no agonistic behaviour as has been witnessed and described for this species in Ajuzie (1998).

Fish mortality

No mortalities were recorded among fish exposed to *P. lima* cell-free medium, even though fish were not fed during the one-week experimental period. This was the same for fish that were in tanks with live *P. lima*, but which were not fed. The implication is that fish might survive toxic blooms by not feeding. Therefore, withholding feeding of trapped fish, as in pens and cages, during harmful algal blooms could prevent mass mortalities of the farmed fish. This is in agreement with the findings of Rensel (1995). Rensel observed that one of the most effective and least costly mitigation practices for finfish

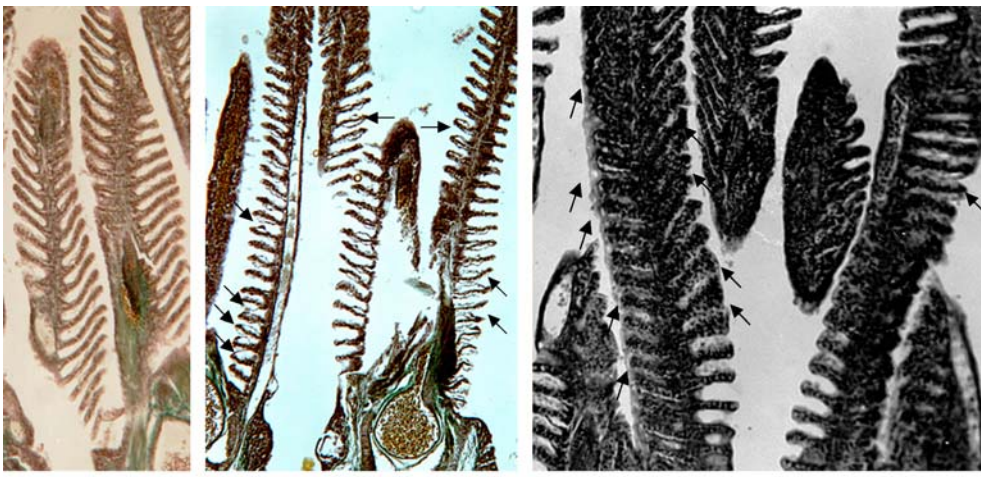

Plate 2 **a**: Normal and healthy gill tissues from control fish. **b**: Affected gills were swollen and showed lifted epithelium (arrows). **c**: Gill epithelial fusion and congestion (arrows)

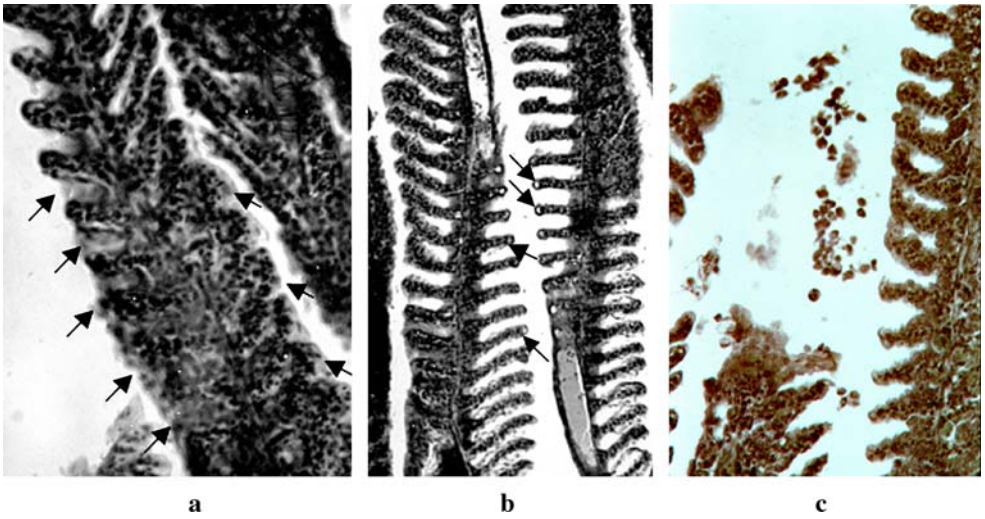

Plate 3 **a**: Mucous and blurred gill tissues (arrows). **b**: Vacuolated tips in affected gills (arrows). **c**: Ruptured lamella in affected gills

aquaculture is to withhold feeding immediately prior to, and during minor harmful algal bloom episodes, arguing that this reduces the digestive demand for oxygen that is still needed for other physiological functions.

Mortalities were recorded in tanks holding treated fish. From the third week, fish fed contaminated *Artemia* nauplii and those that were fed the commercial diet in tanks holding living *P. lima* cells progressively emaciated till they died. Fish emaciation and death occurred after periods of non-feeding. For example, during the first two weeks, fish in tanks harbouring *P. lima* continuously fed on the introduced commercial fish feed. During feeding, they also ingested the re-suspended cells of *P. lima*. During the third week, however, the rate of feeding was reduced and some fish even stopped feeding. Histological examinations of gills and liver of treated fish revealed diseased and degenerated tissues.

Failures in gill respiratory and osmoregulatory functions may have contributed to fish death. Gill swellings and the subsequent separation of lamellar epithelia from the gill vessels could have affected gaseous exchange to the extent that death resulted, since the secondary lamellae of gills are the principal respiratory tissues (Laurent 1984; Ajuzie and Houvenaghel 2003). Lifting of the gill lamellar epithelium

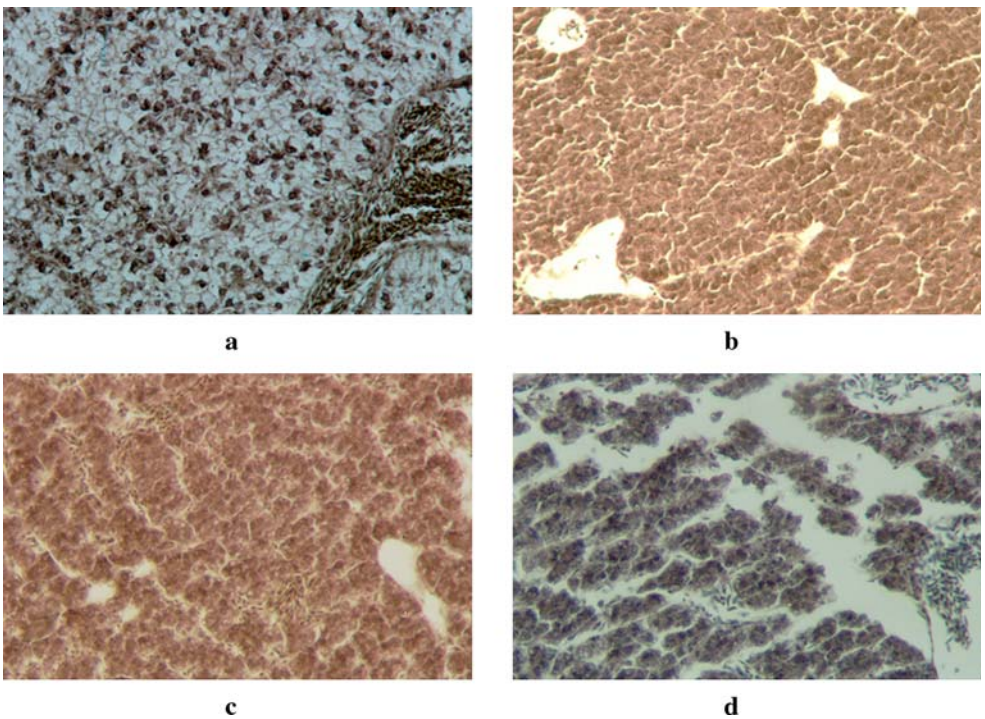

Plate 4 **a**: Normal and healthy liver tissue from control fish. **b** and **c**: Swollen and congested parenchyma in affected liver. **d**: Affected liver suffered parenchymal necrosis and erosion

will impair oxygen transfer as a result of the increased distance between water and secondary lamellar capillaries. Also, gill swellings will reduce water space between adjacent lamellae, and thus the amount of oxygen available to the gill. The secondary lamellar structure is thus, optimized for exchange with the environmental medium with short diffusion distance (Pärt et al. 1982; Ajuzie and Houvenaghel 2003).

Secreted mucus appeared to have overwhelmingly covered the respiratory epithelium of the primary and secondary gill lamellae causing the aorta blood to become hypoxic. Hypoxia is capable of inducing a cascade of events that can disrupt the normal metabolic systems of fish and cause their death (Yang and Albright 1992; Ajuzie and Houvenaghel 2003). Since *P. lima* has no noticeable spines, it might be that OA and DTX produced by the PL2V *P. lima* strain (Pillet et al. 1995; Barbier et al. 1999; Bravo et al. 2001) caused the damage observed on the gills of affected fish. Fish could absorb biotoxins through the gills (Hughes and Perry 1976; Colin et al. 1979; Pärt et al. 1982; Haya et al. 1990). However, further work is needed to ascertain the role(s) of OA and DTXs in organ pathologies observed in fish exposed to *P. lima* cells.

Prorocentrum lima caused fatal degeneration of hepatic tissues among treated fish. This appeared in the form of liver necrosis and generalized loss of liver tissue architecture. Hydropic degeneration, hypertrophy, and dissociation of the hepatocytes may have led to the dissolution of the parenchymal architecture, loss of tissue integrity and ultimately loss of liver functions. Apart from the classical DSP toxins of OA and DTX, *P. lima* might produce hepatotoxin(s) as well. Microcystins, known hepatotoxins, induced similar pathological effects in fish liver (Phillips et al. 1985; Kent et al. 1988; Kent 1990; Råbergh et al. 1991; Andersen et al. 1993). Affected fish could have died from haemodynamic shock, resulting from congestion of the liver.

Both the gill and liver poisonings witnessed here, suggest that *P. lima* strain PL2V is ichthyotoxic. Jones et al. (1982) proposed a hypothesis involving ichthyotoxins when a bloom of *Gyrodinium aureolum* damaged the gills of farmed salmon and caused their death. Similarly, Lush et al. (1998) suggested that cells of the toxic dinoflagellate *Alexandrium minutum* are ichthyotoxic when they caused degenerative and mortal changes in the gills of fish. These results are at variance with the observations of Kohler et al. (1989) who reported that no reaction when ocean surgeon *Acanthuris bahianus* were fed various quantities of *P. lima*. Perhaps the *P. lima* employed by Kohler and co-workers was a less toxic strain, compared to the highly toxic PL2V strain employed in this work. Various workers including Lee et al. (1989) and Bravo et al. (2001) have demonstrated that toxicity in *P. lima* varies with strains. It could also be that the ocean surgeon is more resistant to *P. lima* than the European sea bass.

Prorocentrum lima may have been involved in fish kills in natural waters, but because of its cryptic nature, it evades sampling during such episodes. During fish kills, sediment, floating structures and materials that include detritus, leaves and wood, as well as other macroplant materials in the vicinity of the event should be sampled and analyzed before ruling out the involvement of *P. lima*. Fish in contact with *P. lima* toxins may die from chronic exposure to this toxic alga. This is not the case for the paralytic shellfish-poisoning species *Alexandrium minutum* or for the ichthyotoxic species *Chattonella* and *Cochlodinium*, which actions are acute. Juveniles of the green back flounder, *Rhombosolea taparina*, exposed to whole cell culture and cell-free medium of *A. minutum* died within three hours (Lush et al. 1998). While yellowtails, *Seriola quinqueradiata*, exposed to *C. antiqua* died within 25–90 minutes (Toyoshima et al. 1985). And juveniles of *Leiognathus nuchalis* exposed to *Cochlodinium* species died within 48 hours (Yuki and Yoshimatsu 1989).

Prorocentrum lima is typically benthic or epiphytic (Bomber et al. 1988; Faust 1993a,b). Thus, it was necessary to stir the medium holding the cells so as to bring them into the water column, prior to feeding. This was done to sort of mimic any climatological and/or hydrological conditions like storms, upwellings, destratification and mixing that could disturb the substratum on which *P. lima* dwells, and, by so doing, bring the cells into the water column. Results from this work show that if any of such events occurs and persists for a while, in the vicinity of *P. lima* populations, juvenile fish can be seriously impacted, as they might directly or indirectly ingest *P. lima* during feeding. Plankton filter-feeding and epibenthic micrograzing are largely a non-selective processes through which predators can ingest toxic algae.

In fish farm operations, the physical structures of cages and pens create room for the development of fouling macroalgae and other organisms on which *P. lima* lives and flourishes as an epiphyte (Lawrence et al. 2000). *Prorocentrum lima* can also hang or adhere directly on the structures. During storms, any hanging *P. lima* might be thrown into (re-suspended in) the medium with caged fish. This might be disastrous, especially if the *P. lima* cells are in high concentrations, and if fish are fed. DSP toxicity outbreaks have been linked to re-suspended *P. lima* in natural waters (Lawrence et al. 1998). Though *P. lima* is not likely to be an acceptable food for juveniles of the European sea bass, it might be picked up in and/or with acceptable food items during feeding.

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