JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Volume 36, No. 1
March 2005

Recurrent Amyloodiniosis on Broodstock of the Brazilian Flounder *Paralichthys orbignyanus*: Dinospore Monitoring and Prophylactic Measures.

PAULO C. ABREU[1]
Departamento de Oceanografia, Fundação Universidade Federal do Rio Grande – FURG. Cx. P. 474, Rio Grande – RS 96201-900 Brazil

RICARDO B. ROBALDO
Departamento de Ciências Fisiológicas, Fundação Universidade Federal do Rio Grande – FURG, Cx. P. 474, Rio Grande – RS 96201-900 Brazil

LUÍS A. SAMPAIO
Departamento de Oceanografia, Fundação Universidade Federal do Rio Grande – FURG, Cx. P. 474, Rio Grande – RS 96201-900 Brazil

ADALTO BIANCHINI
Departamento de Ciências Fisiológicas, Fundação Universidade Federal do Rio Grande – FURG, Cx. P. 474, Rio Grande – RS 96201-900 Brazil

CLARISSE ODEBRECHT
Departamento de Oceanografia. Fundação Universidade Federal do Rio Grande – FURG, Cx. P. 474, Rio Grande – RS 96201-900 Brazil

Abstract

Broodstock of the Brazilian flounder *Paralichthys orbignyanus* (Valenciennes, 1839) kept in the laboratory suffered recurrent heavy infestations by the ectoparasitic dinoflagellate *Amyloodinium cf. ocellatum*. Between 10 January and 26 February 2003 we monitored *A. cf. ocellatum* dinospore (infectious motile stage) abundance in a maturation system in order to predict amyloodiniosis outbreaks. Though daily water exchange rate of the tank containing the specimens was 150% of total tank volume (2,500 L), by 15 January the dinospore abundance in the tank reached 1,800 cells/L and on 25 January 7,200 cells/L. There was a subsequent small decrease in dinospore abundance, but by the end of the study period counts were still around 3,000 cells/L. Infested fish were successfully treated with copper sulfate (1.5-mg Cu/L for 24 h during 7d). Observation of the biofilm from the bottom of the tank showed a high number of resting cysts (tomonts) of *A. cf. ocellatum* after treatment. Apparently, the copper sulfate forced the detachment of the trophonts (feeding parasitic growth stage), and generated the high number of tomonts at the bottom of the tank. The copper sulfate concentration used in the treatment was not effective to kill the tomonts. After a disease outbreak in March 2002 and fish recovery, the biofilm with tomonts at the bottom of the tank was removed by brushing and the use of hydrochloric acid (HCl 30% v/v). After this, no infestation occurred for at least a month. Meanwhile, fish in a nearby tank, where biofilm was not removed, had three amyloodiniosis outbreaks. Our results show that the water exchange rate applied was not sufficient to eliminate the dinospores from the water column, or to remove and eliminate the tomonts from the biofilm. We suggest that cleaning the biofilm of tanks after treatment of infested fish should be considered as a prophylactic measure in order to avoid recurrent amyloodiniosis.

[1]*Corresponding author*

Amyloodiniosis, also known as "velvet," "rust," "gold dust disease," or "coral fish disease," is caused by the ectoparasitic dinoflagellate *Amyloodinium ocellatum* (Brown 1931) (Order Blastodinida, Family Oodinidae), which infests gills and skin of both marine and brackish water fish (Lom and Dyková 1992). The life cycle of *A. ocellatum* comprises a feeding parasitic growth stage (trophont), and two free living stages—a resting cyst (tomont) and a disseminative and infectious motile stage (dinospore). After some time the trophont detaches from the fish and falls to the bottom, where it forms a cyst before cell division. The encysted tomont undergoes repeated divisions. Each tomont may release as many as 256 dinospores, which are capable of infesting susceptible fish by swimming towards and attaching to the external surface of the fish on contact (Brown and Hovasse 1946). The dinospore has a characteristic red stigma located at the hyposome, close to the sulcus.

Dinospores remain infective for at least 15 d (Lom and Dyková 1992). They firstly infest the gills, which become swollen, oedematous and hyperplastic after suffering a massive infestation from developing trophonts (Paperna 1980; Lom and Dyková 1992). Gill infestation results in the fish respiring rapidly due to respiratory difficulties. Other typical clinical signs of parasitic dinoflagellate infestation in fishes are anorexia, equilibrium loss, lethargy, darkened color, and "flashing" behavior (Montgomery-Brock et al. 2001). Besides gill infestation, trophonts also occur on the fish's skin causing a velvety appearance. However, accurate diagnosis can only be conducted by microscopic observation of skin or gill scrapes (Reed and Francis-Floyd 1994; Ramos and Oliveira 2001). The signs mentioned above appear only in an advanced stage of illness, and fish require adequate treatment in order to reduce mortality. In closed systems, infestation levels can build up rapidly. Without appropriate management actions, fish mortalities by *Amyloodinium* can be heavy (Landsberg et al. 1994). Thus, this disease represents a serious problem to intensive fish mariculture and aquaria (Lawler 1977; Paperna 1980; Noga et al. 1991; Eiras 1994; Landsberg et al. 1994, 1995).

Flounder are a valuable global commodity characterized by an expanding market, which fisheries will not be able to sustain in the long term. The culture of several flounder species is established in Asian and European countries, but this represents a small percentage of the market. The total sale of flounder in 2000 was 1,050,426 metric tons (FAO 2000a, 2000b), while aquaculture production supplied only 2.5% of this amount, most of it (80.6%) in Korea and Japan (FAO 2000b). Due to the strong economic potential of this market, interest in flounder aquaculture has increased in the Americas in recent years (Benetti et al. 2001).

Among several species of flatfish that could be produced in Brazil, the Brazilian flounder *Paralichthys orbignyanus* (Valenciennes 1839) is one of the best choices, especially due to its high market prices and its wide salinity tolerance, which would permit its production in large estuarine regions in Southern Brazil (Sampaio et al. 2001; Sampaio and Bianchini 2002). For these reasons studies on the farming potential of *P. orbignyanus* are currently being performed at the Aquaculture Marine Station of the Departament of Oceanography at the Fundação Universidade Federal do Rio Grande – FURG (Southern Brazil). During these studies we have observed recurrent amyloodyniosis outbreaks in broodstock and fingerlings kept in captivity. Since infestations begin with dinospore dispersion, we initiated a monitoring program for *A. ocellatum* dinospores in a *P. orbignyanus* maturation system. We aimed to evaluate the efficiency of prophylactic measures in use in our laboratory and to try to predict the next amyloodiniosis outbreak. In this paper we present the first results of this study and propose simple preventative measures to avoid the dissemination of this dinoflagellate.

Material and Methods

Paralichthys orbignyanus broodstock were caught in the surf zone at Cassino Beach (32°S, 52°W), using a beach seine net and immediately transferred to the laboratory. There the brood fish were anesthetized with benzocaine (50 ppm), weighed, measured, and sexed. Afterwards, they were treated prophylactically with formalin (50 mg/L for 40 min) and freshwater bath (for 1 h) in 300-L tanks and transferred to 1,000-L quarantine tanks where they stayed under observation for 1 wk, before being changed to the maturation tanks.

Maturation Tank 1, where dinospore abundance was monitored, received five females and four males of *P. orbignyanus*. Similar numbers of fish were distributed in two other tanks, located in the same room.

The maturation system consisted of octagonal tanks (2,500-L each), where the water exchange rate was equal to 150% of total volume a day. The water used to fill the tanks was collected in the Cassino Beach, close to the Aquaculture Marine Station - FURG, and filtered through two sand filters before entering into the tanks. Besides the water exchange, the water quality was maintained by a recirculation apparatus (capable to filter 450 L/h), composed of a biological filter (crushed shells), a cartridge filter (5 μm), and a UV lamp sterilizer (36,000 $\mu W/cm^2.sec$). Water exchanges were performed continuously during the month of January 2002. In February 2002, due to problems with the sea water pump, the water was not totally renewed, but kept under a semi-static regime using the recirculating apparatus only. The daily routine of tank maintenance consisted of siphoning the bottom of the tank to remove uneaten feed and feces, and measuring temperature and salinity, using a calibrated YSI 33 SCT (Yellow Springs Instruments Co., USA).

For the first few days, flounders were fed on live grey mullets *Mugil platanus*, blotched pompanos *Trachinotus marginatus* and silver whiting *Menticirrhus littorales*. Afterwards, the flounders were fed *ad libitum* with frozen chopped fish. Fish used as live food were also previously treated with formalin bath (50 mg/L) for 40 min, followed by a freshwater bath for 20 min in 100-L tanks.

Every time the flounders showed clinical signs such as rapid respiration, anorexia, and irregular whitish spots on the dorsal surface of the skin, material was collected by scraping fish skin and gills and observed under light microscope (200–400 x final magnification). When *A. ocellatum* was found, fish were treated with baths of copper sulfate (1.5 mg/L). A decrease of salinity was also part of the treatment. Before each treatment, the recirculation system was isolated, the water flow was stopped, and the water volume was reduced to 500 L. We then added 10 mL of a 75 g/L solution of CuSo4.5H2O to the water in the tank. Fish stayed in this solution for 24 h. Afterwards, the tank was drained to a minimal volume, and clean water and copper sulfate solution were added again. This operation was repeated for at least 7 d. At any time when the signs of amyloodiniosis disappeared, the treatment was interrupted, water flow was re-established and the recirculation system was reconnected to the tank.

On 10 January 2002 we initiated a monitoring program to detect dinospores of *Amyloodinium ocellatum* in the water just after treatment and recovery of fish that were ill by 3 January. The study lasted until 26 February 2002. Water samples (100 mL) were collected in amber glass bottles and fixed with formalin at a final concentration of 2% $^{v}/_{v}$ (Throndsen 1978). Subsamples (50 mL) were poured into settling chambers, and cells were counted after 24 h along the entire bottom of the settling chambers at 200X or 400X final magnification, using an inverted phase contrast microscope Axiovert Zeiss (Germany) according to Utermöhl (1958). For identification of *A. ocellatum* dinospores and trophonts, we consulted Kudo (1966) and Lom and Dyková (1992).

Results

Water temperature during the entire experiment varied between 25 and 27.5 C (Fig. 1A). The salinity was quite constant between January and the beginning of February varying between 30 and 33 ppt. The decrease in salinity observed after 8 February (minimum 15 ppt) was part of the treatment of infested fish (Fig. 1B). Figure 1C shows the rates of water exchange (percent of total volume) in the maturation tank during January and February 2002. During January, the water exchange rate was kept as 150% total volume/d. By 2 February the pump that supplied salt water to the laboratory broke down, resulting in an interruption in the water supply on this day, as well as between 4 and 6 February and 13, 20 and 21 February. During the rest of February, water was exchanged at lower rates, varying from 30 to 100% of the total volume per day (Fig. 1C).

Table 1 shows the chronology of amyloodiniosis outbreaks for tank 1, where dinospore abundance was monitored, and for nearby tank 2, and demonstrates a strong similarity between both tanks. *Amyloodinium* infestations were observed one to two times a month from December 2001,

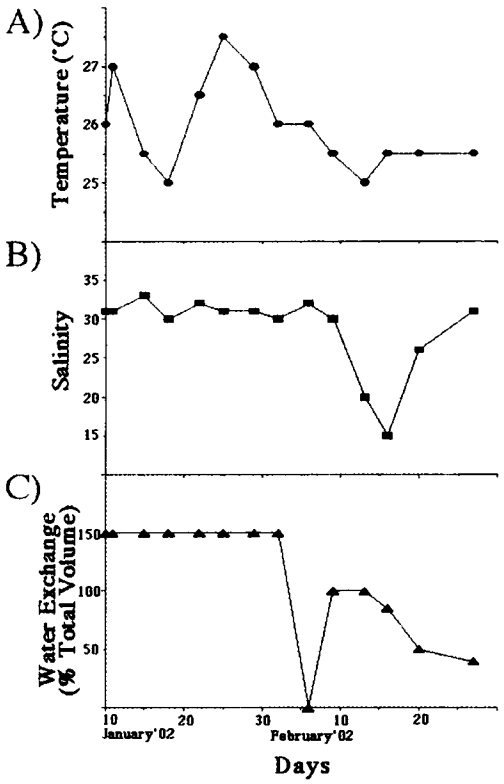

FIGURE 1. *Variation of A) water temperature (C), B) salinity, and C) water exchange (% of total volume) between 10 January and 26 February 2002 in tanks 1 and 2.*

occurring almost simultaneously in tanks 1 and 2 (Table 1). On 2 March we cleaned the biofilm in tank 1 and after this no amylodiniosis outbreak was observed in the tank for more than a month, while fish in tank 2 showed three acute infestations during the same period.

After each amyloodiniosis outbreak fish were treated with copper sulphate and recovered rapidly, without any perceivable damage. Dinospore abundance throughout the study is shown in Fig. 2. Despite the higher levels of water exchanges, dinospores were found in high numbers a few days after the beginning of the study. For instance, by 15 January, dinospore abundance was around 1800 cells/L. This number increased to a maximum of 7,200 cells/L on 25 January. After this, there was a small decrease in dinospore number until the end of February, but it was never less than 2000 cells/L (Fig. 2). The lack of data between 7 and

TABLE 1. *Dates of amyloodiniosis outrbreaks in tank 1 (dinospore monitoring) and in nearby tank 2 (ca. 1 m distant of tank 1). Dinospore abundance was monitored in tank 1 between 10 January and 26 February 2002. On 2 March 2002, biofilm was removed from tank 1*

Tank 1	Tank 2
17 December 2001	17 December 2001
03 January 2002	28 December 2002
07 February 2002	07 February 2002
01 March 2002	01 March 2002
02 March 2002 - BIOFILM CLEANING	25 March 2002
-	18 April 2002
-	29 April 2002
02 May 2002	08 May 2002

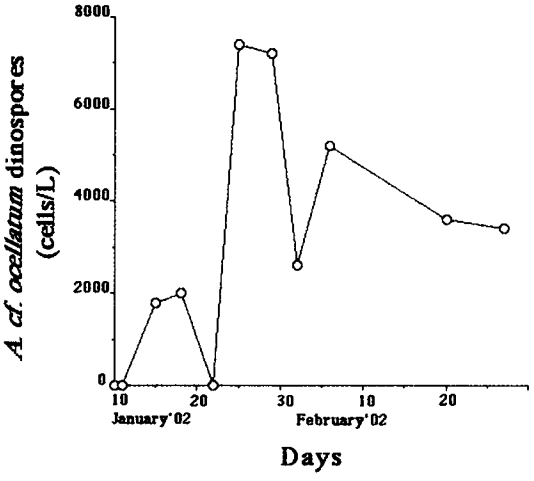

FIGURE 2. *Variation of* A. cf. ocellatum *dinospores (cells/L) between 10 January and 26 February 2002 in tank 1.*

20 February was due to fish treatment after an amyloodiniosis outbreak occurred on 7 February 2002. This outbreak followed problems with water pumping and represented a lag of 13 d between maximum dinospore abundance and the appearance of deleterious symptoms. On the other hand, the infestation observed on 1 March occurred after the stabilization of dinospore abundance and ca. 15 d after the previous fish infestation and recovery (Table 1, Fig. 2).

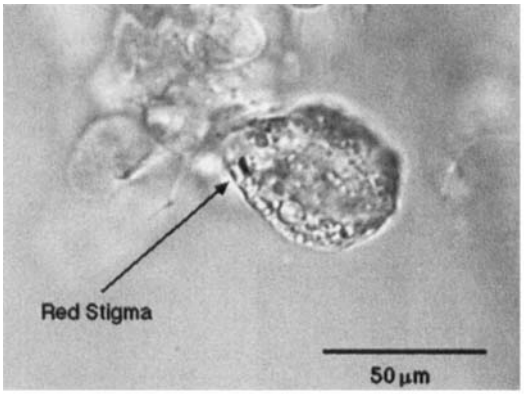

FIGURE 3. *Trophont of* Amyloodinium cf. ocellatum *attached to the gill of* Paralichthys orbignyanus. *The arrow indicates the red stigma. Scale bar = 50 µm.*

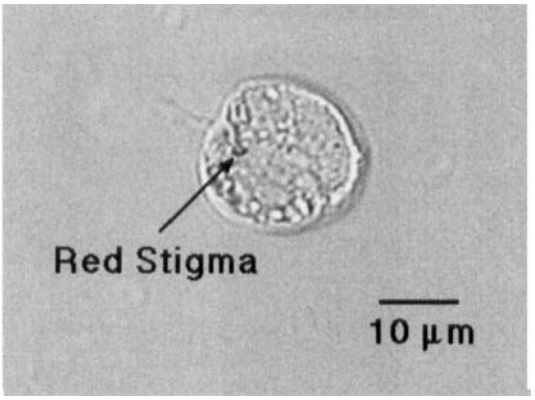

FIGURE 4. *Dinospore of* Amyloodinium cf. ocellatum. *The arrow indicates the red stigma characteristic of this genus. Scale bar = 10 µm.*

In this study, trophonts width (27.5–35 µm) and length (33–100 µm) fall in the size range described for *A. ocellatum* (Fig. 3). However, the magnitude of dinospore sizes varied from 20–25 µm x 10–15 µm (Fig. 4), being larger than the described species (11.6–15.4 x 10.4–14.5 µm) (Lom and Dyková 1992). Therefore, we will refer to the dinoflagellate found in this study as *Amyloodinium cf. ocellatum.*

Discussion

Successful aquaculture depends on numerous biological, environmental and economic factors, but prevention and control of disease is of great importance in all production phases. *Amyloodinium ocellatum* epizootics have been reported around the world with massive mortality in a number of farmed fish species (Paperna and Baudin-Laurencin 1979; Paperna 1980, 1987; Aiello and D'Alba 1986; Alvarez-Pellitero et al. 1995; Rigos et al. 1998). In addition it has also been reported as a matter of concern for flounder broodstock (Benetti et al. 2001). The significance of this epizootic disease relies on the fact that *A. ocellatum* is euryhaline, has a short life cycle and a resistant stage (tomont), which is not affected by most chemical treatments currently in use (Lom and Dyková 1992; Schwarz and Smith 1998). Moreover, this parasite is non-specific to most fish species and life history stages. It is clear that recurrent amyloodiniosis that affects adults, juveniles, and larvae of *P. orbignyanus* in our aquaculture systems could threaten any future flounder production and jeopardize current economical investments. Therefore, to find methods that guarantee the health of reared fish is imperative.

Similarly important is to have the ability to rapidly and correctly identify the three life stages of *A. ocellatum* in aquaculture systems, in order to eliminate and avoid this parasite. However, this is not a simple task. The dinospores observed in this study, with a size range varying between 20–25 x 10–15 µm, were larger than the described species (11.6–15.4 x 10.4–14.5 µm) (Lom & Dyková 1992). Dinospore size variations have been reported in the literature, but most of them were smaller than those found in this study (Landsberg et al. 1994). Such differences in size could represent a new species of *Amyloodinium,* although measured trophonts (27.5–35 µm width x 33–100 µm length) fall in the size range described for *A. ocellatum* (Lom and Dyková 1992). Landsberg et al. (1994) suggest that there may be more than one species or strain of *Amyloodinium* and propose that dinospore and trophont morphology and ultrastructure should be considered together in order to allow proper identification of parasitic dinoflagellates. Considering the identification uncertainties, it would be more appropriate to conduct a further genetic study to resolve the possible identification problem of the dinoflagellate found in this study, as well as to determine the potential variation

amongst species. Meanwhile, we will refer to the dinoflagellate found in this study as *Amyloodinium cf. ocellatum.*

The contamination of aquaculture facilities by infectious organisms results primarily from water pumped into the systems (Reed and Francis-Floyd 1994). It is unlikely that dinospores or tomonts were introduced in our tanks through the water supply, since sea water used in the systems goes through two sand filters before entering in the tanks. It is more probable that *A. cf. ocellatum* was introduced through the *P. orbignyanus*, or the live fish offered to them as food, even considering that all fish were treated with a formalin and freshwater bath before being placed in the tank.

Some prophylactic measures are indicated to avoid the outbreak of this disease in aquaculture systems. Schwarz and Smith (1998) consider that flushing is one of the most effective measures to minimize *A. ocellatum* infestation. The authors also observe that filtration is a very good method to physically remove tomonts. However, in our study, water flushing and the filter of the recirculation apparatus were not effective to eliminate dinospores and tomonts from the tank.

Other prophylactic measures used against *A. ocellatum* are ultraviolet radiation and freshwater bath. Both methods are supposed to eliminate dinospores in the water (Reed and Francis-Floyd 1994; Noga 1996). However, the UV lamp present in the recirculating apparatus of the maturation tank seemed to have no great effect on dinospores. Similarly, the freshwater bath used in this study was not capable of eliminating dinospores, probably because *A. ocellatum* is a euryhaline organism, (Reed and Francis-Floyd 1994). However, the efficiency of both methods should be tested in further experiments.

A variety of chemical treatments can be applied effectively against amyloodiniosis. However, it must be highlighted that most substances used for treatment of this disease are not approved for use in food fish (Schwarz and Smith 1998; FDA 2001). Among the chemicals reported for the treatment of amyloodiniosis, benzalkonium chloride has been suggested as efficient in dislodging trophonts. The antimalarial chloroquine has also been used with some success in amyloodiniosis eradication, though its use is not widespread (Reed and Francis-Floyd 1994). More recently, hydrogen peroxide has been used in the control of this ectoparasite with good results (Montgomery-Brock et al. 2001). Formalin, nitrofurazone, furanace, malachite green, and acriflavin were tested *in vitro*, showing no immediate lethal, but gradual inhibitory effect on dividing tomonts (Paperna 1984).

Recent information concerning the immune response of fish to sublethal exposure to *A. ocellatum* (Smith et al. 1994; Cobb et al. 1998; Cecchini et al. 2001) and the antibiotic effect of histone-like proteins produced by some fish against this ectoparasite (Noga et al. 2001) point to the development of more natural and less hazardous treatments in the near future.

Despite all the above cited elements, the most common chemical reported to eliminate amyloodiniosis infestation is ionic copper (copper sulfate) at a final concentration of 0.2 mg/L. To be successful, copper must be used for a long time, at least 3 wk (Paperna 1984; Reed and Francis-Floyd 1994), and the concentration of this element must be precisely determined in order not to damage the fish, since higher concentrations may affect fish gills and be hepatoxic (Scott 1993).

In the present study, we implemented a treatment with a higher copper sulfate concentration (1.5-mg Cu/L) and shorter period (7 d) than that suggested by Reed and Francis-Floyd (1994). The reason for this is that copper speciation, and consequently its toxicity, depends upon the water chemistry. Copper ligands (Cl^-, SO_4^{2-}, natural organic matter, $S_2O_3^{2-}$, sulfide, Br^-, and $B(OH)_4^-$), or competitors (Na^+, Mg^{2+}, Ca^{2+}, K^+, and Sr^{2+}), can significantly affect copper speciation and consequently the availability of free copper ions in the water (DiToro et al. 2000). Considering the presence of diverse copper ligands and competitors in salt water, the final free ion copper concentration in the treatment water could be lower than that predicted. On the other hand, an initial condition with a high copper concentration, when tanks contained little water, could cause great damage to the fish. Therefore the exposure time to this element was reduced to 7 d.

Even the standard procedure using copper is referred to as being only moderately effective to control *Amyloodinium*. This is mainly because copper sulfate, as well as other chemicals, is ef-

fective at eliminating dinospores and trophonts, but their action on tomonts is not thoroughly recognized (Paperna 1983; Schwarz and Smith 1998; Reed and Francis-Floyd 1994).

Actually, we observed that the exposure of fish to copper forced trophonts to detach from fish and fall rapidly to the bottom of the tank where they then became tomonts. Afterwards a large number of tomonts stayed adhered to the biofilm (an organic matrix colonized by microorganisms observed on any submersed surface) in the bottom of the tank. It is more than likely that the rapid increase in dinospore numbers observed in the first few days of monitoring resulted from the eclosion of tomonts present in the bottom of the tank.

Most probably this is the main reason for the recurrent amyloodiniosis infestation in our maturation system. Considering the "sticky" characteristics of the biofilm, even a strong water flux would not be able to remove all tomonts from the tank. Complete tomont cleaning was only achieved when the biofilm was physically (brushing) and chemically (strong hidrochloric acid -HCl) removed after 2 March 2002. It should be noted that after biofilm cleaning, fish in tank 1 did not present amyloodiniosis infestation for more than 1 mo. Meanwhile, fish in the nearby tank 2 went through three disease outbreaks (Table 1). We consider that the disease signs observed in fish from tank 1 on 2 May 2002, probably resulted from the transference of dinospores from tank 2 due to water splash, or from the use of common cleaning tools.

Conclusions

With respect to the main objective of this study, i.e., monitor the dinospore abundance in order to predict infestation, we can say that this procedure was not very efficient. For instance, the appearance of disease symptoms on 7 February occurred almost 1 mo after the beginning of the experiment and 13 d after the maximum dinospore abundance. On the other hand, the disease outbreak observed on 1 March occurred only two weeks after fish recovery of 7 February outbreak, following a condition where dinospore abundance was quite stable and not as high as before. We consider that the control of tomont abundance and their development stage at the bottom of the tanks would be more efficient in order to warn about an imminent dinospore bloom.

Considering the results outlined above, and the fact that most chemicals used to treat amyloodiniosis are not suitable for use in fish production for human consumption, we propose a few prophylactic measures which should help to avoid amyloodiniosis infestation in aquaculture tanks: 1) Fish should be treated in a different tank to avoid the formation of tomonts in the rearing tanks; 2) Tanks where fish showed disease symptoms should have the biofilm removed by brushing and the use of strong hydrochloric acid (HCl); 3) The tomont abundance and its development stage should be monitored in the biofilm at the bottom of tank; 4) The presence of trophonts or tomonts in the live fish offered as food to the flounders should be controlled; and 5) Tanks should be separated and have isolated equipment to avoid cross-contamination.

Acknowledgments

This study was supported by FAPERGS (Proc. 99/1443.00), and Sul-Química. P. Abreu, R. Robaldo, C. Odebrecht, and A. Bianchini had financial support from the Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq. We appreciated very much the comments on a previous version of this manuscript of Prof. Dr. Jorge Eiras (University of Porto, Portugal), Dr. Jan Landsberg (Florida Marine Research Institute, USA).

Literature Cited

Aiello, P. and A. D'Alba. 1986. Amyloodinium ocellatum infestation in yellowtail, *Seriola dumerili*, intensively reared in Sicily, Italy. Bulletin European Association of Fish Pathology 6:110–111.

Alvarez-Pellitero, P., A. Sitja-Bobadilla, and A. Franco-Sierra. 1995. Protozoan parasites of gilthead sea bream, *Sparus aurata* L. from different culture systems in Spain. Journal of Fish Diseases 18:105–115.

Benetti, D. D., S. W. Grabe, M. W. Feeley, O. M. Stevens, T. M. Powell, A. J. Leingang, and K. L. Main. 2001. Development of aquaculture methods for southern flounder, *Paralichthys lethostigma*: I. Spawning and larval culture. Journal of Applied Aquaculture 11:113–133.

Brown, E. M. and R. Hovasse. 1946. *Amyloodinium ocellatum* (Brown), a peridinian parasitic on marine fishes. A complementary study. Proceedings of the

Zoology Society of London 116:33–36.

Cecchini, S., M. Saroglia, G. Terova, and F. Albanesi. 2001. Detection of antibody response against *Amyloodinium ocellatum* (Brown, 1931) in serum of naturally infected European sea bass by an enzyme-linked immunosorbent assay (ELISA). Bulletin of European Association of Fish Pathology 21:104–108.

Cobb, C., M. G. Levy, and E. J. Noga. 1998. Development of immunity by the tomato clownfish *Amphiprion frenatus* to the dinoflagellate parasite *Amyloodinium ocellatum*. Journal of Aquatic Animal Health 10:259–263.

DiToro, D., H. E. Allen, H. L. Bergman, J. S. Meyer, R. C. Santore, and P. Paquin. 2000. The biotic ligand model. A computational approach for assessing the ecological effect of metals in aquatic systems. International Copper Association, Ltd., New York, New York, USA.

Eiras, J. 1994. Elementos de Ictioparasitologia. Fund. Engo. Antônio Almeida, Porto, Portugal.

FAO. 2000a. Fishery statistics. Capture production, volume 90/1.

FAO. 2000b. Fishery statistics. Aquaculture production, volume 90/2.

FDA (U.S. Food and Drug Administration). 2001. HACCP guidance— Fish and fishery products hazards and controls guidance, 3rd edition.

Kudo, R. R. 1966. Protozoology. C. Thomas Publ., Springfield, U.S.A.

Landsberg, J. H., K. A. Steidinger, B. Blakesley, and R. L. Zondervan. 1994. Scanning electron microscope study of dinospores of *Amyloodinium cf. ocellatum*, a pathogenic dinoflagellate parasite of marine fish, and comments on its relationship to the Peridiniales. Disease Aquatic Organisms 20:23–32.

Landsberg, J. H., K. A. Steidinger, and B. A. Blakesley. 1995. Fish killing dinoflagellates in a marine aquarium. Pages 65–70 *in:* Lassus, P., G. Arzul, E. Erard-Le-Denn, P. Gentien, and C. Marcaillou-Le-Baut, editors. Harmful marine algal blooms. Lavoisier Paris, France.

Lawler, A. R. 1977. The parasitic dinoflagellate *Amyloodinium ocellatum* in marine aquaria. Drum and Croaker 17:17–20.

Lom, J. and I. Dyková. 1992. Protozoan parasites of fishes. Elsevier, Amsterdam.

Montgomery-Brock, D., V. T. Sato, J. Brock, and C. Tamaru. 2001. The application of hydrogen peroxide as a treatment for the ectoparasite *Amyloodinium ocellatum* (Brown 1931) on the Pacific Threadfin *Polydactylus sexfilis*. Journal of World Aquaculture Society 32:250–254.

Noga, E.J. 1996. Fish disease. Diagnosis and treatment. Year Book, Inc., St. Louis, Missouri, USA.

Noga, E. J., Z. Fan, and U. Silphaduang. 2001. Histone-like proteins from fish are lethal to the parasitic dinoflagellate *Amyloodinium ocellatum*. Parasitology 123:57–65.

Noga, E. J., S. A. Smith, and J. H. Landsberg. 1991. Amyloodiniosis in cultured hybrid striped bass (*Morone saxatilis* x *M. chrysops*) in North Carolina. Journal Aquatic Animal Health 3:294–297.

Paperna, I. 1980. *Amyloodinium ocellatum* (Brown, 1931), (dinoflagellida) infestation in cultured marine fish at Eilat, Red Sea: Epizootology and pathology. Journal of Fish Diseases 3:363–372.

Paperna, I. 1983. Review of diseases of cultured warm-water marine fish. Rapport P.-v. Réunion Conseil Internationel de Exploration de Mer 182:44–48.

Paperna, I. 1984. Chemical control of *Amyloodinium ocellatum* (Brown 1931) (Dinoflagellida) infections: in vitro tests and treatment trials with infected fishes. Aquaculture 38:1–18.

Paperna, I. 1987. Solving parasite-related problems in cultured marine fish. International Journal of Parasitology 17:327–336.

Paperna, I. and F. Baudin-Laurencin. 1979. Parasitic infections of sea bass *Dicentrachus labrax* and gilthead sea bream, *Sparus aurata* in mariculture facilities in France. Aquaculture 16:173–175.

Ramos, P. and J. M. Oliveira. 2001. Amilodiniosi em pregado, *Psetta maxima* (L.). Revista Portuguesa de Ciencias Veterinarias 95:201–205.

Reed, P. and R. Francis-Floyd. 1994. *Amyloodinium* infections of marine fish. Fact sheet VM-90. College of Veterinary Medicine, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida, USA.

Rigos, G., P. Christophilogiannis, M. Giahnishi, A. Andriopoulou, M. Koutsodimoy, I. Nengas, and M. Alexis. 1998. *Amyloodinium occelatum* infestation on shap-snout sea bream, Puntazzo puntazzo Cetti. Bulletin European Association of Fish Pathology 18:198–200.

Sampaio, L. A. and A. Bianchini. 2002. Salinity effects on osmoregulation and growth of the euryhaline flounder *Paralichthys orbignyanus*. Journal Experimental Marine Biology and Ecology 269:187–196.

Sampaio, L. A., A. Bianchini, and V. R. Cerqueira. 2001. Growth of juvenile Brazilian flounder, *Paralichthys orbignyanus*, cultured at different salinities.

Journal Applied Aquaculture 11(1/2):67–75.

Schwarz, M. H. and S. A. Smith. 1998. Getting acquainted with *Amyloodinium ocellatum*. Commercial fish and shellfish technology. Virginia Cooperative Extension. Fact Sheet. Publ. 600-200, Virginia, U.S.A.

Scott, P. 1993. Therapy in aquaculture. *In*: L. Brown, editor. Aquaculture for veterinarians. Pergamon Press, Oxford, UK.

Smith, S. A., M. G. Levy, and E. J. Noga. 1994. Detection of anti-*Amyloodinium ocellatum* antibody from cultured hybrid striped bass (*Morone saxatilis* X *M. chrysops*) during an epizootic of amyloodiniosis. Journal Aquatic Animal Health 6:79–81.

Throndsen, J. 1978. Preservation and storage. Pages 69-74 *in* A. Sournia, editor. Phytoplankton manual. UNESCO, Paris, France.

Utermöhl, H. 1958. Zur Vervollkommnurng der quantitativen phytoplankton Methodik. Int Ver Theor Angew Limnologie 9:1–3.