Chapter 38

Amyloodiniosis

Paola Beraldo and Michela Massimo

Section of Animal and Veterinary Sciences, Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Udine, Italy

38.1 Overview

Amyloodinium ocellatum (AO) is a cosmopolitan dinoflagellate protozoan, which is present in several aquatic habitats (sea and brackish water) at both tropical and temperate regions. This dinoflagellate can infect elasmobranchs and teleosts as well as crustaceans and flatworms; moreover, almost all fish that live within its ecological range are susceptible to the infection. The parasite represents a serious problem for fish in captivity (reared and in aquarium) (Landsberg et al., 1994), since amyloodiniosis (also called marine velvet disease) can lead the host to death in less than 12 h (Lawler, 1980) with acute morbidity and mortality around 100%. However, these two parameters considerably vary on the basis of farming condition, parasite burden, fish species, and season considered (Kuperman et al., 2001; Mladineo, 2006; Saraiva et al., 2011; Bahri, 2012; Guerra-Santos et al., 2012; Moreira et al., 2013).

38.2 Etiological agent

AO is the unique species belonging to *Amyloodinium* genus (class Dinophyceae, order Thoracosphaerales, and family Thoracosphaeraceae). This dinoflagellate has a very low species specificity, being able to infect teleosts as well as elasmobranchs (Lawler, 1980). Furthermore, cumulative evidence show it has been isolated from four aquatic organisms phyla: Chordata, Arthropoda (Aravindan et al., 2007), Mollusca (Souza, 2015), and Platyhelminthes (Colorni, 1994).

The lifecycle of *A. ocellatum* is direct and divided into three phases (Fig. 38.1). Salinity and temperature values strongly modulate AO pathogenicity and lifecycle duration. In general, the lifecycle can be completed in 5−7 days when the temperature is comprised between 23 and 27°C and salinity between 30 and 35 ppt. However, the parasite can express its virulence also in extreme conditions, in particular, lethal outbreaks were registered at high temperatures (more than 35°C) in both highly saline water (46 ppt) (Kuperman and Matey, 1999) and brackish environments (7 ppt) (Beraldo et al., 2017). The parasitic stage is represented by the sessile trophont. In this phase, the protist is pear-shaped, enclosed in a cellulose wall, and exhibits specific structures (rhizoids, tentacle-like processes) that enable it to strictly anchor to host epithelia (predominantly gill or skin) (Fig. 38.2). If the infection is severe, trophonts can also be found on eyes, fins, and in the oral cavity (Lawler, 1980; Kuperman and Matey, 1999; Cruz-Lacierda et al., 2004; Byadgi et al., 2019). However, in the European sea bass (ESB), trophonts were detected mainly in the epithelium of the oropharyngeal cavity (Beraldo et al., 2017; Massimo et al., 2017a). Based on data deriving from literature, trophonts feed directly from the host cells, probably by using the stomopode that is supposed to be the AO trophic organ by which digestive enzymes are released (Lom and Lawler, 1973), thus exacerbating the rhizoid lesions (Fig. 38.2). Trophont size can vary considerably, with early trophonts measuring 27 × 23 μm, while mature ones 130 × 60 μm and more.

Two to six days after feeding, the trophont detaches from the host and encysts on inert substrates (pond/tank bottom or seabed) transforming into the tomont: the reproductive stage. In this phase, the protozoan is round-shaped and encapsulated in a thick cellulose wall, which confers it an exceptional resistance to unfavorable conditions and to several therapeutic treatments. The protozoan reproduces asexually and potentially; in two to four days, a single tomont can generate up to 256 new dinospores, based on the nutritive status of the trophont (Paperna, 1984a). The dinospore (8−13.5×10−12.5 μm) is the infective and free-swimming stage characterized by the presence of two flagella. After adhesion to a new host, the dinospore transforms into a trophont within 5−20 min (Noga and Bower, 1987).

Aquaculture Pathophysiology - Volume I. https://doi.org/10.1016/B978-0-12-812211-2.00038-X

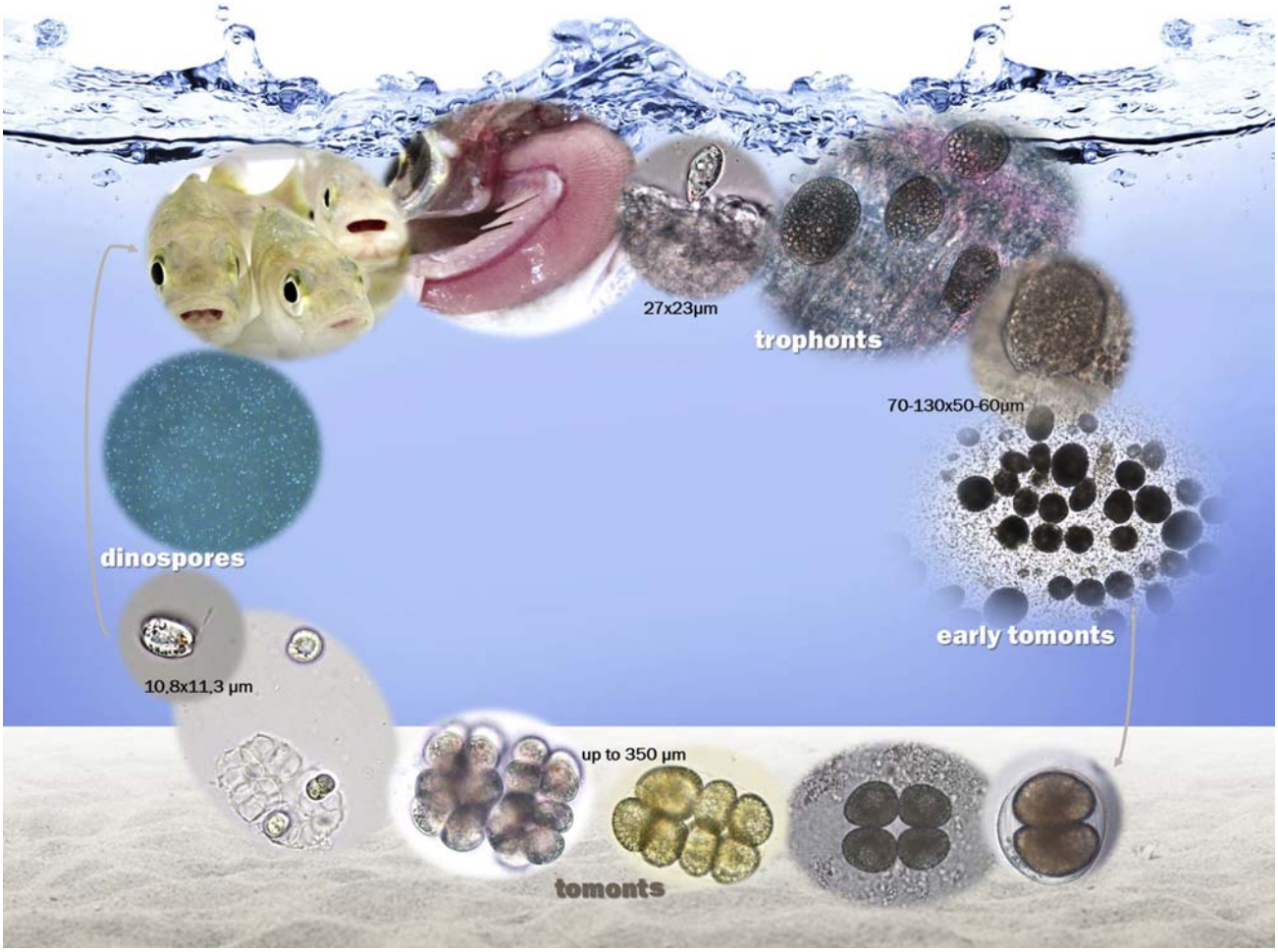

FIGURE 38.1 Diagrammatic representation of the lifecycle of *Amyloodinium ocellatum* (Original, P. Beraldo).

38.3 Clinical signs and gross pathology

The protozoan pathogenicity is associated to the trophont attachment to host tissues (Fig. 38.3B and C). Constantly moving while anchored thus causing physical injuries to epithelial cells (Noga and Bower, 1987), trophonts inflict serious damage to the host, potentially leading to its death in 12−48 h when the parasite burden is very high (over 200 trophonts/gill filaments). Gills and the entire oropharyngeal cavity are the primary sites of the infection in teleosts, but heavy infestations may also involve the skin, fins, and eyes.

A. ocellatum inflicts moderate-to-intense tissue reactions associated with serious gill hyperplasia, inflammation, hemorrhage, and necrosis, whose severity is parasite burden-dependent. However, some mortalities were documented also in subclinical or mild infestations as a probable consequence of the osmoregulatory impairment and secondary microbial infections due to the serious epithelial damage (Noga, 2012). The symptoms of amyloodiniosis is indicated by some sudden host behavioral changes, which are more evident when the disease is severe.

Amyloodiniosis is characterized by clinical signs such as apathy, dyspnea, and increased respiratory rate with labored breathing and gathering at the water surface (Fig. 38.4). Jerky movements/spastic swimming and pruritis have also been documented as typical symptoms of the infection (Brown, 1934; Brown and Hovasse, 1946; Lawler, 1980; Kuperman and Matey, 1999; Noga, 2010; Vivanco-Aranda et al., 2018), the latter is probably due to the effect of toxic/irritants substances (hypothetically digestive enzymes) released by trophonts (Lom and Lawler, 1973; Paperna, 1980). Poor appetite, anorexia, and lethargy are frequently associated with heavy and prolonged infections because of stress and probably of the neuropeptide Y reduced production (Nozzi et al., 2016). An additional clinical sign could be the dusty appearance of the skin (hence the name marine velvet disease). Nevertheless, this is not a common finding as, in ESB but also in massive

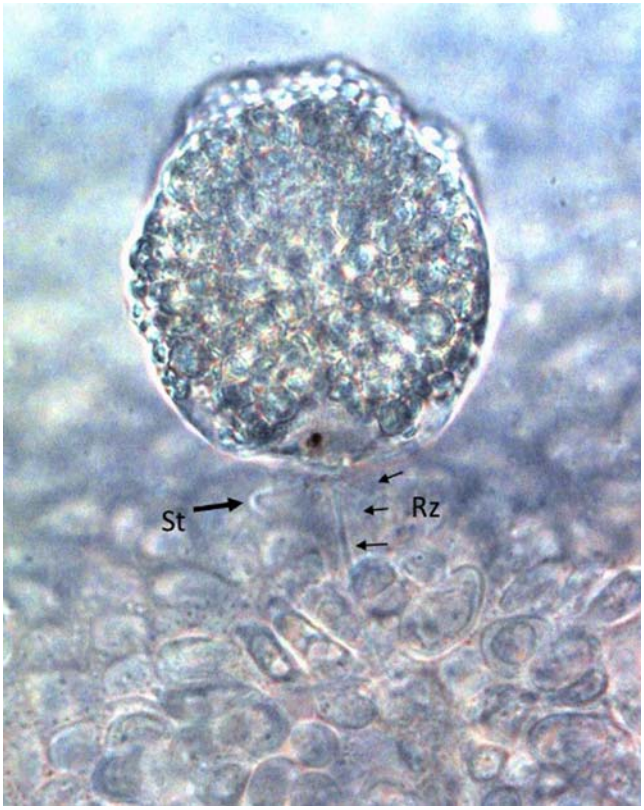

FIGURE 38.2 A trophont detaching from the gill epithelium, some rhizoids (Rz) and the stomopode (St) are visible.

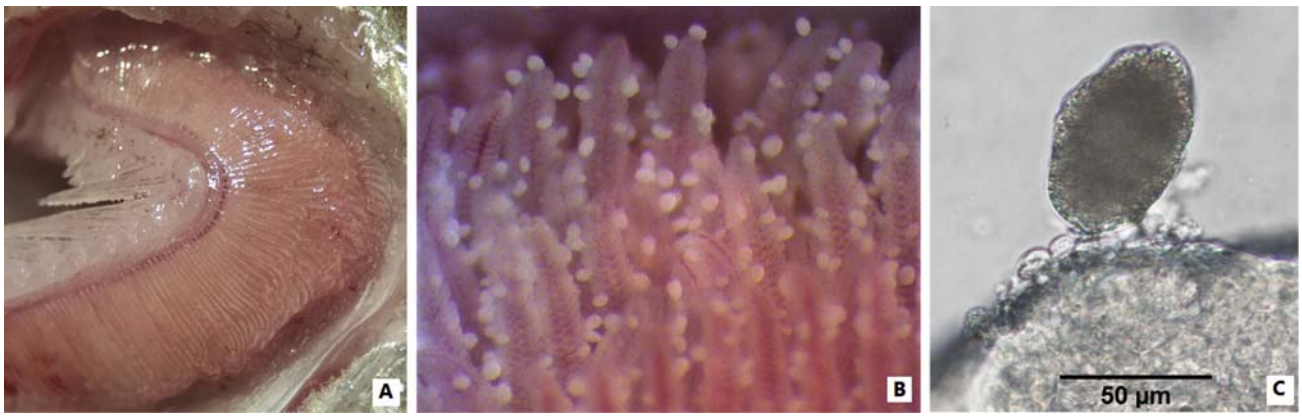

FIGURE 38.3A-B *A. ocellatum* heavy infection in European sea bass gills. (A) Diffuse anemia and hyperplasia of apex gill filament; (B) trophonts attached to the gill filaments; (C) trophont attached to the gill epithelium by rhizoids and stomopode.

infections, fish often die without obvious gross skin alterations (Noga and Levy, 2006; Beraldo et al., 2017). Marked diffuse gill anemia (Fig. 38.4A) and filiform hemorrhages are also present in heavy infected ESB (Byadgi et al., 2019).

38.4 Histopathology

The histopathological pattern is parasite density-dependent; temperature and the oxygen concentration can increase the severity of the disease. In mild infections (no more than 10 trophonts/primary lamella) or at the onset of the disease, no significant lesions are observed, except for inflammatory cells around the single trophont adhesion site. On the other hand, in heavy infections (more than 100−200 trophonts/primary lamellae), a severe and diffuse epithelium degeneration of the

FIGURE 38.4 Surface swimming with labored breathing of an *A. ocellatum*-infected European sea bass juvenile.

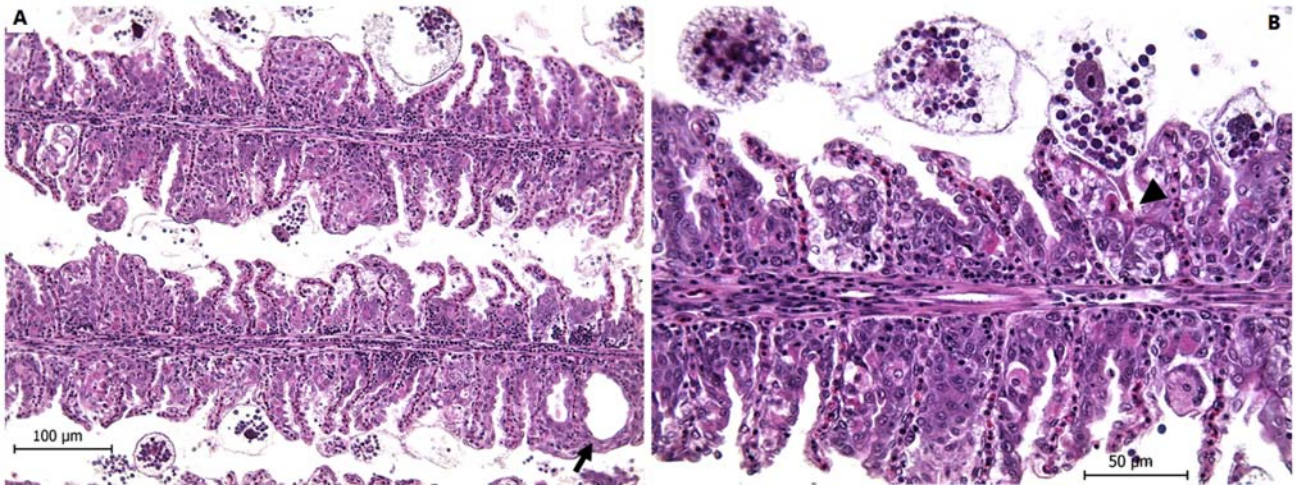

FIGURE 38.5A-B European sea bass infected gills. (A) Epithelial damage, hyperplasia with lacunae formation between lamellae (arrow) and cellular infiltrate along the vascular axis. (B) Magnification of the previous figure, a trophont in adhesion is visible (arrow). H-E.

oropharyngeal cavity is evident, characterized by hydropic degeneration of gill epithelial and ionocytes (chloride cells) with necrosis and edema (Fig. 38.5A and B). In general, there is a marked hyperplasia of the gill epithelium, especially in the distal third of primary lamellae (Brown, 1934; Paperna, 1980; Kuperman and Matey, 1999; Kuperman et al., 2001; Cruz-Lacierda et al., 2004; Saraiva et al., 2011; Bahri, 2012; Guerra-Santos et al., 2012; Ramesh Kumar et al., 2015; Vivanco-Aranda et al., 2018).

The same histological pattern can be observed in pseudobranchs, while marked epithelial hyperplasia was the primary alteration of buccal cavity, pharynx, and gill arches epithelium (Fig. 38.6) (Byadgi et al., 2019). A marked gill epithelial hyperplasia can induce a pattern in which synechiae are easily visible at lamellae tips (Fig. 38.5A). Alongside the primary gill lamellae, lymphocytes, macrophages, mast cells are identifiable (Fig. 38.5A); the same cellular infiltrate can be evident in the buccal cavity and pharynx with abundant mucus cells in the epithelium, and rodlet cells often present in the secondary gill lamellae epithelium. *A. ocellatum* trophonts feed and anchor on multiple epithelial cells simultaneously, inducing extensive damage of the vascular system as well as provoking the rupture of pillar cells and formation of lamellar aneurysms or microhemorrhages. These changes were observed at necropsy in the form of anemia in gills (Byadgi et al., 2019).

Histologically, *A. ocellatum* causes mainly a gill inflammatory pattern that can be described as hyperplastic parasitic branchitis, although the hyperplastic changes depend on the course of infection.

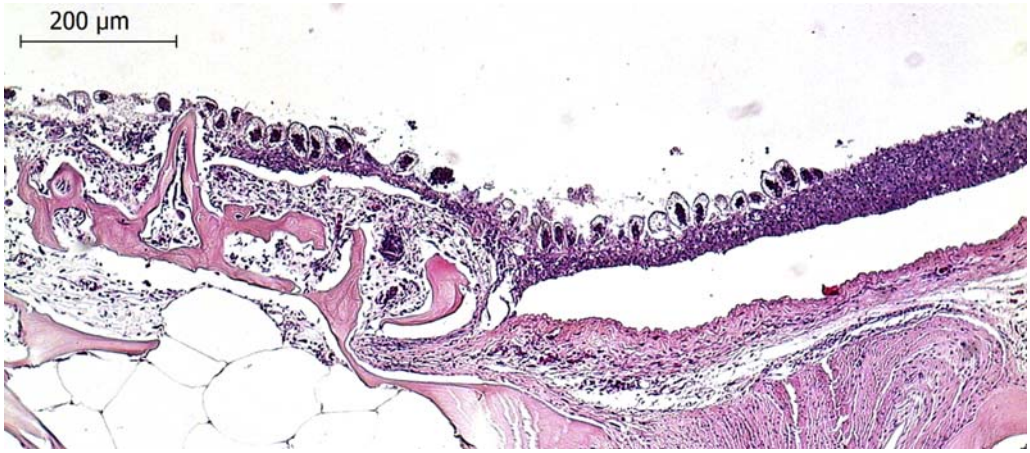

FIGURE 38.6 Buccal cavity floor of European sea bass showing a multitude of attached trophonts causing a marked epithelial hyperplasia. H-E.

38.5 Disease risk factors

Environmental factors can strongly promote fish infestation by ectoparasites, and, in fact, the severity of AO infection is strongly related to water temperature, salinity, and oxygen concentration (Kuperman and Matey, 1999; Kuperman et al., 2001; Pereira et al., 2011). Paperna (1984a) demonstrated that 18−30°C is the optimal temperature range for the parasite development; within this range, *A. ocellatum* can complete its lifecycle producing infectious dinospores. Tomont division and tomit reproduction are progressively inhibited by the temperature decreasing (at 15°C, the reproduction process stops after some divisions of the tomonts, although it can restart if the incubation conditions return to the optimal range, albeit with less efficiency in dinospores produced) until it stops completely with the parasite death at ≤8°C. At over 30°C, the reproductive process success declines and yielded dinospores can be less mobile and infective. Anyway, this dinoflagellate is more pathogenic at higher temperatures (Kuperman et al., 2001; Beraldo et al., 2017), namely during the warmer months. *A. ocellatum* is tolerant to different salinities, and this tolerance is proportionally dependent on the temperature (Paperna, 1984a; Kuperman and Matey, 1999; Kuperman et al., 2001). Tomonts division can potentially happen within the 1−78 ppt salinity range (highest tolerance at 22−27°C); however, infective and vital dinospores are originated only when salinity is comprised between 4 and 50 ppt, at the extremes of this interval dinospores are less active (Paperna, 1984a).

The effect of the dissolved oxygen concentration on different parasite stages has not been fully studied, but low oxygen levels could indirectly reinforce the negative impact of *A. ocellatum*, having trophonts destroy gill tissues, thus compromising the fish's respiratory functions (Kuperman et al., 2001).

Furthermore, some atmospheric events, such as storms and typhoons (Dequito et al., 2015), must be taken into account as possible promoters in the environmental spreading of the parasite. In fact, dinospores can be transported in aerosol droplets (Roberts-Thomson et al., 2006), thus contaminating other nearby facilities (Dequito et al., 2015).

Amyloodiniosis is generally more frequent and severe in lagoon-based rearing sites (such as confined areas of coastal lagoons, natural and managed deltas, and semiclosed bays and estuaries, encompassing polders with earthen ponds), characterized by high water temperatures (28−30°C), poor water exchange (typical of these farms), and low oxygen concentration (1.5−2 ppm). Normally, cage-reared fish are not affected by the infection (Diamant, 2001). Nevertheless, in the presence of shallow seabeds and poor water recirculation, this farming typology could also be involved as documented by Rigos et al. (1998).

38.6 Pathogen isolation and identification

The amyloodiniosis *intra vitam* diagnostic process should start from the observation of the clinical signs and prior to fish anesthesia, and by employing nonlethal techniques to identify the protozoan in skin and gills. A cytological approach by means of wet mount examinations of skin scrapes and gill biopsies is recommended to detect trophonts (Fig. 38.7). Anesthetized small-size fish can be directly observed under the microscope by gently lifting the opercular structure. The same approach is also adopted for *postmortem* fish, even if it is recommended that fish are just dead, as parasites often

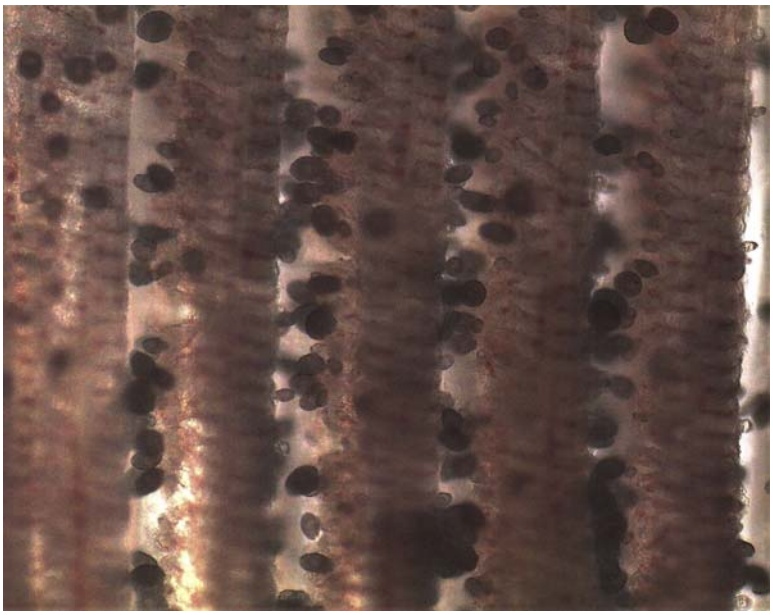

FIGURE 38.7 Several *A. ocellatum* trophonts on European seabass gills.

detach shortly after host death (Noga and Levy, 2006; Noga, 2012; personal data). Anyway, trophonts are best observed using indirect illumination, such as by shining a flashlight on the top of the fish in a darkened room (Noga, 2012). Some droplets of diluted Lugol's iodine on skin/gills improve AO detection, since the iodine reacts with the starch-containing trophonts, coloring them in dark brown/black (Noga, 2012).

38.7 Molecular diagnostics

Recently developed molecular approaches (polymerase chain reaction [PCR] and loop-mediated isothermal amplification [LAMP]) can provide accurate, early detection of *A. ocellatum* in water and gill tissue samples, also in course of subclinical infections that usually go undetected (Levy et al., 2007; Picón-Camacho et al., 2013; Bessat and Fadel, 2018). Anyway, their application is still not applicable on a large scale.

Molecular diagnosis of *A. ocellatum* is based on primers AO18SF (5′ GACCTTGCCCGAGAGGG 3′) and AO18SR (5′ GGTGTTAAGATTCACCACACTTTCC 3′) for PCR amplification of a 248 bp segment of the 3′ end of the LSU rDNA gene (Levy et al., 2007; Byadgi et al., 2019). Multiple sequence alignment using the CLUSTALw confirmed that sequenced AO was conserved with different geographic isolates from Mediterranean Sea (DQ490256.1), Red sea (DQ490257.1), Fujian-China (KU761581.1 and KR057921.1), and Southern Mississippi-USA (JX905204.1) (Byadgi et al., 2019).

Enzyme-linked immunosorbent assay can detect the specific anti-AO antibody levels (Smith et al., 1992, 1993, 1994; Cobb et al., 1998a,b; Cecchini et al., 2001) in fish recovering from natural/experimental amyloodiniosis episodes or for the purpose of monitoring levels of protection in susceptible populations, as elevated antibody titers have been associated with resistance (Cobb et al., 1998a,b).

38.8 Disease control

Numerous efforts have been performed in order to control amyloodiniosis in many different fish species (Noga, 2012), but no treatment has proved univocal to eliminate the parasite from the host nor to be totally effective or legal worldwide, but at most the disease can be controlled.

The early diagnosis and consequently a prompt treatment is crucial due to the exponential reproductive capacity of *A. ocellatum* during warmer months. However, only dinospores are really susceptible to some drugs, while trophonts and tomonts are more resistant (Lawler, 1977; Paperna, 1984b; Noga, 2012; Bessat and Fadel, 2018).

Copper sulfate (0.75−1 g/m^3 for almost two weeks by dripping on ponds/tanks to maintain constant the copper concentration) is one of the most effective treatments to kill dinospores (tomonts and trophonts are not very susceptible),

and it is widely used because of its inexpensiveness and availability (Paperna, 1984b; Alvarez-Pellitero et al., 1995; Cecchini et al., 2001; Noga, 2010; Massimo et al., 2017a). The free copper ion is unstable in seawater, so copper levels should be monitored closely with a copper test kit; instead, chelated copper is more stable in water even if more difficult to monitor (Noga, 2010). Although not banned, copper sulfate is not registered for treatment against *A. ocellatum* everywhere.

The antimalarial chloroquine diphosphate (5−10 mg/L water) is effective against dinospores (Bower et al., 1987; Ramesh-Kumar et al., 2015), and its oral administration in cultured red drum (*Sciaenops ocellatus*) suggested promising results in terms of oral medication (Lewis et al., 1988). However, chloroquine is very expensive and is not likely to be approved for food fish.

A prophylactic strategy is the application of medicated baths by adding formalin (4 mg/L of 36% formaldehyde for 7 h or 1 h to the concentration of 50 mg/L) or hydrogen peroxide (75 and 150 mg/L for 30 min and after six days again) to the water, most of all for fish newly introduced in farm (Paperna, 1984b; Ramos and Oliveira, 2001; Montgomery-Brock et al., 2001; Fajer-Ávila et al., 2003; Cruz-Lacierda et al., 2004).

Freshwater bath is really effective to detach trophonts from skin and gill epithelium due to the sudden osmotic shock (Smith et al., 1993; Cruz-Lacierda et al., 2004; Abreu et al., 2005; Roberts-Thomson et al., 2006; Benetti et al., 2008; Bonucci Moreira et al., 2013). Nevertheless, freshwater bath procedure remains impracticable for the majority of the marine fish farms, and some fish species cannot tolerate a similar treatment. Alternatively, some authors proposed the quarantine as a valid procedure to reduce the infection risk or possible cross-transmissions (Blaylock et al., 2001; Ramos and Oliveira, 2001; Diamant, 2001). Another prophylactic approach could be the molecular diagnostic technique proposed by Picón-Camacho et al. (2013). The *Amyloodinium* LAMP assay, developed in their study, showed to be a novel tool for the sensitive detection of *A. ocellatum* in water and gill tissue samples, for the purpose of assisting in the early detection in aquaculture systems.

More eco-friendly and suggestive was the treatment proposed by Oestmann et al. (1995). The authors suggested the usage of nauplii of brine shrimp *Artemia salina* as a bioremediation measure against the protozoan; although the results appear successful, this treatment is not applicable.

Some plant-derived compounds have been investigated *in vitro* against *A. ocellatum* dinospores (Massimo et al., 2017a,b; Tedesco et al. 2020), as part of ParaFishControl research activities (Horizon 2020 project). In particular, 2^{I},4^{I}-dihydroxychalcone and tomatine exhibited effective inhibitory properties, but their very high costs make their usage prohibitive in farming realities.

At date, no indirect prophylactic strategies exist through a vaccine formulation, but within the project mentioned above, the evaluation of the protective effects of fragmented dinospores administered intracoelomatically in juveniles of ESB is in progress (Byadgi et al., 2018).

References

Abreu, P.C., Robaldo, R.B., Sampaio, L.A., Bianchini, A., Odebrecht, C., 2005. Recurrent amyloodiniosis on broodstock of the Brazilian flounder *Paralichthys orbignyanus*: dinospore monitoring and prophylactic measures. J. World Aquacult. Soc. 36, 42−50.

Alvarez-Pellitero, P., Adilla, A.S.-B., Franco-Sierra, A., Palenzuela, O., 1995. Protozoan parasites of gilthead sea bream, *Sparus aurata* L., from different culture systems in Spain. J. Fish. Dis. 18, 105−115.

Aravindan, N., Kalavati, C., Aravindan, S., 2007. Protozoan parasites in commercially important shrimp species from northeast cost of Andhra Pradesh India. J. Exp. Zool. 10, 9−20.

Bahri, S., 2012. Protozoan and myxozoan infections in wild gilthead seabream (*Sparus aurata* L.) from North Lake of Tunis, Tunisia. Acta Parasitol. 57, 114−121.

Benetti, D.D., Orhun, M.R., Sardenberg, B., O'Hanlon, B., Welch, A., Hoenig, R., Zink, I., Rivera, J.A., Denlinger, B., Bacoat, D., Palmer, K., Cavalin, F., 2008. Advances in hatchery and grow-out technology of cobia *Rachycentron canadum* (Linnaeus). Aquacult. Res. 39, 701−711.

Beraldo, P., Byadgi, O., Massimo, M., Bulfon, C., Volpatti, D., Galeotti, M., 2017. Grave episodio di amyloodiniosi in giovanili di branzino (*Dicentrarchus labrax*): analisi dei determinanti di malattia e rilievi anatomopatologici. In: Conference proceedings of the XXIII Convegno nazionale S.I.P.I. Società Italiana di Patologia Ittica, Lecce, 5-6th of October 2017, p. 30.

Bessat, M., Fadel, A., 2018. Amyloodiniosis in cultured *Dicentrarchus labrax*: parasitological and molecular diagnosis, and an improved treatment protocol. Dis. Aquat. Org. 129, 41−51.

Blaylock, R.B., Overstreet, R.M., Lotz, J.M., 2001. Health management in red snapper (*Lutjanus campechanus*) culture. Bull. Natl. Res. Inst. Aquacult. (Suppl. 5), 5−9.

Bonucci Moreira, C., de Oliveira Hashimoto, G.S., Rombenso, A.N., Candiotto, F.B., Laterça Martins, M., Tsuzuki, M.Y., 2013. Outbreak of mortality among cage-reared cobia (*Rachycentron canadum*) associated with parasitism. Revista Brasileira de Parasitologia Veterinária 22 (4), 588−591. https://doi.org/10.1590/S1984-29612013000400021.

Bower, C.E., Turner, D.T., Biever, R.C., 1987. A standardized method of propagating the marine fish parasite, *Amyloodinium ocellatum*. J. Parasitol. 73, 85−88.

Brown, E.M., 1934. On *Oodinium ocellatum* Brown, a parasitic dinoflagellate causing epidemic disease in marine fish. Proc. Zool. Soc. Lond. 104, 583−607.

Brown, E.M., Hovasse, R., 1946. *Amyloodinium ocellatum* (Brown), a Peridinian parasitic on marine fishes. A complementary study. Proc. Zool. Soc. Lond. 116, 33−46.

Byadgi, O., Beraldo, P., Massimo, M., Bulfon, C., Galeotti, M., Volpatti, D., 2018. The first pilot study on amyloodiniosis vaccine efficacy trial in European seabass (*Dicentrarchus labrax*). Atti del XXIV Convegno Nazionale SIPI (Società Italiana di Patologia Ittica) 58, 11−13 ottobre 2018, Torino (Italia).

Byadgi, O., Beraldo, P., Volpatti, D., Massimo, M., Bulfon, C., Galeotti, M., 2019. Expression of infection-related immune response in European sea bass (*Dicentrarchus labrax*) during a natural outbreak from a unique dinoflagellate *Amyloodinium ocellatum*. Fish Shellfish Immunol. 84, 62−72.

Cecchini, S., Saroglia, M., Terova, G., Albanesi, F., 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). Bull. Eur. Assoc. Fish Pathol. 21, 104−108.

Cobb, C.S., Levy, M.G., Noga, E.J., 1998a. Development of immunity by the tomato clownfish *Amphiprion frenatus* to the dinoflagellate parasite *Amyloodinium ocellatum*. J. Aquat. Anim. Health 10, 259−263.

Cobb, C.S., Levy, M.G., Noga, E.J., 1998b. Acquired immunity to amyloodiniosis is associated with an antibody response. Dis. Aquat. Org. 34, 125−133.

Colorni, A., 1994. Hyperparasitism of *Amyloodinium ocellatum* (Dinoflagellida: Oodinidae) on neobenedenia melleni (monogenea: capsalidae). Dis. Aquat. Org. 19, 157−159.

Cruz-Lacierda, E.R., Maeno, Y., Pineda, A.J.T., Matey, V.E., 2004. Mass mortality of hatchery-reared milkfish (*Chanos chanos*) and mangrove red snapper (*Lutjanus argentimaculatus*) caused by *Amyloodinium ocellatum* (Dinoflagellida). Aquaculture 236, 85−94.

Dequito, A.Q.D., Cruz-Lacierda, E.R., Corre Jr., V.L., 2015. A case study on the environmental features associated with *Amyloodinium ocellatum* (Dinoflagellida) occurrences in a milkfish (*Chanos chanos*) hatchery. AACL Bioflux 8, 390−397.

Diamant, A., 2001. Cross-infections between marine cage-cultured stocks and wild fish in the northern Red Sea: is the environment at risk? Presented at the Risk analysis in aquatic animal health. In: Proceedings of an International Conference, Paris, France, 8−10 February, 2000. Office International des Épizooties, Paris, pp. 202−208.

Fajer-Ávila, E.J., Abdo-de la Parra, I., Aguilar-Zarate, G., Contreras-Arce, R., Zaldívar-Ramírez, J., Betancourt-Lozano, M., 2003. Toxicity of formalin to bullseye puffer fish (*Sphoeroides annulatus* Jenyns, 1843) and its effectiveness to control ectoparasites. Aquaculture 223, 41−50.

Guerra-Santos, B., Albinati, R.C.B., Moreira, E.L.T., Lima, F.W.M., Azevedo, T.M.P. de, Costa, D.S.P., Medeiros, S.D.C. de, Lira, A.D., 2012. Parameters hematological and histopathologic alterations in cobia (*Rachycentron canadum* Linnaeus, 1766) com amyloodiniose. Pesqui. Vet. Bras. 32, 1184−1190.

Kumar, P.R., Nazar, A.K.A., Jayakumar, R., Tamilmani, G., Sakthivel, M., Kalidas, C., Balamurugan, V., Sirajudeen, S., Thiagu, R., Gopakumar, G., 2015. *Amyloodinium ocellatum* infestation in the broodstock of silver pompano *Trachinotus blochii* (Lacepede, 1801) and its therapeutic control. Indian J. Fish. 62, 131−134.

Kuperman, B.I., Matey, V.E., 1999. Massive infestation by *Amyloodinium ocellatum* (Dinoflagellida) of fish in a highly saline lake, Salton Sea, California, USA. Dis. Aquat. Org. 39, 65−73.

Kuperman, B.I., Matey, V.E., Hurlbert, S.H., 2001. Parasites of fish from the salton sea, California. U.S.A. Hydrobiol. 466, 195−208.

Landsberg, J.H., Steidinger, K.A., Blakesley, B.A., Zondervan, R.L., 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. Dis. Aquat. Org. 20, 23−32.

Lawler, A.R., 1977. Dinoflagellate (*Amyloodinium*) Infestation of Pompano. Disease Diagnosis and Control in North American Marine Aquaculture, pp. 257−264.

Lawler, A.R., 1980. Studies on *Amyloodinium ocellatum* (Dinoflagellata) in Mississippi sound: natural and experimental hosts. GCR 6. https://doi.org/10.18785/grr.0604.08.

Levy, M.G., Poore, M.F., Colorni, A., Noga, E.J., Vandersea, M.W., Litaker, R.W., 2007. A highly specific PCR assay for detecting the fish ectoparasite *Amyloodinium ocellatum*. Dis. Aquat. Org. 73, 219−226.

Lewis, D.H., Wenxing, W., Ayers, A., Arnold, C.R., 1988. Preliminary studies on the use of chloroquine as a systemic chemotherapeutic agent for amyloodinosis in red drum (*Sciaenops ocellatus*). Contrib. Mar. Sci. 30 (Suppl. l), 183−189.

Lom, J., Lawler, A.R., 1973. An ultrastructural study on the mode of attachment in dinoflagellates invading gills of Cyprinodontidae. Protistologica T IX, 293−309.

Massimo, M., Beraldo, P., Bulfon, C., Volpatti, D., Byadgi, O., Galeotti, M., 2017a. *Amyloodinium ocellatum*: milestones, doubts and future goals. In: Book of Abstracts XVIII International Conference on Diseases of Fish and Shellfish − EAFP (European Association of Fish Pathologists), vol. 248, pp. 4−8. September 2017, Belfast (UK).

Massimo, M., Beraldo, P., Bulfon, C., Volpatti, D., Byadgi, O., Galeotti, M., 2017b. Effetti *in vitro* di 16 composti di origine vegetale sulla motilità di dinospore di *Amyloodinium ocellatum*. In: Proceeding of XXIII Convegno Nazionale Società Italiana di Patologia Ittica, 5-6 ottobre 2017, Lecce.

Mladineo, I., 2006. Check list of the parasitofauna in Adriatic sea cage-reared fish. Acta Vet. 56, 285−292.

Montgomery-Brock, D., Sato, V.T., Brock, J.A., Tamaru, C.S., 2001. The application of hydrogen peroxide as a treatment for the ectoparasite *Amyloodinium ocellatum* (Brown 1931) on the Pacific threadfin Polydactylus sexfilis. J. World Aquacult. Soc. 32, 250−254.

Moreira, C.B., Hashimoto, G.S. de O., Rombenso, A.N., Candiotto, F.B., Martins, M.L., Tsuzuki, M.Y., 2013. Outbreak of mortality among cage-reared cobia (*Rachycentron canadum*) associated with parasitism. Rev. Bras. Parasitol. Vet. 22, 588−591.

Noga, E.J., 2012. Amyloodinium ocellatum. In: Fish Parasites: Pathobiology and Protection. CABI, Wallingford, pp. 19−29. https://doi.org/10.1079/9781845938062.0019.

Noga, E.J., 2010. Fish Disease: Diagnosis and Treatment, second ed. Wiley-Blackwell.

Noga, E.J., Bower, C.E., 1987. Propagation of the marine dinoflagellate *Amyloodinium ocellatum* under germ-free conditions. J. Parasitol. 73, 924−928.

Noga, E.J., Levy, M.G., 2006. Phylum Dinoflagellata. In: Fish Diseases and Disorders. Protozoan and Metazoan Infections, vol. 1. CABI, Wallingford, pp. 16–45.

Nozzi, V., Strofaldi, S., Piquer, I.F., Crescenzo, D. di, Olivotto, I., Carnevali, O., 2016. *Amyloodinum ocellatum* in *Dicentrarchus labrax*: study of infection in salt water and freshwater aquaponics. Fish Shellfish Immunol. 57, 179–185.

Oestmann, D.J., Lewis, D.H., Zettler, B.A., 1995. Clearance of *Amyloodinium ocellatum* dinospores by *Artemia salina*. J. Aquat. Anim. Health 7, 257–261.

Paperna, I., 1984a. Reproduction cycle and tolerance to temperature and salinity of *Amyloodinium ocellatum* (Brown, 1931) (Dinoflagellida). Ann. Parasitol. Hum. Comp. 59, 7–30.

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

Paperna, I., 1980. *Amyloodinium ocellatum* (Brown, 1931) (Dinoflagellida) infestations in cultured marine fish at Eilat, Red Sea: epizootiology and pathology. J. Fish. Dis. 3, 363–372.

Pereira, J.C., Abrantes, I., Martins, I., Barata, J., Frias, P., Pereira, I., 2011. Ecological and morphological features of *Amyloodinium ocellatum* occurrences in cultivated gilthead seabream *Sparus aurata* L.; a case study. Aquaculture 310, 289–297.

Picón-Camacho, S.M., Thompson, W.P., Blaylock, R.B., Lotz, J.M., 2013. Development of a rapid assay to detect the dinoflagellate *Amyloodinium ocellatum* using loop-mediated isothermal amplification (LAMP). Vet. Parasitol. 196, 265–271.

Ramos, P., Oliveira, J.M., 2001. Amyloodiniosis in turbot, *Psetta maxima* (L.). Rev. Port. Ciencias Vet. 96, 201–205.

Rigos, G., Christophilogiannis, P., Giahnishi, M., Andriopoulou, A., Koutsodimoy, M., Nengas, I., Alexis, M., 1998. *Amyloodinium ocellatum* infestation on sharpsnout sea bream, *Puntazzo puntazzo* Cetti [*Diplodus puntazzo*]. Bull. Eur. Assoc. Fish Pathol. 18, 198–200.

Roberts-Thomson, A., Barnes, A., Fielder, D.S., Lester, R.J.G., Adlard, R.D., 2006. Aerosol dispersal of the fish pathogen, *Amyloodinium ocellatum*. Aquaculture 257, 118–123.

Saraiva, A., Jerónimo, D., Cruz, C., 2011. *Amyloodinium ocellatum* (Chromalveolata: Dinoflagellata) in farmed turbot. Aquaculture 320, 34–36.

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

Smith, S.A., Levy, M.G., Noga, E.J., 1992. Development of an enzyme-linked immunosorbent assay (ELISA) for the detection of antibody to the parasitic dinoflagellate *Amyloodinium ocellatum* in *Oreochromis aureus*. Vet. Parasitol. 42, 145–155.

Smith, S.A., Noga, E.J., Levy, M.G., Gerig, T.M., 1993. Effect of serum from tilapia *Oreochromis aureus* immunized with the dinospore *Amyloodinium ocellatum* on the motility, infectivity and growth of the parasite in cell culture. Dis. Aquat. Org. 15, 73–80.

Souza, N.A. de, 2015. Resposta metabólica da ostra *Crassostrea gigas* na presença do parasita *Amyloodinium ocellatum*. PhD Thesis. Universidade do Algarve.

Tedesco, P., Beraldo, P., Massimo, M., Fioravanti, M.L., Volpatti, D., Dirks, R., Galuppi, R., 2020. Comparative Therapeutic Effects of Natural Compounds Against *Saprolegnia* spp. (Oomycota) and *Amyloodinium ocellatum* (Dinophyceae). Front. Vet. Sci. 7, 83. https://doi.org/10.3389/fvets.2020.00083.

Vivanco-Aranda, M., Río-Zaragoza, O.B. del, Lechuga-Sandoval, C.E., Viana, M.T., Rombenso, A.N., 2018. Health response in yellowtail *Seriola dorsalis* exposed to an *Amyloodinium ocellatum* outbreak. Cienc. Mar. 44, 267–277.