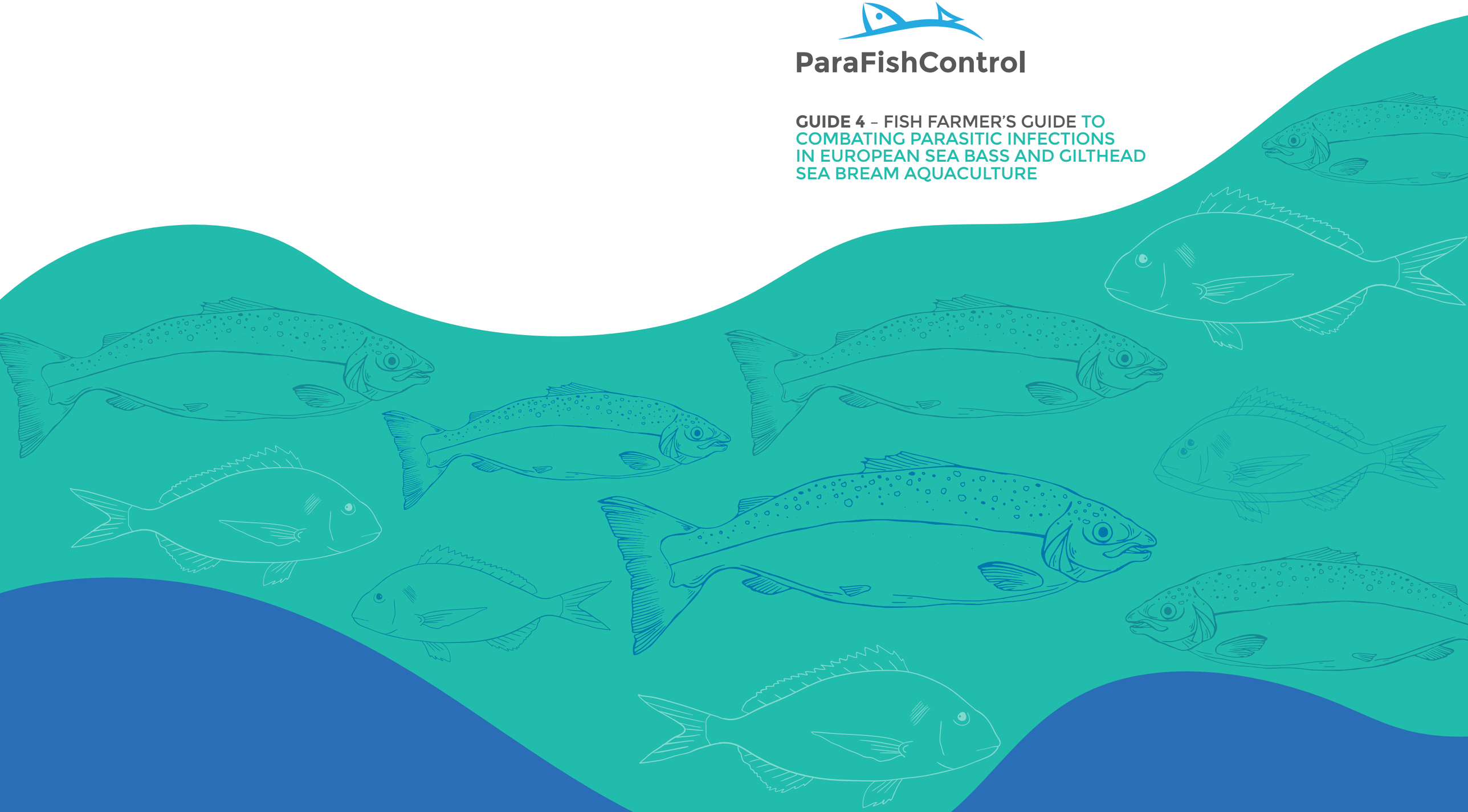
ParaFishControl

GUIDE 4 – FISH FARMER'S GUIDE TO COMBATING PARASITIC INFECTIONS IN EUROPEAN SEA BASS AND GILTHEAD SEA BREAM AQUACULTURE



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A Series of ParaFishControl Guides to Combating Fish Parasite Infections in Aquaculture. **Guide 4**

ParaFishControl

> "An ounce of prevention is worth a pound of cure."
>
> *Benjamin Franklin*

European sea bass and gilthead sea bream are the main fish species farmed in the Mediterranean region. They are produced in land-based extensive and intensive grow-out systems and sea cages. Millions of fingerlings are produced and traded in the region, with potential impacts on health control. Parasitic diseases are a serious constraint on production. This guide provides useful information about the biological background of five parasites, their diagnostics and control measures.

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Introduction

Mediterranean marine finfish aquaculture is characterized by different systems and technologies. These include industrial hatcheries, land-based extensive and intensive grow-out systems up to sea cages, and mainly focus on producing European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*). Greece, Spain, Italy, Croatia and France are the main producers of these species in Europe. Additionally, several hundred million juveniles of both species were produced in EU countries in 2018. Despite recent attempts to diversify Mediterranean aquaculture, other species such as meagre (*Argyrosomus regius*), sole (*Solea* spp.), red porgy (*Pagrus pagrus*) and other sparids contribute less than 5% to overall production.

On a global basis, European sea bass (*Dicentrarchus labrax*, ESB) and gilthead sea bream (*Sparus aurata*, GSB) are farmed almost entirely in 19 Mediterranean countries, although more than 90 % is concentrated in Turkey, Greece, Egypt, Spain, Tunisia and Italy. These six countries export largely to EU markets, accounting for almost 350,000 tonnes (Source: FAO, 2016). In the EU, production amounted to 82,443 tons and 93,609 tons for ESB and GSB respectively in 2018 (Source: FEAP, 2019). Total juvenile production (more than 1,200 million fingerlings) is concentrated in just five countries, as more than 90% of fingerlings are produced in Greece, Turkey, Spain, Italy and France. This implies an important trade of fingerlings in the region, with potential impacts on health control (Source: FEAP 2019 and MedAid project). This production means that more than 600 million bacterial and viral vaccine doses are used each year (Source: P. J. Midling, Aquamedic AS). However, no vaccine is available for parasitic diseases and very few treatments are licensed.

"An ounce of prevention is worth a pound of cure". Although originally coined by Benjamin Franklin in relation to firefighting measures in Philadelphia, the phrase has come to indicate more generally that preventing a problem is better than solving it. This also holds true in relation to animal and human health problems.

This can be well understood and appreciated by those who are faced with parasitic diseases of farmed fish. The aetiology is frequently complex and involves, in addition to the parasite itself, several environmental and host-related cofactors, and human safety regulations. This often makes treatments inconclusive. Therefore, the application of biosecurity measures to decrease the risks of infection and disease outbreak is essential.

Control of parasitic diseases in aquaculture, therefore, requires a holistic approach which considers all factors involved, based on a comprehensive understanding of the life cycle and transmission routes of parasites, and of the abiotic and biotic factors that can alter host-parasite interactions.

In this guide, some of the main parasites causing diseases in ESB and GSB are illustrated in a concise and schematic way. This will enable fish farmers to find updated information on the biological cycle and routes of transmission, disease clinical presentation, diagnosis, main risk factors for parasite introduction and parasitic disease occurrence, as well as recommendations for their management and control. In particular, five parasites have been addressed: the dinoflagellate *Amyloodinium ocellatum*, responsible for amyloodiniosis in ESB and GSB; *Enterospora nucleophila*, which causes emaciative microsporidiosis in GSB; *Enteromyxum leei*, an enteric myxozoan that causes enteromyxosis in GSB; S*paricotyle chrysophrii*, a gill monogenean responsible for sparicotylosis in GSB; and *Ceratothoa oestroides*, an isopod crustacean infecting ESB and, to a lesser extent, GSB. Diagrammatic charts of other ecto- and endo-parasites are also provided.

This manual also provides indications on how to monitor and combat some parasitic diseases in ESB and GSB farms, increasing awareness of the main risks for parasite entry/transmission and related control measures. Proper application of these measures will require farm-tailored biosecurity plans with the support of qualified fish health professionals and diagnostic centres. This manual is not comprehensive but serves as an easily comprehended and necessary support during the daily handling and management of these fish. The guidance provided for individual parasites reflects the current state of knowledge for these pathogens, obtained in the framework of the **ParaFishControl** project, which has expanded scientific knowledge on the main parasites of fish farmed in European countries. **ParaFishControl** has enhanced the understanding of risk factors leading to parasite infection and development of parasitic disease in aquaculture. The project provides useful tools to combat and mitigate parasites with an IPMS (Integrated Parasite Control Strategies) approach that targets parasite control in a sustainable way by including treatment only in combination with biosecurity and hygiene measures.

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1. Fish farmer's guide to combating *Enteromyxum leei* infections

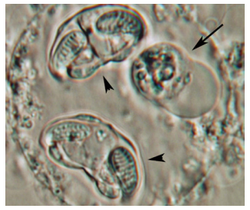

Figure 1. Photomicrograph from a fresh intestinal smear with a large trophozoite harbouring two *Enteromyxum leei* myxospores (arrowheads) and a secondary or accompanying cell (arrow). Photo: A. Sitjà-Bobadilla, CSIC.

Introduction

Enteromyxum leei (Figure 1) is a myxozoan parasite that infects the intestinal tract of fish and associated organs, such as the gall bladder and liver. This endoparasite is responsible for enteromyxosis, a disease that causes emaciation. In sparids, this disease is also known as "razor blade syndrome" or "knife-syndrome", due to the appearance of severely emaciated individuals. It has significant impacts on gilthead sea bream (*Sparus aurata*) intensive farms at Mediterranean and Atlantic sites, and it has caused the stagnation or abandonment of wide-scale production of valuable fish such as red porgy (*Pagrus pagrus)* or sharpsnout sea bream (*Diplodus puntazzo* - the most susceptible host), across the Eastern Mediterranean and Adriatic seas.

Biological life cycle

Although the life cycle of myxozoans generally involves two alternating hosts, a fish and an aquatic invertebrate, direct spontaneous fish-to-fish transmission has been demonstrated only for species belonging to the genus *Enteromyxum* in several marine fish. In particular, *E. leei* is transmitted directly from fish to fish by cohabitation, waterborne contamination or by eating infected material. The infective stages are not the myxospores, but rather developmental stages which are released from the infected fish in faecal casts, together with intestinal epithelial debris. This release can be intense as the parasites cause inflammation and destroy the intestine epithelium. This unique mode of horizontal transmission favours the spread of this parasite in farmed fish stocks. Although the survival time of isolated developmental stages (infective for fish) in seawater is estimated to be less than 24 hours, it is long enough for direct transmission, especially in conditions of high biomass density, poor water exchange, and presence of infected carcasses. On the contrary, myxospores are more resistant parasite stages that can survive for longer periods but are not infectious to fish. Myxospores are presumed to require a suitable invertebrate host (still unidentified) to complete development and sexual reproduction (Figure 2).

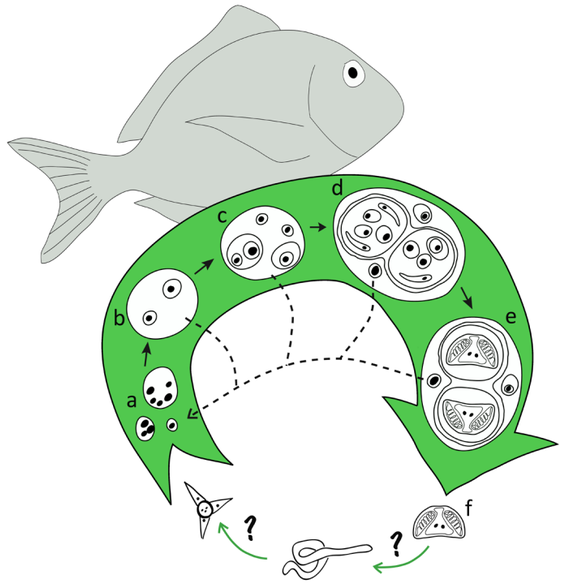

Figure 2. Life cycle of *Enteromyxum leei.* In the intestinal epithelium of the fish, proliferative (a-c) and sporogonic (d-f) development occurs. Stages a-e are responsible for the invasion and dispersion within the fish, as well as for transmission to other fish, when released through faeces. Whether the mature spore starts an alternate cycle infecting an invertebrate host is currently unknown. Drawing: I. Estensoro, CSIC.

Seasonality

Water temperature is a critical risk factor in the transmission and onset of enteromyxosis. Under culture conditions, the minimum temperature for developing clinical enteromyxosis in gilthead sea bream usually ranges from 18° C to 22 °C, and outbreaks in some farms have only been observed above 20 °C. Optimal development is between 20-25 °C. Disease onset is largely delayed or even suppressed at temperatures below 15 °C. The limited parasite multiplication rate and infective dose reached in the water during winter seems too low to establish the infection, however, it can remain latent during the cooler period and re-emerge when water temperature increases. This has important epizootiological consequences, since fish that test negative during winter can become a source of the parasite when water temperature rises. A high-water temperature (>30 °C) seems to have preventive or curative effects in some fish species.

Age / mean weight susceptibility

E. leei has a wide host and geographical range within marine fish (at least 60 species from 22 different families, mainly Perciforms), and even freshwater fish have been infected experimentally. The most susceptible host is sharpsnout sea bream, producing up to 100 % mortalities among juveniles. Other highly susceptible species include red porgy and red sea bream (*Pagrus* spp.), Japanese flounder (*Paralichthys olivaceus*), or fugu (*Takifugu rubipres*). In gilthead sea bream, *E. leei* infection produces a chronic disease in juveniles and adults, with mortalities depending on host and environmental factors. Other farmed marine fish such as European sea bass or Senegalese sole (*Solea senegalensis*) show minor clinical signs, but they can transmit the disease to other species with higher susceptibility that are farmed nearby. Many ubiquitous wild fish species such as labrid and gobids are susceptible.

Risk predisposing factors

Enteromyxosis caused by *E. leei* has been described in all types of farming facilities (Recirculated Aquaculture Systems (RAS), raceways, concrete or PVC tanks, earth ponds and sea cages). Facilities with high water temperature throughout the production cycle (in (sub)tropical areas or using heated water), with low water flow and water exchange rate, with water intake influenced by water effluents, or with poor management strategies, are more prone to reach high intensities of infection. In gilthead sea bream farms, the main risk or aggravating factors are: high biomass densities; poor water exchange and / or reuse of contaminated water; recirculation systems; extended culture cycles for production of large fish; infrequent removal of dead fish and / or their inappropriate disposal; and low feeding rates which may increase cannibalism. Enteromyxosis has also been associated with overfeeding and with high fat content diets. Some diets high in vegetable oils have also been shown to induce a worse disease outcome in gilthead sea bream.

A) What clinical signs should alarm me?

External signs

In gilthead sea bream, clinical *E. leei* infection is characterized by anorexia and weight loss until emaciation and / or cachexia, which is evident for the marked atrophy of epaxial muscle and prominent head bones in severely affected fish (Figure 3A). Accumulation of fluid (ascites) with opportunistic bacteria may turn into abdominal swelling in some cases (Figure 3B). Direct mortality is largely dependent on the culture model and aggravating factors, ranging from a low-level dropping mortality (e.g. in certain sea cages), to heavy sustained losses (e.g. in raceways or closed systems with heated water). Most often, the infection causes a significant growth retardation and mortality in adult specimens (generally >100 g) in cage and land-based farms. A decrease in the feed conversion rate (FCR) should be an alarming sign. On the contrary, in sharpsnout sea bream, enteromyxosis has an acute disease course without clear external signs except for a heavy mortality starting a few weeks after being seeded in enzootic on-growing systems (up to 100% in first-year fish, <80 g).

Figure 3. A: Strongly emaciated gilthead sea bream infected by *Enteromyxum leei*, notice the absence of skeletal muscle and the typical "razor blade-syndrome". Photo: O. Palenzuela, CSIC; B: Acute abdominal distension due to accumulation of ascitic fluid in an *Enteromyxum leei*-infected gilthead sea bream. Photo: A. Sitjà-Bobadilla, CSIC.

Internal lesions: Thin, transparent, intestinal walls, sometimes with focal congestion and accumulation of liquid in the lumen (Figure 4). Sometimes ascites can be present, and the gall bladder can display alterations.

Figure 4. Gross internal signs of enteromyxosis in gilthead sea bream infected by *E. leei*. The absence of visceral fat and transparent intestinal wall is visible. Photo: O. Palenzuela, CSIC.

B) How to detect the parasite at farm level

1. Monitoring plan (what to measure and how often) and trigger level for action

Intestinal scrapings from the rectum epithelium (either fresh or stained) with easily recognized spores can be observed using a light microscope (Figures 1 and 5). However, developmental stages are more difficult to distinguish and can be misdiagnosed, particularly at low intensity of infection. Therefore, monitoring of the infection by PCR is recommended for all batches, before their introduction to a new farm and throughout the production cycle, in order to promptly detect the infection and apply control measures. There is no threshold level of parasite burden related to the emergence of mortality / morbidity, though a clear relationship between the intensity of the infection and weight loss has been shown. For epidemiological purposes, the most appropriate sampling periods are warmer months, except for farms with high water temperature throughout the production cycle. Otherwise, samples must be collected and submitted for diagnosis as soon as clinical signs related to enteromyxosis are seen.

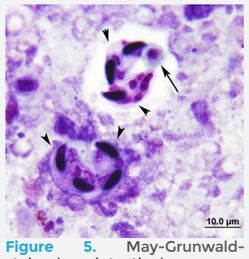

Figure 5. May-Grunwald-stained intestinal smear showing myxospores of *E. leei* (arrowheads) and a secondary cell (arrow). Polar capsules are strongly stained. Photo: A. Sitjà-Bobadilla, CSIC.

2. Recommendations for the submission of samples to be diagnosed

Whole fresh fish should be delivered within 24 hours to the lab for microscopical examination and PCR. For PCR / qPCR, rectal ampoule can be preserved in 80 % ethanol, while, for histology, the posterior part of the gut must be fixed in 10 % buffered formalin. Non-lethal molecular diagnostic tests, using rectal swabs, may also be used.

3. Contact laboratories

- Fish Pathology Group, Instituto de Acuicultura Torre de la Sal, (IATS-CSIC) Castellón, Spain.
- Fish Pathology Lab, DIMEVET, University of Bologna, Ozzano Emilia (BO), Italy.

C) Action plan for prevention and control

1. Prevention and farm management

In land-based open systems, micro-filtration of inflow water (mesh size < 5µm) can reduce the risk of introduction of parasitic stages infective for fish. Furthermore, regular maintenance of water channels and pipes can control the settlement of (hypothetical) invertebrate hosts. Infective stages are not particularly mechanically or physically resistant: tanks can be cleaned with freshwater and / or routinely used surface disinfectants. In these facilities, it is essential to avoid recirculation or re-use of the water through proper design and positioning of water intake and effluent points. Serial reuse raceways pose a serious risk and should be avoided. In cage systems, cages should be preferably exposed to moderate seawater current, respecting a wide distance between cages containing different batches. Nets should frequently be cleaned or changed in high-risk periods.

In all farming systems, quarantine and PCR checks of the fish prior to contact with other fish in the facility should be performed. Fish stocks from enzootic areas should be tested before introduction to disease-free sites. Furthermore, parasitological checks throughout the production cycle should be performed to promptly detect the infection and implement proper control measures. Routine hygienic disposal of the water and disinfection of transport vehicles and farm equipment helps to reduce the risk of parasite transmission. Feeding fish with inadequate diets based on high vegetable inclusion should be avoided. Instead, farmers should select high-quality protein feeds. Overfeeding and extreme overcrowding should also be avoided. Deceased fish should be separated and removed as soon and as frequently as possible, by means of mortality traps and / or automated suction devices.

If enteromyxosis is diagnosed, avoid stressing procedures (such as handling and transport), reduce biomass density, increase the frequency of removal of dead fish and the level of hygienic measures. Depending on the age of the fish and the severity of the infection, culling the stock and administration of specific functional feeds can be considered. Fish that recover from enteromyxosis are resistant to reinfection, which enables the development of immunoprophylaxis tools in future.

2. Treatment

There are currently no registered treatments effective against *Enteromyxum leei*. A combination of Amprolium and Salinomycin has been shown to be partially effective in some trials. Some infeed nutraceutical mitigation solutions are currently available, such as sodium butyrate BP-70® (Norel), Sanacore®GM (Adisseo) and Shield™ (Skretting).

3. Management of co-infections

Gilthead sea bream affected by enteromyxosis could be co-infected by other pathogenic parasites such as the gill monogenean, *Sparicotyle chrysophrii* and/or the gut microsporidian, *Enterospora nucleophila*, which need to be addressed with targeted measures (see the specific sections of this guide). Furthermore, infectious diseases due to opportunistic bacteria could easily develop in the affected sea bream, requiring an appropriate diagnostic approach and antibiotic therapy.

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2. Fish farmer's guide to combating *Ceratothoa oestroides* infections

Figure 6. Pair of *Ceratothoa oestroides* in the buccal cavity of a wild bogue (arrowhead: gravid female; long arrow: male). Photo: Fish Pathology Lab, University of Bologna.

Introduction

Ceratothoa oestroides is a crustacean isopod parasitizing several marine fish species inside the host buccal cavity (Figure 6). In aquaculture, *C. oestroides* infects European sea bass and to a lesser extent gilthead sea bream and meagre (*Argyrosomus regius*) in sea cages in certain parts of the Mediterranean. The infection causes severe clinical signs and mortality outbreaks, particularly in fry and fingerlings, while in larger, market-size fish, it reduces growth. Even if *C. oestroides* is supposed to be hematophagous, this has not yet been proven or refuted. Although the intrabuccal development of the parasite induces deformities of the fish lower jaw, this does not cause rejection at harvest. However, each fish needs to be checked for parasites, and the latter needs to be manually extracted in order to meet EU regulations on the hygiene of food of animal origin (EC, 853/2004).

Biological life cycle

The life cycle of this parasite is direct (Figure 7). *C. oestroides* is a protandric hermaphrodite (female develops from the male and stops further development of other males). Adult male and female mate in the host buccal cavity, and embryos develop in the female marsupium, moulting through two "pullus" stages (I-II stages). The first pullus (I stage) can be found only in the marsupium, whereas the second pullus (II stage) hatches as a "manca" stage, which is released out of the marsupium to find a new host. If a host is not found quickly, it will sink to the bottom and die. Under suitable conditions a mature female can release 450-550 manca stages. After attachment, manca will become an offspring-releasing female in 29 days at 20.5 °C. The life cycle duration of other related isopods has been established experimentally to be about two months at 24 °C. In *in vivo* experiments performed in flow-through systems with natural oscillations of seawater temperature, paired *C. oestroides* took more than 3 months to produce the first batch of manca (from August to October), while the next batch was produced in May the following year.

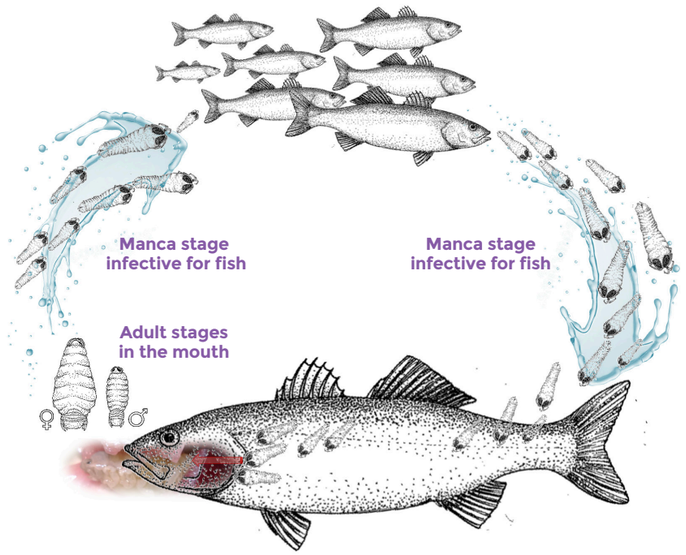

Figure 7. Scheme of the life cycle of *Ceratothoa oestroides*. Drawing: M.L. Fioravanti, University of Bologna.

Seasonality

Although parasite infection is present all year round, fecundity and hatching rate of the parasite increases with water temperature. Therefore, the infection is most prominent during the summer, with peaks between June and August.

Age / mean weight susceptibility

In general, fry and young fish of all species are susceptible to infection by the larval stages of the parasites. Adult, reproductive parasites are sessile and cannot re-infect new fish but they are found attached in the buccal cavity of larger on-growing fish.

Risk predisposing factors

Juvenile fish introduced from hatcheries into sea cages are typically parasite-free. Therefore, exposure to *C. oestroides* happens at the farm level due to the transfer of the parasite from wild fish (bogue with, *Boops boops*) and other sparids are effective reservoirs) to farmed ones, or from already infected adult farmed fish to newly introduced fingerlings (Figure 8). Predisposing factors are: proximity of susceptible fry to on-growing fish carrying adult isopods; presence of large numbers of wild fish around the cages; cages in areas characterized by weak sea currents and high farming activity; infrequent cleaning of the nets and high biomass density.

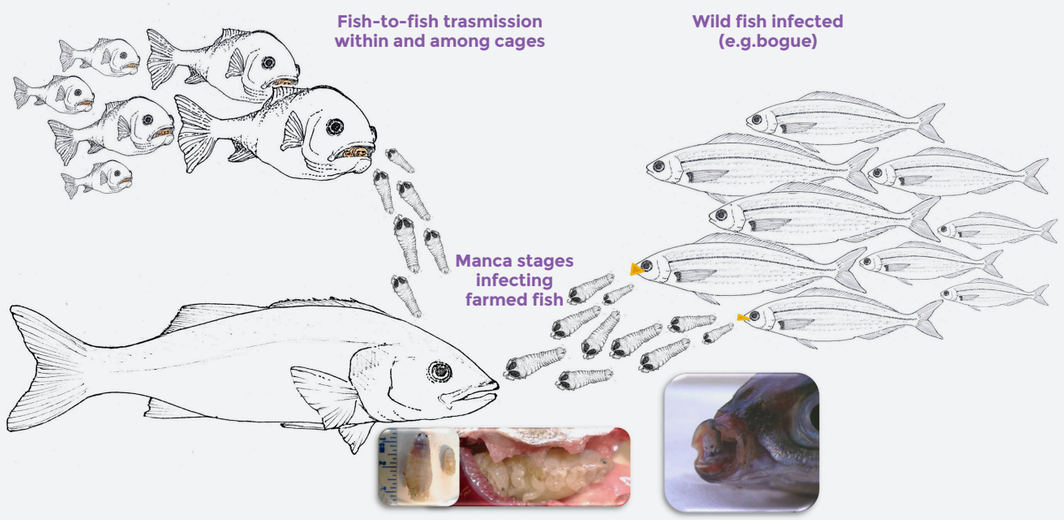

Figure 8. Transmission routes of C. oestroides in sea cages. Drawing: M.L. Fioravanti, University of Bologna.

A) What clinical signs should alarm me?

External signs

Larval stages of *C. oestroides* that attack small fish cause the most damage including severe ulcers, gill lesions and extensive granulomatous lesions in the eyes that results in blindness or total loss of the eyeball. Smaller European sea bass are especially affected, resulting in mortality of 5-20 %. Fish infected with adult parasites do not show serious pathology, but lesions can be present at the upper and lower jaws and the tongue. Growth of the market size infected caged fish can be reduced by 20 % compared to uninfected specimens. In highly infected small fish, manca stages can be observed attached to the body surface, under the operculum, in gills and the buccal cavity. Eventually, excess of manca will fall off and only two individuals will settle in the buccal cavity. Infected larvae and fingerlings will show sluggish swimming, darkened pigmentation, large heads and thin body, with a 5-20 % mortality rate. Fish infected with adult parasites do not show serious pathology, except for a deformed ventral oropharyngeal part (lower jaw), which is the result of a growing female *C. oestroides* pushing down the soft tissues of the host. Sometimes, external skin can show small patchy haemorrhagic spots caused by secondary bacterial infections.

Physical observations

Checking the buccal cavity, parasites can be easily identified. In early infection, manca will be present in the gill cavity.

Internal lesions

No internal lesions are generally associated with *C. oestroides* infection.

B) How to detect the parasite at farm level

Monitoring plan (what to measure and how often) and trigger level for action

Special care should be given in warm season when transferring larvae and fingerlings into sea cages, as released manca can be observed swimming and attaching to the newly- seeded fish. Early in the morning before the first feeding, a planktonic net can be pulled through the cages from 1-1.5 m of the depth up to the surface to check for newly released manca. Although there is not an established trigger level for action, as it depends on the farm production characteristics (fish density, number of cages, closeness of cages, etc.), if high numbers of manca (e.g. >100 pulli per net trawl) are observed, then farmers should consider treatment or other zooprophylactic measures.

Harvested commercial fish should be checked for adult parasites, as they must be manually de-loused before selling to retailers and often fish with deformed lower jaw (parasitized) are rejected. However, at harvest, the level of infection in commercial-size fish is usually low and does not fully reflect the situation in small-size fish. Monitoring of wild fish for the presence of *C. oestroides* in warmer periods can be also done but provides limited information of the epidemiological status in farmed fish.

2. Recommendations for the submission of samples to be diagnosed

Prepare air-dried or methanol-fixed This ectoparasite is easily diagnosed at farm level, so sample submission is not required.

3. Contact laboratories

This parasite is identifiable at all standard fish disease laboratories.

C) Action plan for prevention and control

1. Prevention and farm management

- Maintaining a wide distance between small fish and adult cages (which could be infected by adult parasites in the buccal cavity).
- Locating cages in sites with higher depths and currents to hamper fish infection by isopod larval stages.
- Periodical fish grading and separation by size.
- Periodic cleaning of cage nets, which diminishes biofouling where manca can temporarily attach, and allows currents and better exchange of water within the cage, helping to dissipate unattached manca.
- Avoidance of high biomass density in cages containing fry or lowering it when infection is detected.
- Manual removal of isopods from fish during handling procedures, such as size sorting and vaccination by injection, can help reducing the parasitic load in the batch.

2. Treatment

Although bath treatments with formalin, hydrogen peroxide, anti-sea lice commercial formulations of organophosphates and pyrethroids have been tested against *Ceratothoa* infections, cost-benefit, safety, environmental and resistance issues must be taken into account. Furthermore, bath treatments in cages are labour-intensive and often stressful for fish in addition to being costly, with short or limited effects, and are not always feasible in open sea conditions. Among in-feed treatments potentially effective against this parasite, emamectin benzoate has been used in the field without consistent results. Diflubenzuron has been experimentally tested with positive results against *C. oestroides* in sea bass, but it is not currently commercially available.

2. Management of co-infections

Infections by bacterial opportunistic pathogens (e.g. *Vibrio* spp.) can easily occur in *Ceratothoa*-infected fish. Administration of medicated feed has limited efficacy since infected fish do not eat, requiring the implementation of preventive measures. Since infections by Rickettsia-like organisms (RLO) have been frequently observed in *Ceratothoa*-highly infected farms, a role of this crustacean in the transmission of RLO has been hypothesized. Co-infections due to other parasites are also possible and should be specifically managed.

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3. Fish farmer's guide to combating *Sparicotyle chrysophrii* infections

Figure 9. *Sparicotyle chrysophrii* in a fresh mount of gilthead sea bream gill. Photo: Fish Pathology Lab, University of Bologna.

Introduction

Sparicotlye chrysophrii (Figure 9) is a monogenean microcotylid gill parasite mostly found in gilthead sea bream (*Sparus aurata*), but also in other sparids. It is widespread throughout the Mediterranean and is probably the most serious pathogenic threat to gilthead sea bream intensive farming in the region. It is amplified in cage farms where it readily infects newly introduced fish and is easily transmitted from cage to cage. Infections result in some mortalities, significant reduction of growth and an increased feed conversion rate. *S. chrysophrii* has also been recorded in gilthead sea bream from the Red Sea and Northeast Atlantic, and from cultured sharpsnout bream (*Diplodus puntazzo*).

Biological life cycle

S. chrysophrii is a hermaphrodite and has a direct life cycle. It produces eggs, passively transmitted by currents, from which the free swimming oncomiracidia hatch and actively search for the host (Fig. 10). The peculiar features of eggs, i.e. high buoyancy and presence of two tendril-like filaments, allow them to entangle in host gills and / or submerged substrates such as nets and biofouling, enabling propagation of the parasite in a sea cage environment. The oncomiracidium hatches mainly during darkness after 5-10 days at 20 °C and survives briefly in seawater without finding a fish host (only 10 % live more than 24 hours, reaching a maximum of 52 hours at 20 °C). The whole life cycle lasts approximately 50 days at 20 °C. Pathogenic effects of the parasite are mainly linked to its hematophagous nature and to the severe gill lesions observed even at low infection intensity.

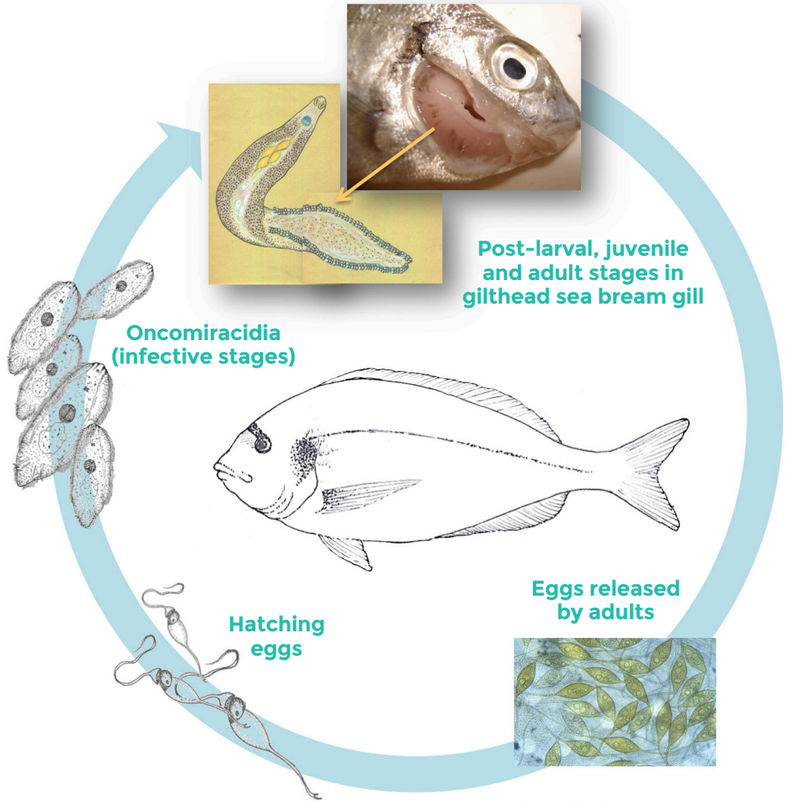

Figure 10. Scheme of the life cycle of S. *chrysophrii*. Drawing: M.L. Fioravanti, University of Bologna.

Seasonality

Seasonality of sparicotylosis differs depending on the geographical area. Although the parasite is reported throughout the year, prevalence and intensity is generally higher in the warm season in the Mediterranean, except for Corsica where highest values are observed in winter. Some mortality outbreaks have been also been reported at water temperature <15 °C in Spain.

Age / mean weight susceptibility

All fish life stages are susceptible, but juveniles are usually more sensitive to *Sparicotyle* infection than adult fish, and can develop clinical signs at lower parasite intensity.

Risk predisposing factors

The parasite is not present in hatchery-reared fry, but the possibility that juveniles are already parasitized when introduced to an on-growing farm must be considered.

The presence of eggs and / or oncomiracidia in water entering the fish farm is a relevant risk factor in intensive on-growing systems, due to the fish-to-fish transmission, and the high rearing densities that favour its transmission and propagation in the farm. In fish farms (Figure 11), low currents, low distance between the sea bottom and cages, close vicinity of cages with newly introduced fish to cages with adult fish, infrequent changes and cleaning of nets (a substrate for egg entanglement) and infrequent removal of dead fish are all relevant risk factors influencing the parasite load and parasite outbreaks. Transfer of *S. chrysophrii* from wild infected sparids to cultured fish is known to occur but the rate of transfer varies depending on the geographic area. Although the role of the wild bogue (*Boops boops*) as a wild reservoir was initially excluded using mitochondrial DNA markers, recent findings using NGS prove the opposite.

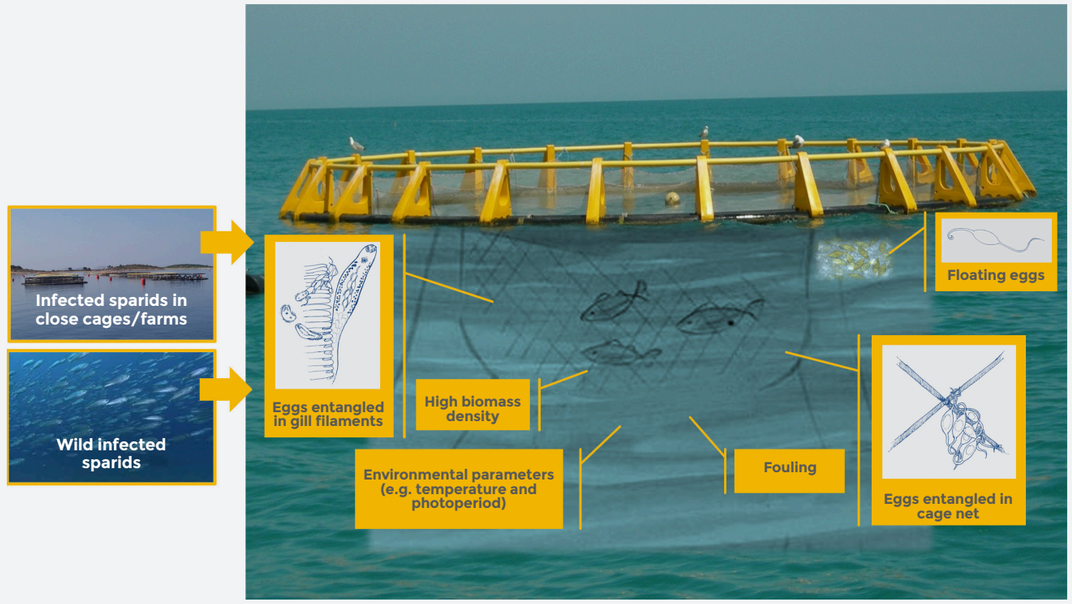

Figure 11. Risk factors favoring the transmission of *S. chrysophrii* in sea cages. Drawing: M.L. Fioravanti, University of Bologna.

A) What clinical signs should alarm me?

External signs

Gilthead sea bream affected by sparicotylosis show pale gills as a sign of anaemia, lethargy due to the lack of oxygen (Figure 12), reduced appetite up to ceasing of feeding (anorexia), growth retardation, increase of FCR by a factor of > 0.4, and mortality at high infection intensity. The ventral part of the fish body can be visibly indrawn. Severe pathology is also observed at low infection intensity of just a few parasites per gill arch.

Physical observations

The most striking sign is a pale appearance of the gills when the operculum is opened. Small and elongated, brownish-coloured parasites can be observed among the gill epithelium.

Internal lesions

Systemic anaemia is frequently observed in the course of acute infections by *S. chrysophrii.* In the visceral cavity, guts can be filled with transparent and viscous liquid if fish do not eat, but adversely can also be filled with half-digested feed.

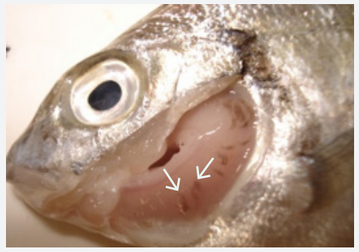

Figure 12. Gilthead seabream with anaemic gills infected by *S. chrysophrii* (arrows). Photo: Fish Pathology Lab, University of Bologna.

B) How to detect the parasite at farm level

1. Monitoring plan (what to measure and how often) and trigger level for action

Farmers should carefully monitor fish with increases in seawater temperature after the wintertime, as the proliferation of *Sparicotyle* usually occurs above 22-23 °C. The gill arches are first checked visually for the presence of the parasite, and then the parasites are counted under the stereomicroscope. Depending on the farm's previous experiences and practice, farmers should then decide whether to treat the fish with formalin baths. Usually levels higher than 1-2 monogeneans per external gill arch should trigger the treatment, since comparative studies of gill arches have shown that this is the preferred site for *S. chrysophrii.* In flow-through or recirculation tanks, submerged equipment parts (nets, pipes, inlets and outlets) should be checked visually for the presence of entrapped eggs, or a piece of net could be intentionally tied up in the tank where released eggs could be collected and subsequently destroyed. This practice depends on technical factors of the system and, if employed, could be adapted depending on the system.

2. Recommendations for the submission of samples to be diagnosed

Since this ectoparasite is easily diagnosed at farm level, submission of samples is not required.

3. Contact laboratories

This parasite is identifiable by all fish diseases laboratories and experts in parasitology.

C) Action plan for prevention and control

1. Prevention and farm management

In any farming system, periodic evaluation of the parasite load through a farm scoring system is essential, especially for newly introduced fish and to determine when treatments have to be done. The use of separate equipment for fingerlings and older fish sizes, and periodic cleaning and disinfection of all the equipment helps in reducing the risk of in-farm transmission of the parasite. The administration of specific diets may help to reduce the parasitic load in the farm in the periods most at risk.

In intensive land-based farms, the treatment of inflow water through mechanical filtration and the periodical fallowing with drying and bottom disinfection of the tank are essential. Structural separation of sectors with newly introduced fish from those with already stocked fish can reduce transmission within the farm. Lowering of biomass density and increasing water exchange can mitigate the infection by reducing the environmental load of eggs and limiting the success of oncomiracidial attachment to the fish.

In sea cage systems, to reduce the likelihood of *S. chrysophrii* spreading into the farm the following is recommended:

- Periodic cleaning / change of the nets to remove entangled eggs (at least 20 times per production cycle).
- Wide distance between cages containing new batches from those with the old ones (also considering sea currents), separation of cages with adult fish and fingerlings.
- If possible, alternate cages with different species (sea bream and sea bass) to increase the distance between infected cages.
- Cage location in sites with higher depths and stronger currents to hamper oncomiracidia settlement.
- Removal of dead fish as frequently as possible (preferably daily).
- Decrease of rearing density to avoid shorter routes of transmission.
- Management of aggregating wild fish population should be considered, since wild sparids influence the disease transfer depending on locations.

2. Treatment

Fish smaller than 20 g and larger than 200 g are normally not treated, and other size categories are treated once during the respective production stage. In vitro trials showed that a 30 min bath in formalin (300 ppm) is 100% effective for eggs, oncomiracidia and adults of *S. chrysophrii*, and hydrogen peroxide (200 ppm) is fully effective for oncomiracidia and adults (efficacy against eggs was not determined). Farms use formalin baths (300 ppm) usually for 60 min, while higher concentrations are used in winter. This should be synchronized with net changing for the best results. However, bath treatments (with formalin, hydrogen peroxide and other substances) are not authorized in several countries. Some *in vivo* trials have been conducted by oral administration of praziquantel, showing its potential efficacy in reducing prevalence and intensity of *S. chrysophrii* but highlighting problems due to the poor palatability of praziquantel medicated feed. Recently, attention has been focused on more practical and safe control strategies such as the use of feed additives. In particular, caprylic acid alone (200 mg/kg b.w.) or combined with iron (0.2 % of diet) and immunostimulants like mannan oligosaccharides (MOS) (0.4 % of diet) showed a good efficacy in decreasing intensity of adults and juveniles in the gills, although it did not reduce prevalence. Addition of organic iron in feed (100-200 ppm) is used in some farms for 5-30 g fish. Integrated control strategies against *S. chrysophrii* also includes the use of feed supplemented with immunostimulants, i.e. mostly vitamin E, selenium, glucans, mannan oligosaccharides (MOS), ß-glucans and nucleotides.

3. Management of co-infections

Secondary bacterial infections (e.g. *Tenacibaculum maritimum*) are common in *S. chrysophrii*-infected gills, requiring the application of targeted control measures against these pathogens. Co-infections by other ecto or endoparasites can also occur in *Sparicotyle*-infected sea bream but have to be specifically managed (see sections on *Enteromyxum leei* and *Enterospora nucleophila*).

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4. Fish farmer's guide to combating *Amyloodinium ocellatum* infections

Introduction

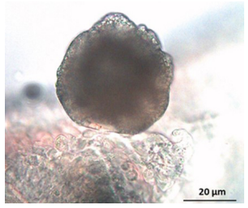

Figure 13. Trophont of *Amyloodinium ocellatum* attached to gill epithelium (Dr Beraldo, University of Udine).

Amyloodinium ocellatum (Brown, 1931) is an ectoparasitic dinoflagellate distributed in marine and brackish-water environments at both tropical and temperate regions worldwide. Thanks to its plasticity, almost all fish (both teleosts and elasmobranchs) living within its ecological range are susceptible to infection, but crustaceans and flatworms can also be infected. In Mediterranean aquaculture, *A. ocellatum* can cause a serious disease called "marine velvet disease" or amyloodiniosis, mainly in European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) farmed in intensive and semi-intensive inland systems. It can cause dramatic losses, primarily during warmer months. Amyloodiniosis can kill the host in less than 12 hours, with acute morbidity and mortality around 100 %, depending on the farming conditions, parasite burden, fish species and season. *A. ocellatum* pathogenicity is related to the attachment to host tissues (mainly gills) of its parasitic stage (trophont, Figure 13), which constantly twists and turns slowly, thus damaging and killing host cells. Gills of heavily infected fish appear pale with filiform haemorrhages (Figure 14A), the histopathology of the epithelium shows a severe alteration of the architecture with hyperplasia and fusion of secondary lamellae, oedema, aneurysms and necrosis (Figure 14B).

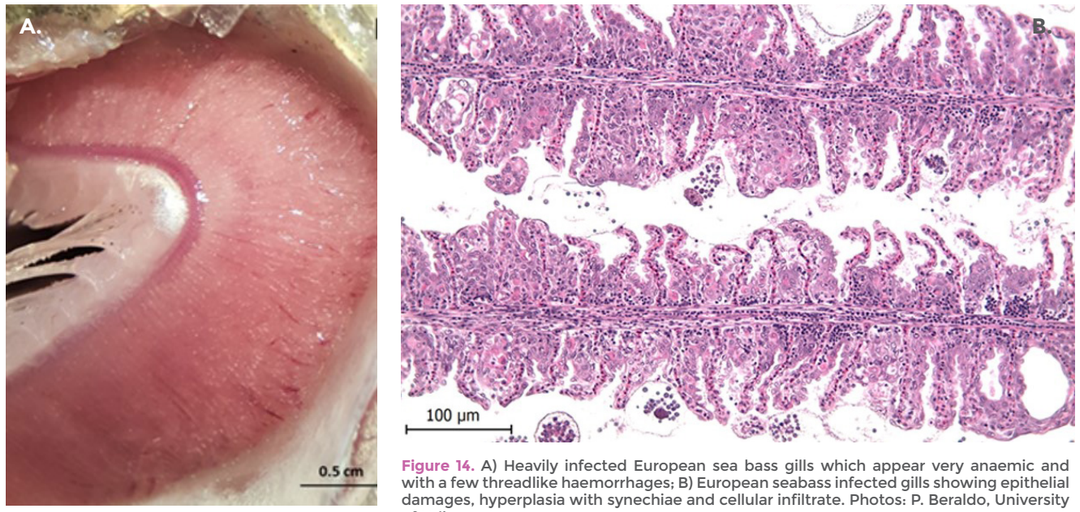

Figure 14. A) Heavily infected European sea bass gills which appear very anaemic and with a few threadlike haemorrhages; B) European seabass infected gills showing epithelial damages, hyperplasia with synechiae and cellular infiltrate. Photos: P. Beraldo, University of Udine.

Biological life cycle

A. ocellatum has a direct life cycle which is divided into three phases (Figure 15). The sessile trophont is the parasitic stage feeding directly from the host cells. In general, trophonts are predominantly present in the gills and skin epithelia, to which they anchor through rizhoids. However, if the infection is severe, trophonts can also present on eyes, fins and in the oral cavity. In European sea bass, trophonts are mainly detected in the gill and oropharyngeal cavity, where, in a burden-dependent manner, they could induce hyperplasia and degeneration of the epithelium. In two to six days, the trophont detaches from the host and encysts on inert substrates transforming into a spherical-shaped reproductive stage, the tomont. Within 2-4 days, tomonts can produce up to 256 dinospores asexually, with a typical dinoflagellate morphology (two flagella). These are the disseminative, infective stages. After adhesion to a new host, dinospores transform into trophonts in 5 to 20 minutes. If salinity and temperature values are favourable (i.e. 23-27 °C and 30-35 ppt), the life cycle can be completed in 5-7 days. In any case, the protozoan can express its virulence under extreme conditions. Indeed, serious outbreaks have been documented at very high temperatures (more than 35 °C) in both hypersaline water (46 ppt) and brackish-water environments (7 ppt).

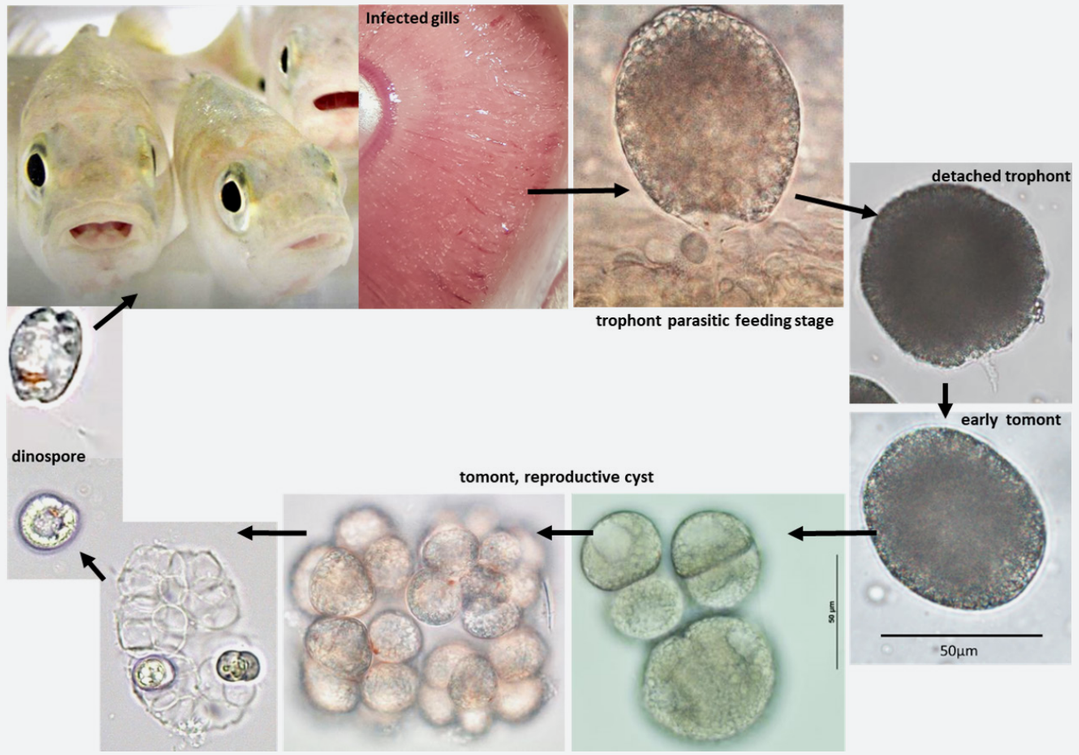

Figure 15. Life cycle of *Amyloodinium ocellatum* in European sea bass. Trophont feeds on the gill and skin epithelium; after reaching a size of 80-100 µm (4-5 days), the trophont loosens its attachment and drops from the fish, encysts as tomont in the substrate and begins dividing. The reproduction process culminates in 2-3 days at 24 ±2 °C with the release from each tomont of several infective motile dinospores. Drawing: P. Beraldo, University of Udine.

Seasonality

Typically, mortality outbreaks are more frequently observed in the warmest months (especially between July and August), when water temperature reaches high values (more than 28 °C) and water exchange and O_2 concentration are poor.

Age / mean weight susceptibility

All fish stages are potentially susceptible to amyloodiniosis. Although early stages can be highly susceptible, they are generally not affected by the infection due to strict biosecurity measures applied in hatcheries, such as effective water filtration systems and proper sanitation procedures.

Risk predisposing factors

In sea cages, the occurrence of amyloodiniosis is highly unlikely due to the distance of the sea bottom from cage nets and water currents, which prevents fish colonization by the infective dinospores. The presence of the parasite in newly introduced batches of fry or juveniles from pre-ongrowing systems cannot be ruled out. In contrast, amyloodiniosis is very frequent in ongrowing land- or lagoon-based semi-intensive and intensive systems in Mediterranean countries. Live fish movements into or between farm sites (transfer of infected fish from one site to another) could be a relevant risk factor for introduction of *A. ocellatum* into aquaculture sites, especially when biosecurity measures, such as parasitological screening of newly-introduced fish and quarantine are not adequately implemented. Furthermore, the intake water, if not properly treated, could bring infective dinospores into the farming system. High water temperature and low dissolved oxygen (DO) concentration are also predisposing factors for infection. The low DO can indirectly reinforce the negative impact of *A. ocellatum*, as trophonts disrupt the gill epithelium in a burden-dependent manner, thus compromising the respiratory function. Furthermore, some atmospheric events, such as storms and typhoons, must be considered as possible promoters in the environmental spreading of the parasite. Dinospores can be transported in aerosol droplets, thus contaminating other nearby facilities. Finally, fish-eating birds may also act as mechanical vectors of the dinoflagellate.

A) What clinical signs should alarm me?

External signs

The typical clinical signs of amyloodiniosis are: jerky movements / flashing; "pruritus", with fish rubbing themselves against surfaces; dyspnoea, characterised by increased respiratory rate with laboured breathing and gathering at the water surface; apathy, decreased appetite up to anorexia, frequently associated with prolonged and severe infections.

Physical observation

Pale gills due to anaemia and filiform haemorrhages are associated with high parasite burden. In European sea bass and gilthead sea bream, the skin typically does not present gross lesions, regardless of the parasite burden.

Note: In other fish species, in particular ornamental fish, amyloodiniosis has been associated with a typical dusty appearance of the skin (hence the name "marine velvet disease").

Internal lesions

No specific internal lesions are related to amyloodiniosis.

B) How to detect the parasite at farm level

1. Monitoring plan (what to measure and how often) and trigger level for action

During daily monitoring of fish, especially during the warmer months and in the rearing systems at the greatest risk of *A. ocellatum* infection, it is important to pay attention to alterations of swimming behaviour, in particular flashing, and respiratory distress. Fresh smears of gills and skin from freshly deadly or euthanized moribund fish should be examined with a light microscope. The parasitological exams should be performed regularly in farms located in endemic areas from spring to autumn, and should be intensified during the warmer period. Due to the infectivity potential of the dinoflagellate, the presence of 5-10 trophonts per gill arch can be considered as a threshold level to start treatments in order to avoid serious outbreaks.

2. Recommendations for the submission of samples to be diagnosed

Whole fish can be collected in plastic bags and transported on ice within a few hours to the laboratory. Since gills, the primary sites of infection, decay quickly, the following samples could be taken and submitted for diagnosis:

- Gill hemibranchs or smaller samples can be fixed in 4% buffered formaldehyde solution for histopathological examination
- Gill smears or biopsies for confirmation of presence and levels of *A. ocellatum* trophonts may be placed in ethanol/RNAlater (weight/volume 1:10) for PCR or qPCR testing

3. Contact laboratories

Most laboratories with expertise in fish diseases diagnosis are capable of performing a qualitative and quantitative diagnosis of amyloodiniosis.

C) Action plan for prevention and control

1. Prevention and farm management

Prevention is essential against amyloodiniosis to avoid catastrophic outbreaks. Several biosecurity measures can be applied in order to limit *A. ocellatum* introduction and spread within the farm:

- Treatment of the intake water with UV irradiation, eventually combined with mechanical filtration.
- Appropriate parasitological controls, especially for newly introduced batches.
- Quarantine of newly introduced batches may contribute to reducing the risk of infection or possible cross-transmissions; freshwater baths, for a couple of minutes, can be applied in order to induce trophont detachment form host epithelia.
- Physical separation of fish batches of different stages or species, especially *A. ocellatum* naïve fish.
- Periodic cleaning of the tank surfaces (paying particular attention to the bottom) by manual or mechanical procedures, to remove encysted tomonts.
- Provide separate equipment for each tank and sector, otherwise disinfect it with freshwater before its use in other tanks or sectors.
- Apply rigorous disinfection protocols of equipment and mandatory adequate management of farms.
- Perform sanitary fallowing by letting the pond or tank bottom dry in the sun before introducing new fish in the rearing site. Drying is lethal to the dinoflagellate however, this procedure is not practical in many fish farms.
- Pay attention to the characteristics of the site where the farm is located (e.g. endemicity of amyloodiniosis) or weathering, which are important aspects influencing presence of the parasite.
- Apply proper anti-bird netting, since fish-eating birds might play a vector role in *A. ocellatum* spreading.
- If feasible, regulate the water temperature and salinity during the periods of highest risks.

Careful monitoring of fish, particularly during periods of high-water temperature, is crucial to control amyloodiniosis, along with prompt treatment decisions when some trophonts are detected on gills or skin. Good hygiene practices and maintenance of good health and welfare levels in farmed fish can help to reduce risks of severe infections. When clinical outbreaks with increased mortality due to *A. ocellatum* occur, the best management practices should include: reduction of stress levels, very frequent cleaning of tank bottoms and high water quality parameters (if possible, by increasing water flow).

Fish surviving amyloodiniosis develop at least a partial immunity, indicating the potential for vaccine development. Recent studies carried out in **ParaFishControl** have obtained promising results on the efficacy of inactivated/fragmented *A. ocellatum* dinospores combined with adjuvant (Montanide ISA 763 A VG) as an intracoelomatic vaccine in European seabass juveniles. However, this vaccine formulation protected fish 30 days post-vaccination, but failed to give a long-lasting protection (6-month post-vaccination). Further studies on *A. ocellatum* vaccine development are ongoing.

2. Treatment

Dinospores are the most susceptible parasitic stages to chemotherapy, while trophonts and tomonts are relatively resistant. For this reason, treatments are mainly directed against the dinospores. To date, copper sulphate remains the most widely used treatment to control *A. ocellatum*'s epidemics in aquaculture, due to the proven dinosporicide properties of free copper ion. The infusion of copper sulphate at 0.75-1 g/m^3 for almost two weeks by dripping on ponds / tanks, maintaining constant copper concentration (the chelated form of the salt is more stable in salt water) can be effective to kill dinospores, while tomonts and trophonts are not very susceptible. However, copper sulphate is not licensed for amyloodiniosis treatment in farmed fish in Europe and other countries, due to its negative effects on the environment and to food safety.

A rapid detachment of the trophonts from the gills can be induced with a fresh-water bath. Although inconclusive, it does help to control the disease. However, some areas have limited access to freshwater. Formalin (4mg/L of 36% formaldehyde for 7 hours or 50mg/L for 1 hour) or other chemicals (hydrogen peroxide, 75 and 150mg/L for 30min and repeated after 6 days) have shown limited success against amyloodiniosis. These treatements cause the trophont to detach from the fish and rapidly encyst, but the development of the parasite restarts when the drug is removed. Therefore, treatment should be maintained until all dinospores are hatched and discarded.

3. Management of co-infections

The simultaneous presence of other ectoparasites and the development of secondary bacterial infections at the sites of parasite attachment often complicate and worsen the course of disease. In this case, the control of other pathogens has to be taken into consideration when mitigating measures and treatment protocols against amyloodiniosis are implemented.

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5. Fish farmer's guide to combating *Enterospora nucleophila* infections

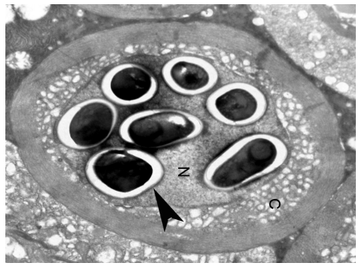

Figure 16. Transmission electron micrograph of an *Enterospora nucleophila*-infected rodlet cell harbouring spores (arrowhead) within its nucleus (N). No stages are visible in the cytoplasm (C). Photo: A Sitjà-Bobadilla, CSIC.

Introduction

Enterospora nucleophila is a microsporidian parasite infecting the teleost fish gilthead sea bream (*Sparus aurata*). It develops primarily within the nuclei of rodlet cells and enterocytes, at the intestinal epithelium, but also in the cytoplasm of other cell types at subepithelial layers, where tiny spores (1.67 x 1.05 µm) are produced (Figure 16).

Biological life cycle

Only parasite development within gilthead sea bream is known. It is currently unknown whether *E. nucleophila's* life cycle involves other hosts (Figure 17), however, it is clear that infection occurs through ingestion of spores.

Figure 17. Life cycle of *Enterospora nucleophila* in the gilthead sea bream. Tiny intranuclear merogonial stages start in rodlet cells and enterocytes (a), develop into intranuclear spores (b) in the digestive tract, which can be engulfed by macrophages and start new intracytoplasmic development (c) and be spread to other organs. Mature spores freed from any infected cell type are released to the water and can infect new fish (d). Drawing: A Sitjà-Bobadilla, CSIC & M.L. Fioravanti, University of Bologna.

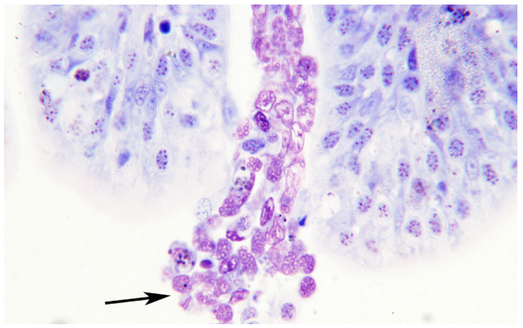

Figure 18. Giemsa-stained intestinal section showing the invasion of epithelial nuclei by *Enterospora nucleophila* and the released stages into the lumen (arrow). Photo: A Sitjà-Bobadilla, CSIC.

Infected cells and free spores are released to the intestinal lumen (Figure 18) and then through the faeces to the water. The parasite is the causative agent of an emerging disease in gilthead sea bream, (emaciative microsporidiosis), a chronic condition manifested as a severe growth retardation, normally accompanied by trickling mortality. Aside from mortality, the main economic impact of this parasite is related to the growth arrestment and size segregation within a batch, which causes inefficient feeding and serious biomass and quality losses at harvest.

Seasonality

Comprehensive epidemiological information for the disease in different culture models is still lacking. In sea cages at the Western Mediterranean, the clinical condition seems to appear more frequently in gilthead sea bream during their first winter.

Age / mean weight susceptibility

All fish stages can be affected, but clinical signs are more evident in juveniles and sub-adult fish. Fry can be affected starting from 0.9 g.

Risk predisposing factors

Gilthead sea bream are generally negative for the parasite from the egg to the fry stage just after weaning, including the live prey they eat during this time, regardless of the water type and rearing system used. The risk of infection is higher during the nursery stages if the incoming water is an open flow from the sea with no filtering system. Water is usually the source of infection and some filtering procedures seem to reduce the risk of infection. After stocking in sea cages, most fish can become positive for the microsporidian within 4 months, which is most likely transmitted through the water from infected cages to nearby ones. The transmission from wild to farmed fish and the involvement of other intermediate organisms cannot be ruled out.

A) What clinical signs should alarm me?

External signs

Infections by *E. nucleophila* are associated with stunted growth of gilthead sea bream stocks, which can be accompanied by low-level, but sustained, trickling mortality (0.1-0.3 % daily, up to 1% at peaks per sea cage). Affected fish normally appear lethargic and cachectic, with other nonspecific signs, such as discoloration and occasional scale loss. As a result of the arrested growth, infected animals can have a wasted appearance and average half the weight of the unaffected stock within the same cage (Figure 19). An unusually large coefficient of variation (CV) in biometrical parameters, especially the weight and condition factor, can be used as likely indicators of disease.

Internal lesions

Internally, it is common to observe a thinned and transparent wall in the intestines, which frequently accumulate clear or greenish fluid and white faeces in the terminal portion. Internal organs can appear pale and accumulation of fluid (ascites) is occasional (Figure 20).

Figure 19. Gilthead sea bream with stunted growth due to *Enterospora nucleophila* (bottom) compared with a non-affected fish of the same age and entrance time in the cage (top). Photo: O. Palenzuela, CSIC.

Figure 20. Pale internal organs of a gilthead infected by *E. nucleophila.* The thinned and transparent intestinal walls, and white faeces in the terminal portion can be seen. Photo: O. Palenzuela, CSIC.

B) How to detect the parasite at farm level

1. Monitoring plan (what to measure and how often) and trigger level for action

Detection of the parasite from microscopical examination of fresh specimens is unreliable due to the small size of the parasite spores and its intranuclear location in fish cells, which can be scarce or absent, even in heavily infected fish. At the farm and routine veterinary consultant level, this kind of monitoring is fruitless. The highest magnification is needed to detect the different stages by histology at light microscope (Figure 21). Therefore, monitoring of the infection by qPCR of intestinal samples and other specific diagnostic methods is recommended for all the batches, from the hatchery to on-growing sectors, since epidemiology of *Enterospora nucleophila* is still largely unknown. In sea cages, systematic tracking of biometrical parameters and growth data, and especially of the coefficient of variation (CV) for the mean weight and condition factor of the stock, can allow the detection of anomalies and trigger an opportunity for a targeted surveillance. There is no threshold level of parasite burden related to the emergence of mortality / morbidity, but a clear relationship between the parasite burden in the intestine detected by qPCR and the worsening of the condition factor has been shown. Thus, fish samples should be promptly submitted for diagnosis as soon as clinical signs referable to emaciative microsporidiosis appear in the farm.

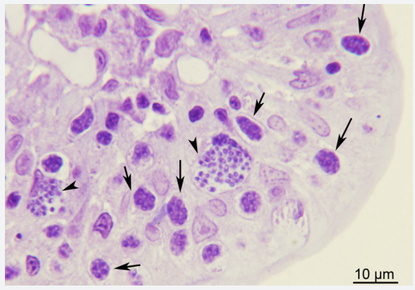

Figure 21. High magnification of a Giemsa-stained intestinal section harbouring intranuclear (arrows) and intracytoplasmic stages (arrowheads) of *E. nucleophila.* Photo: A Sitjà-Bobadilla, CSIC.

2. Recommendations for the submission of samples to be diagnosed

Samples of fry / juveniles up to 10 g can be submitted fresh within 24 hours or preserved whole in >80 % ethanol for qPCR and / or in 10 % buffered formalin for histology and / or ISH, if possible, opening the belly to let in the fixative. From larger fish, the whole gut (from end of stomach to rectum) can be extracted, preserved as described above and delivered to the diagnostic lab. The parasite is detected by qPCR in the intestine at higher rates than by conventional histology.

3. Contact laboratories

Several European labs can do histological diagnosis, for specialized molecular diagnosis:

- Fish Pathology Group, Instituto de Acuicultura Torre de la Sal, (IATS-CSIC), Castellón, Spain
- Fish Pathology Lab, DIMEVET, University of Bologna, Ozzano Emilia (BO), Italy

C) Action plan for prevention and control

1. Prevention and farm management

Water is the source of infection and some filtering procedures seem to reduce the risk. However, wide-scale characterization of the epidemiology in hatchery-nurseries under different filtering and RAS systems is lacking. There does not seem to be vertical transmission and the tests conducted on live feed in different hatcheries have resulted negative for the parasite. Fish as small as 0.9 g have been found to harbour the parasite without noticeable signs, so fish can be seeded in sea cages carrying the parasite and act as a source of infection. The seeding of smaller juveniles in late summer and autumn appears to be related with a higher incidence of clinical infections in the lot, at some cage-based facilities.

2. Treatment

There are currently no approved therapies for *E. nucleophila*. Microsporidian infections relevant for human and animal medicine are normally treated with Albendazole, Metronidazole or Fumagillin, but the use of these drugs in aquaculture settings is not regulated and their effectiveness for treating gilthead sea bream microsporidiosis is unknown.

3. Management of co-infections

Gilthead sea bream affected by *Enterospora* could be co-infected by other pathogenic parasites such as the gill monogenean, *Sparicotyle chrysophrii* or the gut myxozoan, *Enteromyxum leei*, which need to be addressed with targeted measures (see the specific sections of this guide). Co-infections by the Apicomplexan, *Cryptosporidium molnari* and other intestinal coccidians are also frequent and can complicate the diagnosis of the disease.

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New cite to add if accepted (now under revision)

Picard-Sánchez, A. *et al.* (2020). Pathology and cellular immune response of gilthead sea bream (*Sparus aurata*) infected by *Enterospora nucleophila* (Microsporidia). *Veterinary Pathology*.

6. Other common parasites in European sea bass

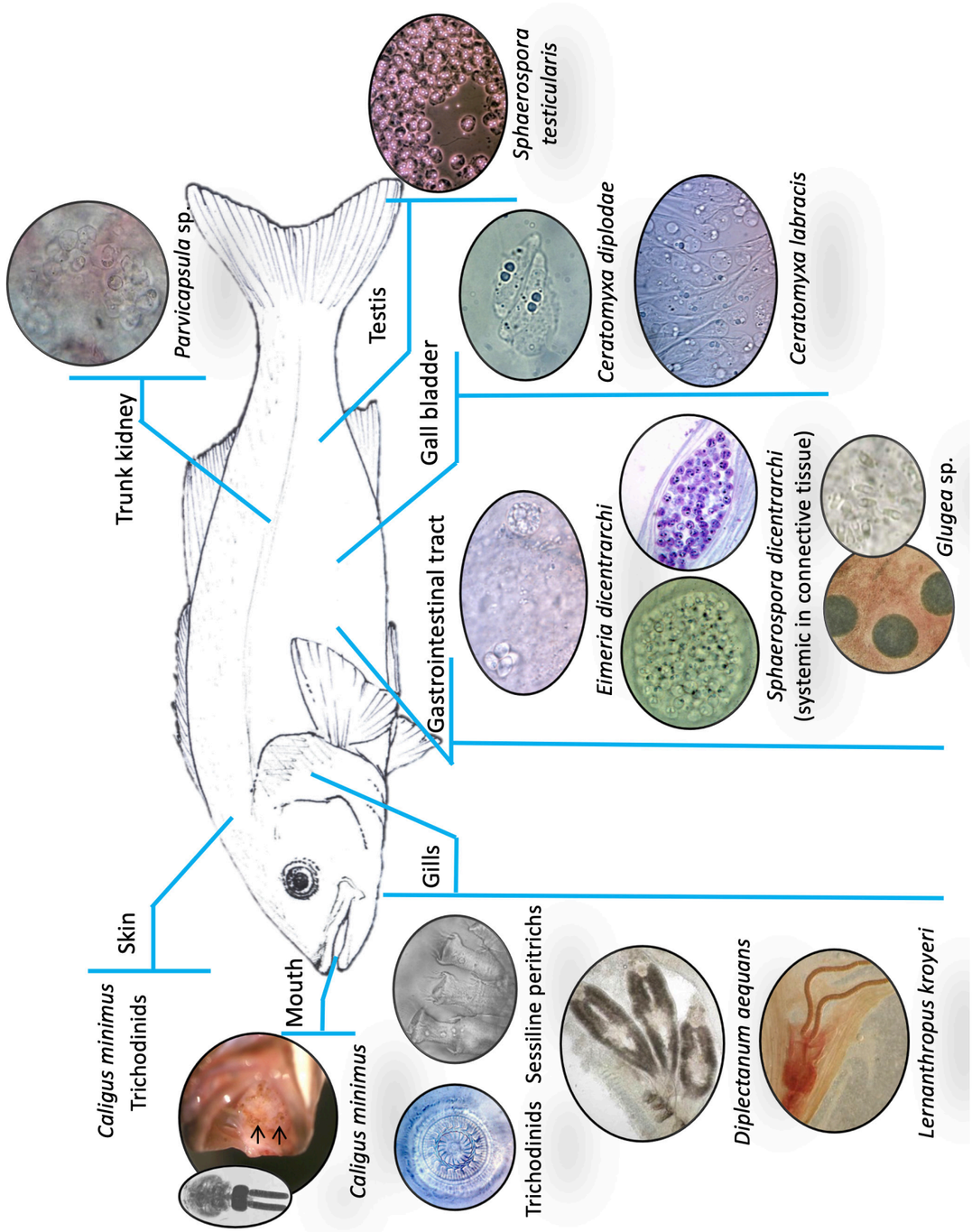

Figure 22. Other common parasites in European sea bass. Drawing and photos: M.L. Fioravanti, University of Bologna and A. Sitjà-Bobadilla (CSIC).

7. Other common parasites in gilthead sea bream

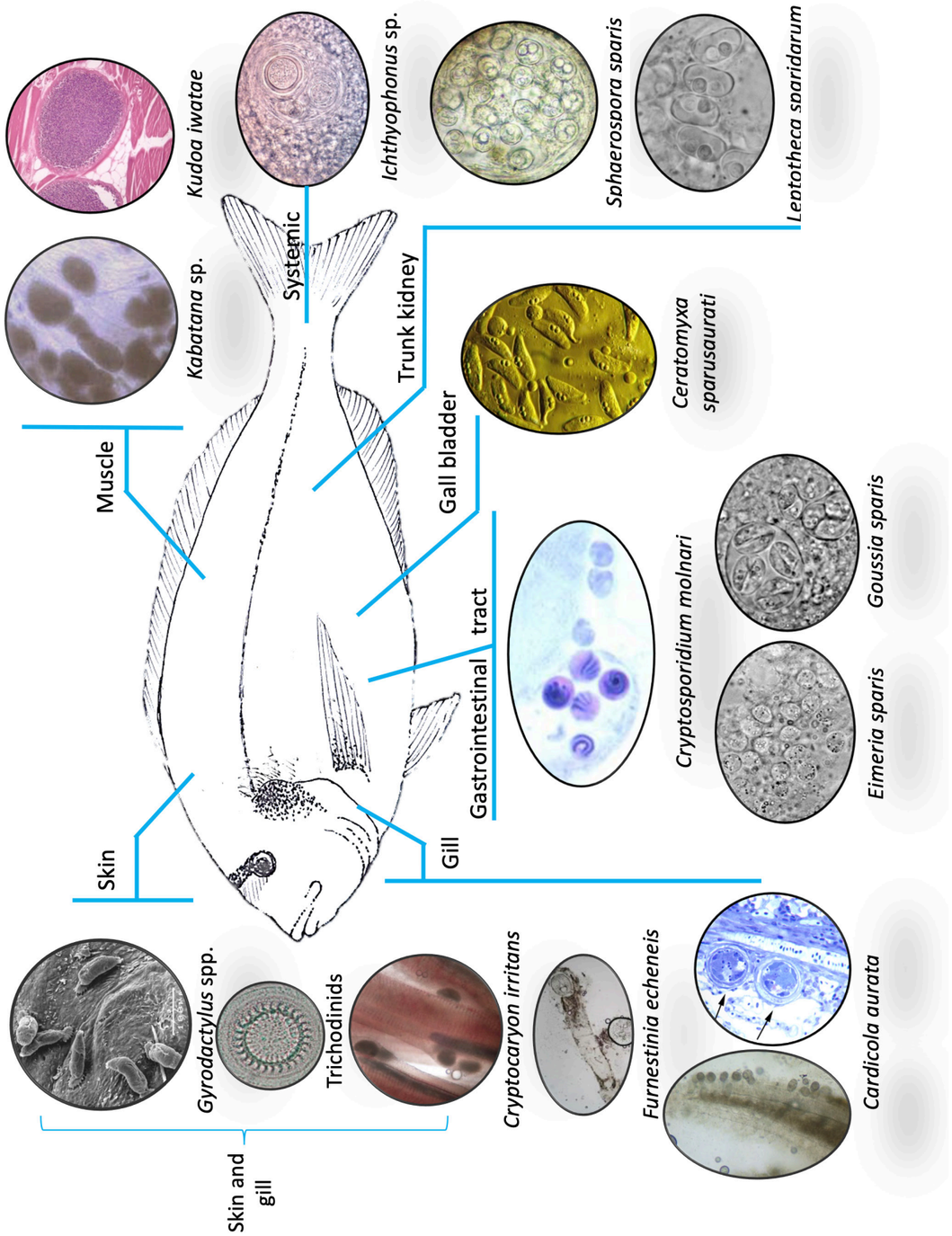

Figure 23. Other common parasites in gilthead sea bream. Drawing and photos: M.L. Fioravanti, University of Bologna and A. Sitjà-Bobadilla (CSIC).

Other ParaFishControl Resources

1. Integrated Pest Management Strategies for *Enteromyxum leei*: bit.ly/2VLZf0k
2. Integrated Pest Management Strategies for S*paricotyle chrysophrii:* bit.ly/3aruByx

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