TECHNICAL MANUAL FOR GREATER AMBERJACK HEALTH

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December, 2018

DIVERSIFY
New species for EU aquaculture

Contents

Introduction

Greater amberjack, *Seriola dumerili* (Risso, 1810) is a leading candidate species for enhancing European aquaculture, showing growth rates ten times higher than the European seabass (Muraccioli *et al.,*2000). *S. dumerili* culture in the Mediterranean region started in the 80s using standard culture conditions, where the feed was first based on fresh fish but quickly progressed to artificial feeds. In recent years, interest for this species in the aquaculture industry is expanding, due to its high demand and market price, rapid growth, excellent fillet quality, and its capacity to accept inert food. Today in Malta, the commercial production of amberjack has reached 500 MT (FAO, 2016) while other aquaculture companies in countries around the Mediterranean have begun to produce this species. Today, mainly as a result of work undertaken in the DIVERSIFY project, a limited but gradually growing commercial activity with hatchery-produced individuals exists in Greece, and the first market size fish have reached the European market in 2018.

One of the major obstacles of integrating a new fish species into the commercial rearing procedures and production is the emerging and new pathologies that may arise. In this technical manual, all major diseases and health-related issues studied and recorded during the course of DIVERSIFY project are presented.

The manual which was prepared in the frame of the DIVERSIFY project is divided in two parts; **Part A**, containing the bacterial diseases and **Part B**, containing diseases caused by parasites.

PART A

BACTERIAL DISEASES

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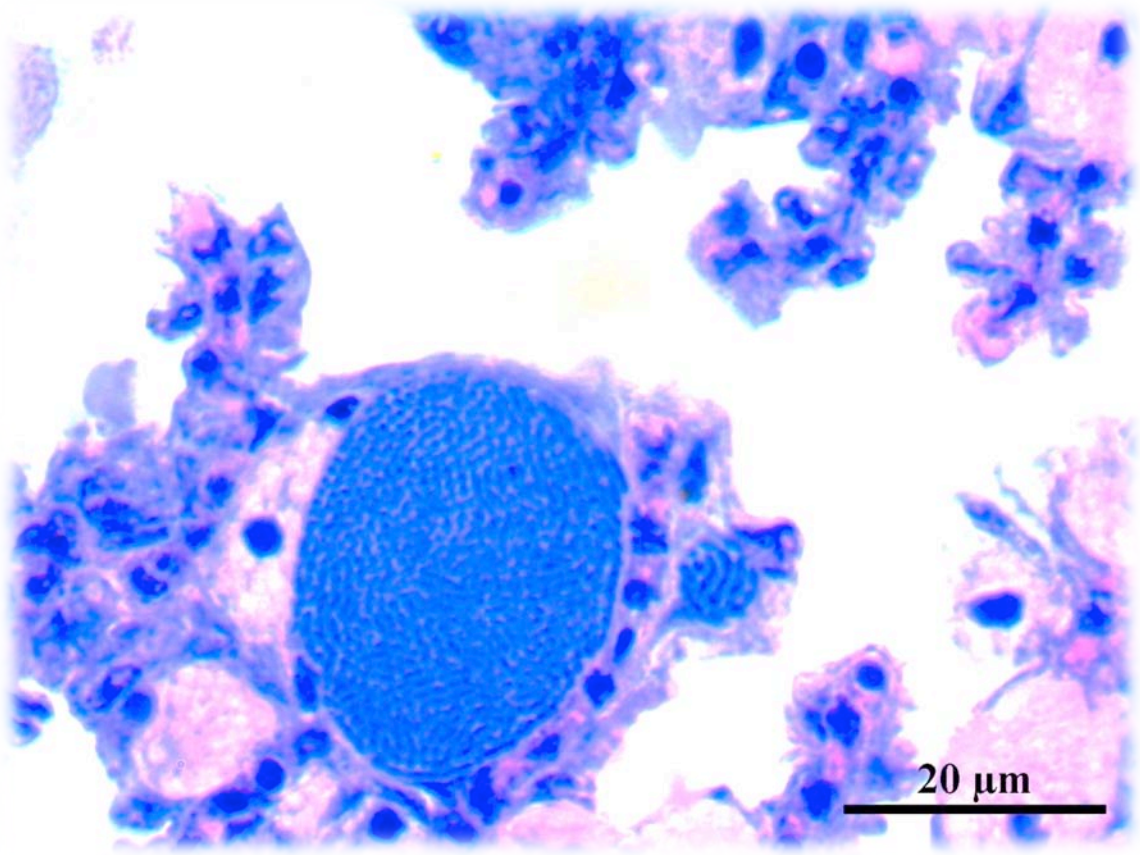
1. EPITHELIOCYSTIS

Pantelis Katharios (HCMR)



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Epitheliocystis disease

Epitheliocystis is an infectious disease, characterized by multiple cysts in the gills **(Figure 1)** that has been shown to cause significant problems and mortality if it occurs at the early life stages of the fish, or during the transition of the fish from the hatchery to the on-growing cages **(Figure 2)** (Nowak and LaPatra, 2006).

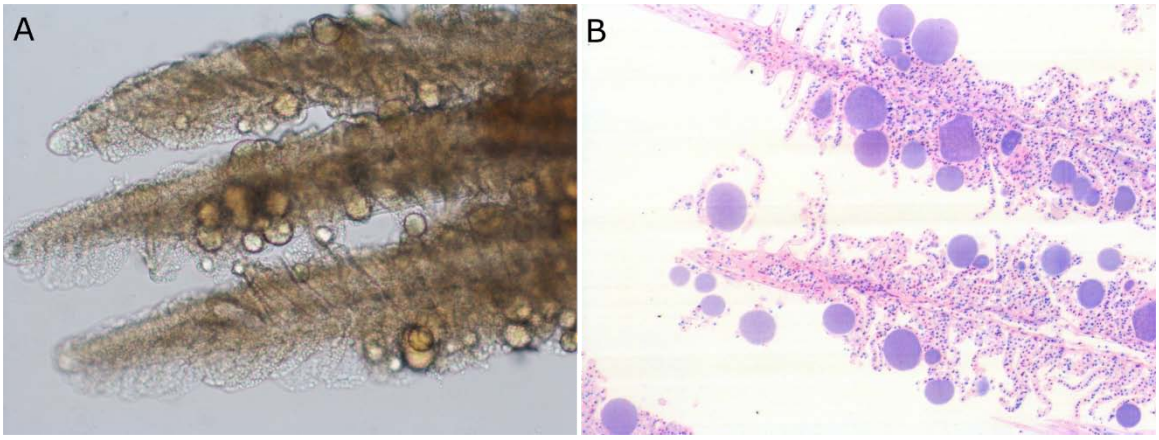

Figure 1. A) Fresh squash preparation of gills showing massive epitheliocystis infection. **B)** The same gills as seen in histological section. Note the characteristic multiple cysts that are epithelial cells containing the epitheliocystis-causing bacteria.

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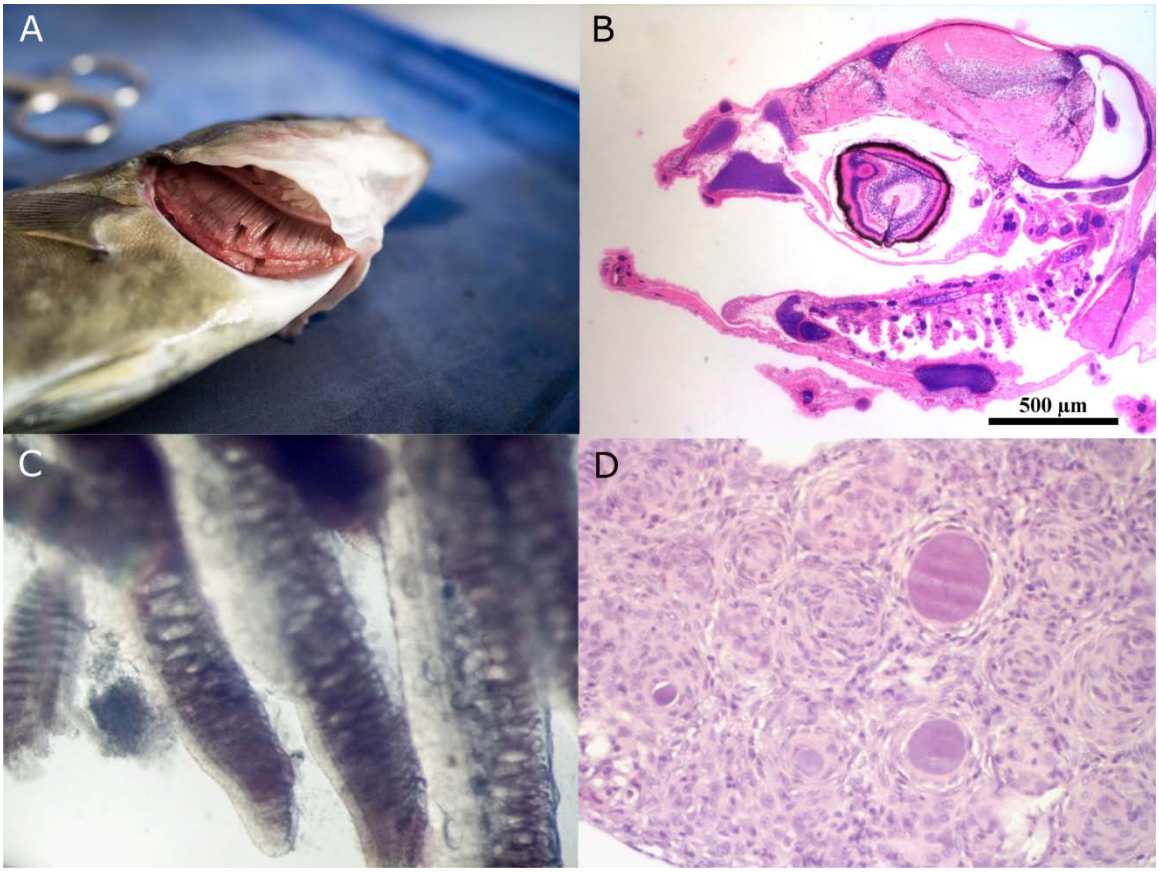

Figure 2. A) Juvenile greater amberjack from sea cages. The gills are affected by epitheliocystis and exhibit mucous overproduction. Using a microscope, the characteristic cysts can be easily seen in a squash preparation of gill tissues **(C)**. Epitheliocystis can become lethal if it affects early life stages as in the fish in **(B)**, where the developing gills are covered with epitheliocystis inclusions. **D)**. Histological section of the same gills showing massive hyperplastic response, bacterial inclusions and granulomatous inflammation (Histological picture of **D** by Dr. Maja Rueten, Pathovet AG)

Despite the fact that the disease is one of the first described in fish generally, little is known about the causative agents and the route of infection. Until recently, the disease was thought to be caused exclusively by chlamydia, however recent studies have expanded the range of the types of bacteria that can cause the disease including representatives from the β- and γ-proteobacteria (Katharios et al., 2015, 2008; Seth-Smith et al., 2015; Seth-Smith et al., 2017). In the DIVERSIFY project we have developed and assessed molecular tools for the early diagnosis of the disease. The tools include molecular PCR probes for all major epitheliocystis-causing agents including Chlamydia, *Endozoicomonas* spp. and *Ichthyocystis* spp. A nation-wide survey was contacted in Greece in order to collect data and samples regarding the epitheliocystis outbreaks in the major farmed fish species that include, European seabass, gilthead seabream and of course greater amberjack. It was shown that at least in Greece, the major pathogen

causing epitheliocystis belongs to the newly described genus *Ca.* Ichthyocystis. In bass and bream aquaculture, the pathogenic species are either *Ca.* Ichthyocystis sparus or *Ca.* Ichthyocystis hellenicum, while in greater amberjack the infectious agent is a related but different and possibly a novel species of the same genus **(Figure 3)**. The disease has been observed at the first months of the fish in the cages, following the same pattern as in other reared species. It can cause mortality that may reach 4-5%, however it can contribute to significantly higher mortality if it co-exists with other pathogens such as Vibrios and monogenean parasites. The disease causes massive granulomatous inflammatory response in the gill tissues which is unique in this species that results in significant impairment of breathing capacity **(Figure 2D)**.

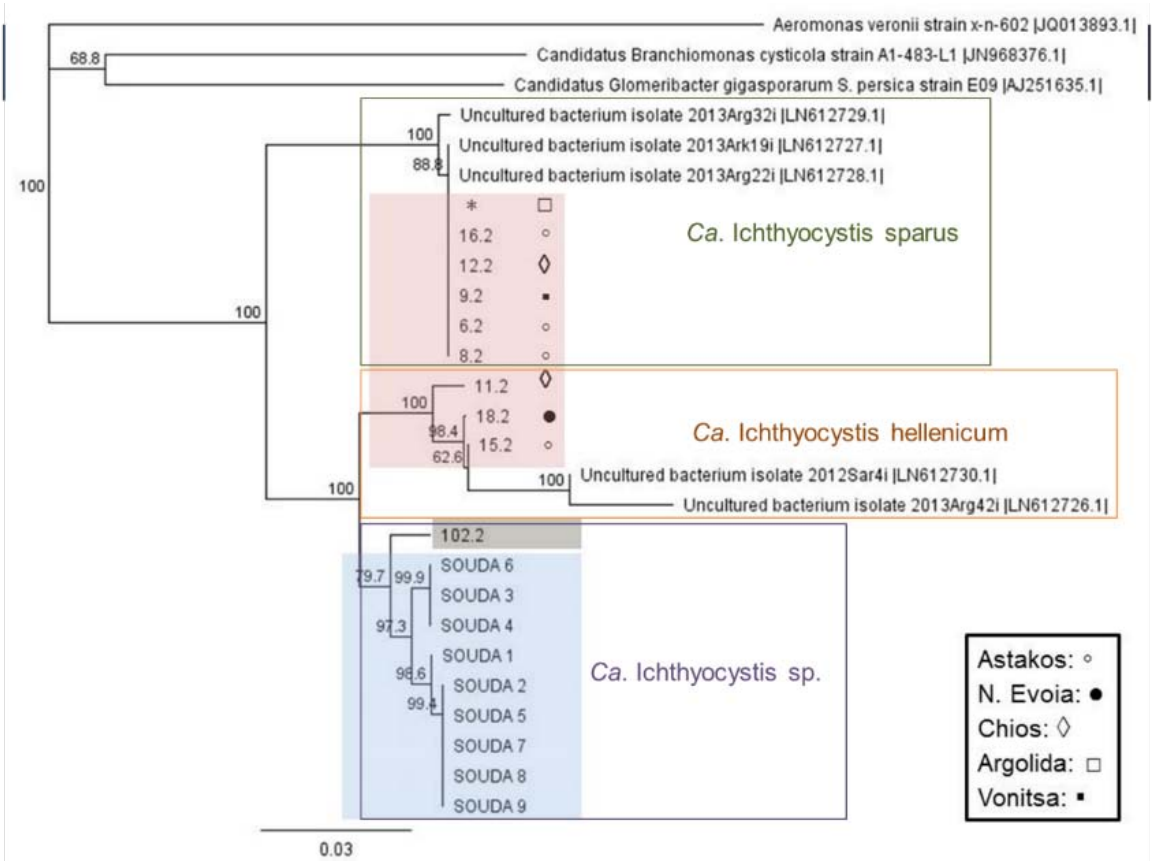

Figure 3. Phylogenetic analysis of epitheliocystis-causing agents in Greece. *Ca.* Ichthyocystis sparus and *Ca.* Ichthyocystis hellenicum have already been reported in gilthead seabream and European seabass. The cluster indicated with blue (from Souda) contains the novel species infecting greater amberjack and is closer to *Ca.* Ichthyocystis hellenicum.

Although, there are anecdotal reports that antibiotics can be used as a treatment, epitheliocystis lesions usually resolve without intervention if the host is not immunocompromised within a couple of weeks. Therefore, it is highly recommended that fish are monitored throughout rearing and especially during their first three to four months in the sea-cages. If epitheliocystis is observed, care should be taken to reduce stress and prevent in other disease that may co-infect the host.

2. VIBRIOSIS (Vibrio harveyi)

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Vibrio harveyi

Vibrio harveyi is one of the most important pathogens for marine fish and invertebrates (Austin and Zhang, 2006). It is a Gram-negative bacterium that can be luminous. It has a very wide phenotypic and biochemical diversity that makes its identification quite problematic, especially if it is based only on biochemical profiling. It is considered a serious pathogen for shrimps causing the disease known as luminous vibriosis. Many different fish species have been affected by the pathogen that may cause persistent infections with severe economic consequences for the aquaculture industry worldwide.

Greater amberjack is susceptible to vibriosis with the principal species being *Vibrio harveyi* (Castillo et al., 2015). The disease presents the typical signs of a bacterial septicemia with haemorrhages in the skin mainly in the tail, anus and behind the opercula. Skin ulcers are often seen at the progressed stages of the disease. The onset of the infection coincides with changes in the water temperature, mostly when temperature is above 23^{o}C. Losses may reach 40% if the disease is not treated early. Since this is a bacterial infection, antibiotics can be of value, however *Vibrio harveyi* may develop antibiotic resistance rapidly, therefore it is extremely important to select the appropriate antibiotic based on antibiogram.

Figure 4. Juvenile greater amberjacks infected by *V. harveyi*

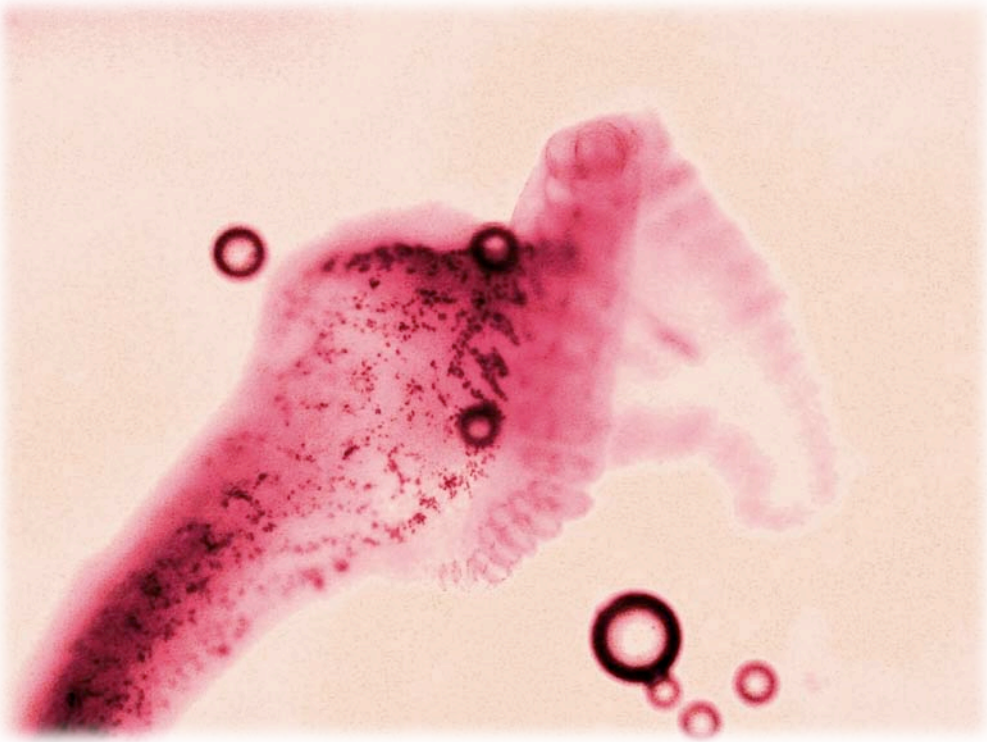
Development of an efficacious vaccine is urgently needed for the control of the disease. One of the major problems in developing a universal vaccine that could be applied in various localities is the heterogeneity of the bacterium that may also be reflected on its antigenicity. Therefore, the application of autogenous vaccines in areas where Vibriosis due to *V. harveyi* is a problem, might provide a solution towards the sustainability of the greater amberjack culture.

PART B

PARASITIC DISEASES

3. ZEUXAPTA SERIOLAE

Pantelis Katharios (HCMR)



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Zeuxapta seriolae

The most significant parasitic disease in cultured greater amberjack is caused by the monogenean, *Zeuxapta seriolae* **(Figure 5A)**, which infects the gills **(Figure 6)** causing severe anemia to the fish **(Figure 7)** (Grau et al., 2003; Mansell et al., 2005; Montero et al., 2004). This parasite is highly host-specific, meaning that it can establish a successful infection only in greater amberjack.

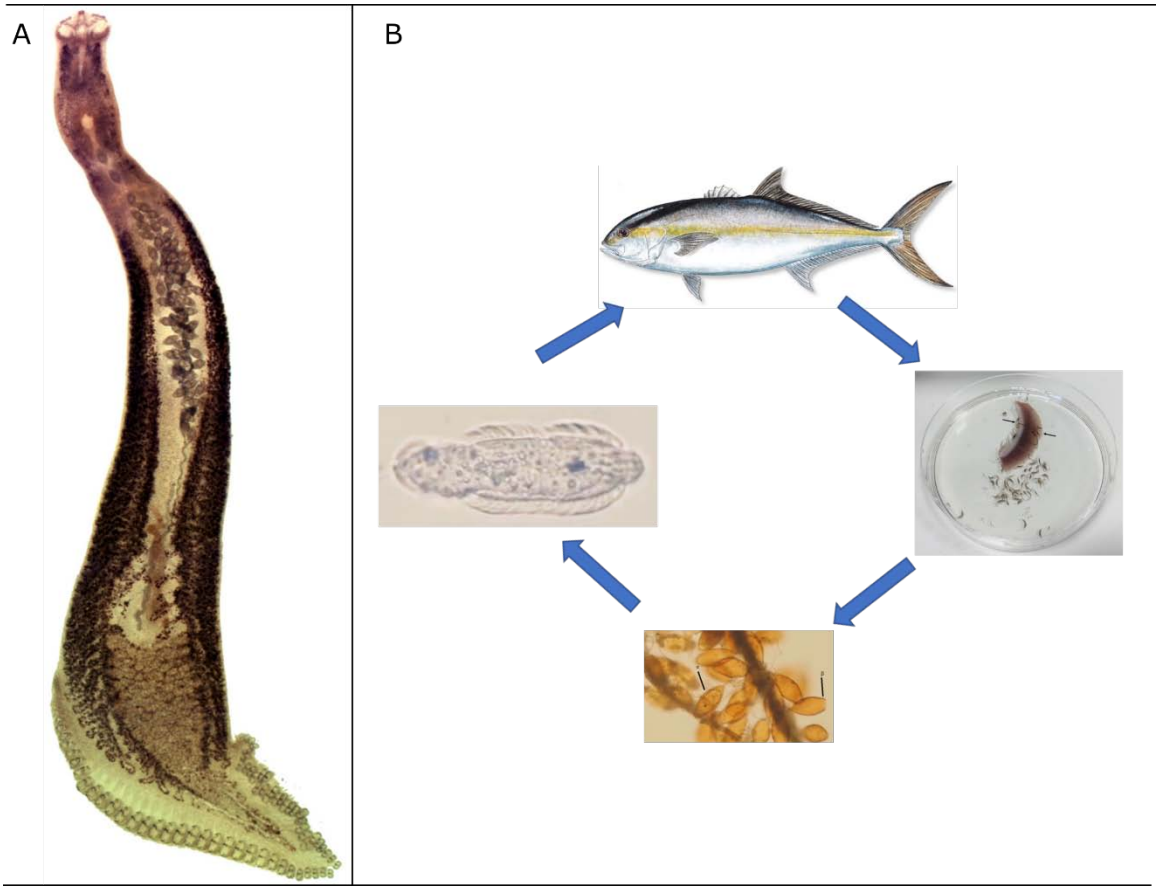


Figure 5. A. Adult parasite at maturity. Note the numerous eggs in the body cavity and the haptor (attaching device) with the clams. **B.** The life cycle of the parasite is direct. Parasites on the gills release large number of eggs which are entangled in a string. The eggs may stay in the gills but most commonly are attached on the cage nets. Oncomicacidia hatch from the eggs and within 24 hours they re-infect the host.

Figure 6. Adult parasites are big and readily seen following visual inspection.

Figure 7. Anemic gills and numerous parasites which are detached from the gills. This fish was preserved in ice that caused the detachment of the parasite.

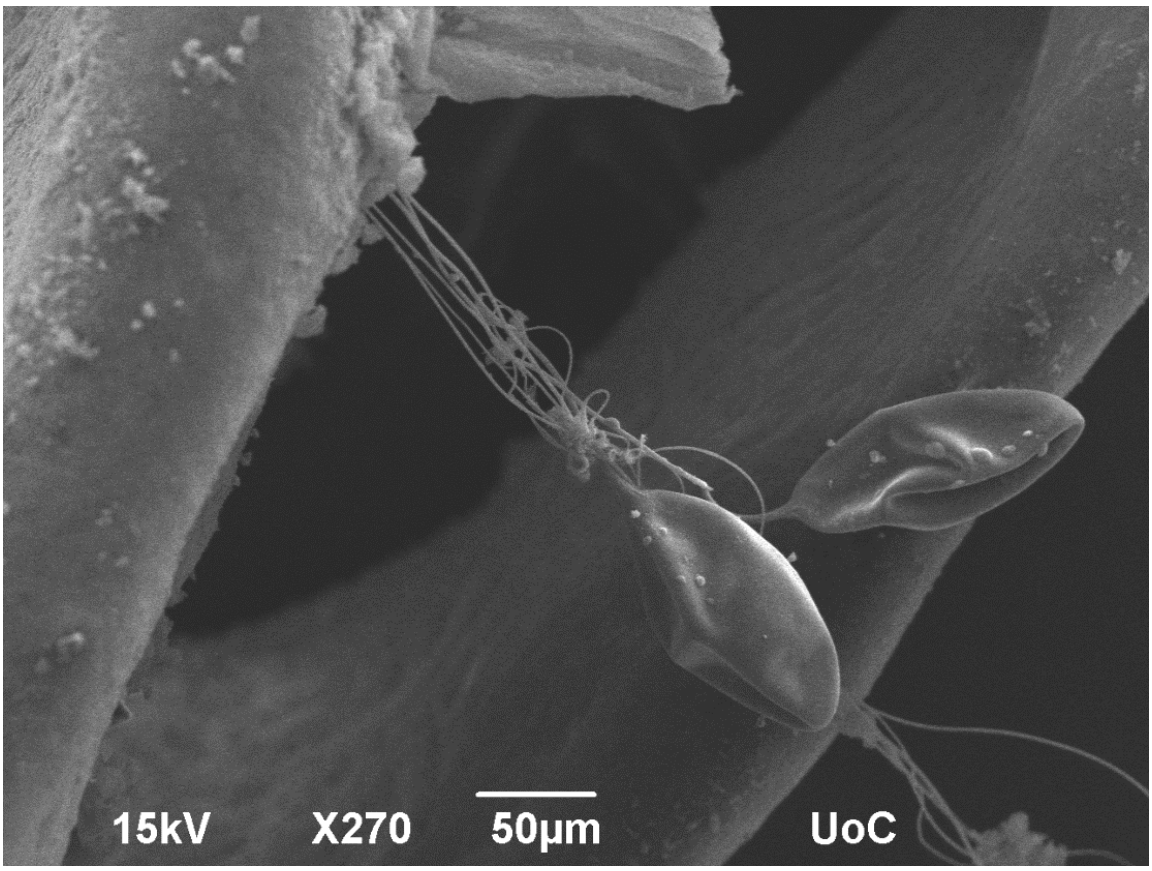


Figure 8. Monogenean eggs as observed using Scanning Electron Microscopy. Note the "string"- like structure with which eggs are entangled.

Zeuxapta seriolae can be found throughout the year however it becomes extremely problematic in the summer months. This is because the propagation of the parasite is temperature-dependent and in temperature above 22^{o}C it can complete its life-cycle within 20-25 days **(Figure 5B)**. When it reaches maturity, the parasite becomes extremely fecund and during an outbreak, millions of eggs are released on a daily basis. The eggs are entangled on a sticky string **(Figure 8)** and big masses of eggs can be dispersed to neighboring cages. Fouled nets are the best substrate for the attachment of eggs and therefore clean nets are a prerequisite for the successful management of the parasite. At hatching, parasitic larvae which are called oncomiracidia have cilia with which they can actively swim in search of a suitable host. Cilia are maintained for 24-48 hours, a critical time-window for the successful infection of the parasite. After this period, the cilia are shed and if the oncomiracidium is not attached on a suitable host, it will die.

Zeuxapta seriolae, is a blood-feeding parasite and the most common clinical sign of the affected fish is gill anemia. Histologically, the parasite may cause hyperplasia and inflammatory response of the host **(Figure 9)**. Infected fish become lethargic and lose appetite. Mortality can be extremely high and may reach 100% if the fish are not treated.

It is also very common to have a secondary infection during a parasitic infection, most likely by opportunistic bacteria.

Bath treatments with hydrogen peroxide have shown great potential to reduce the impact of infestation and control the parasite. Dose as low as 75 ppm of hydrogen peroxide for a 30-min bath can be effective. However, hydrogen peroxide can be highly toxic to the fish and its toxicity increases with the water temperature. Therefore, this intervention should be applied with great cautious and under veterinary supervision. The parasite can be controlled only with repeated treatments with a two-week interval in order to break the life cycle. However, if wild greater amberjacks are near the culture area, it is very possible to have a re-infection of the cultured fish very fast. Therefore, it is highly recommended to monitor gill health at a monthly basis. The most important factor for the appropriate management of the disease is to avoid year-class overlap, and if this is not possible, to treat all stocks simultaneously in order to eliminate all possible reservoirs of reinfection.

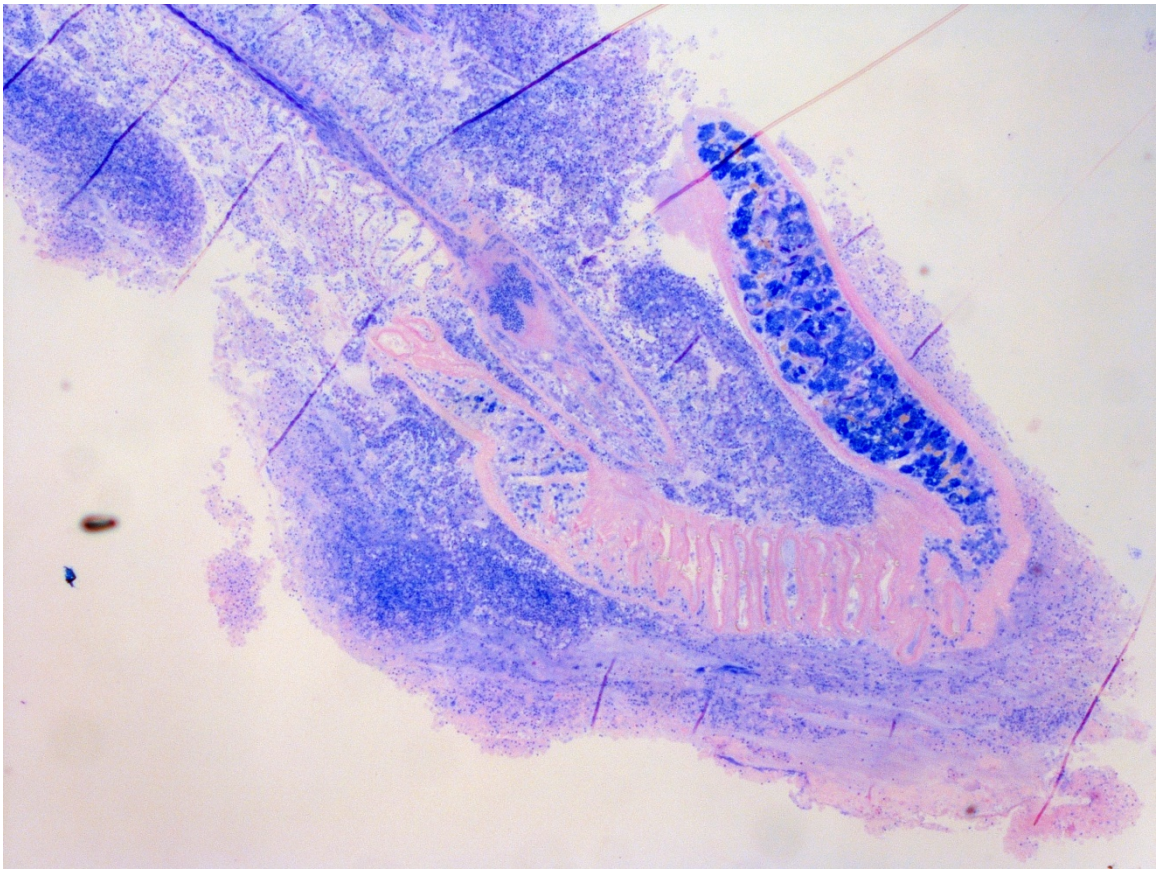
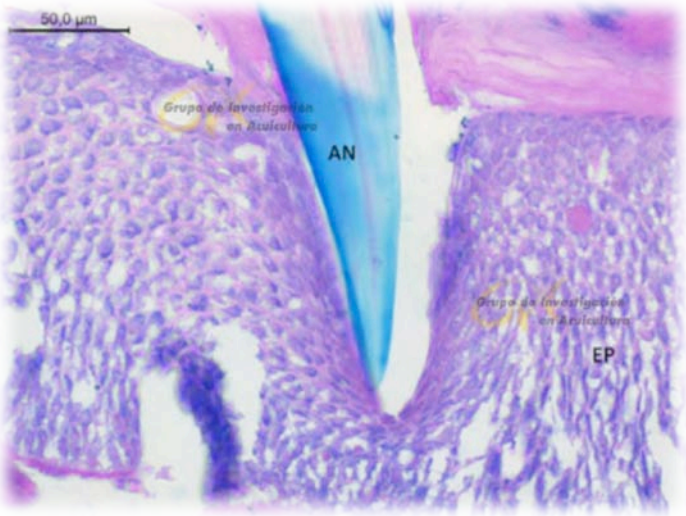


Figure 9. Histological section of a greater amberjack gill infected with Zeuxapta seriolae. The parasite has induced a big inflammatory response from the host.

4. NEOBENEDENIA GIRELLAE

Daniel Montero, Alvaro Fernandez-Montero (FCPCT) and Pantelis Katharios (HCMR)



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Neobenedenia girellae

Neobenedenia girellae is a capsalid monogenean parasite that infects the skin of the fish causing damage of the epidermis and ulceration and may lead to a secondary infection by opportunistic bacteria (Hirazawa et al., 2010). The parasite can cause significant mortality to the fish if left untreated.

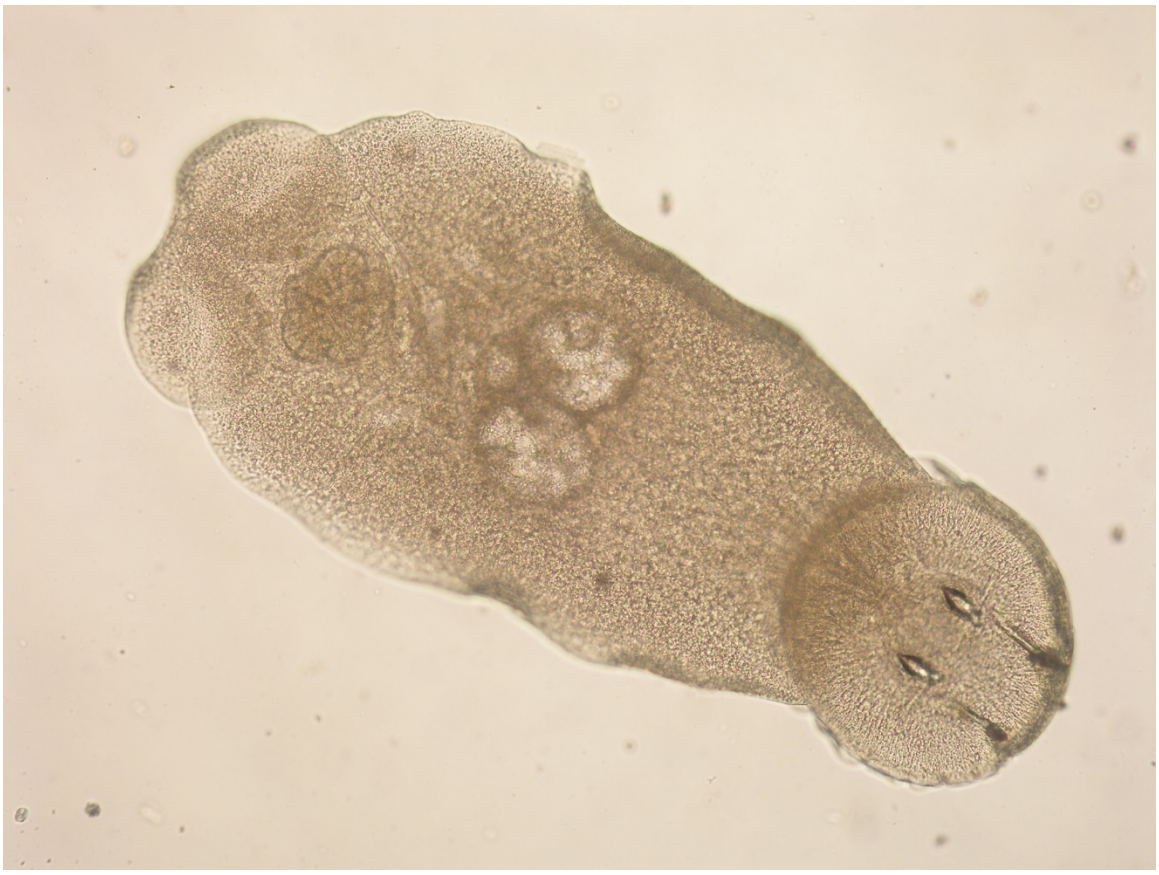


Figure 10. *Neobenedenia* sp. squash preparation.

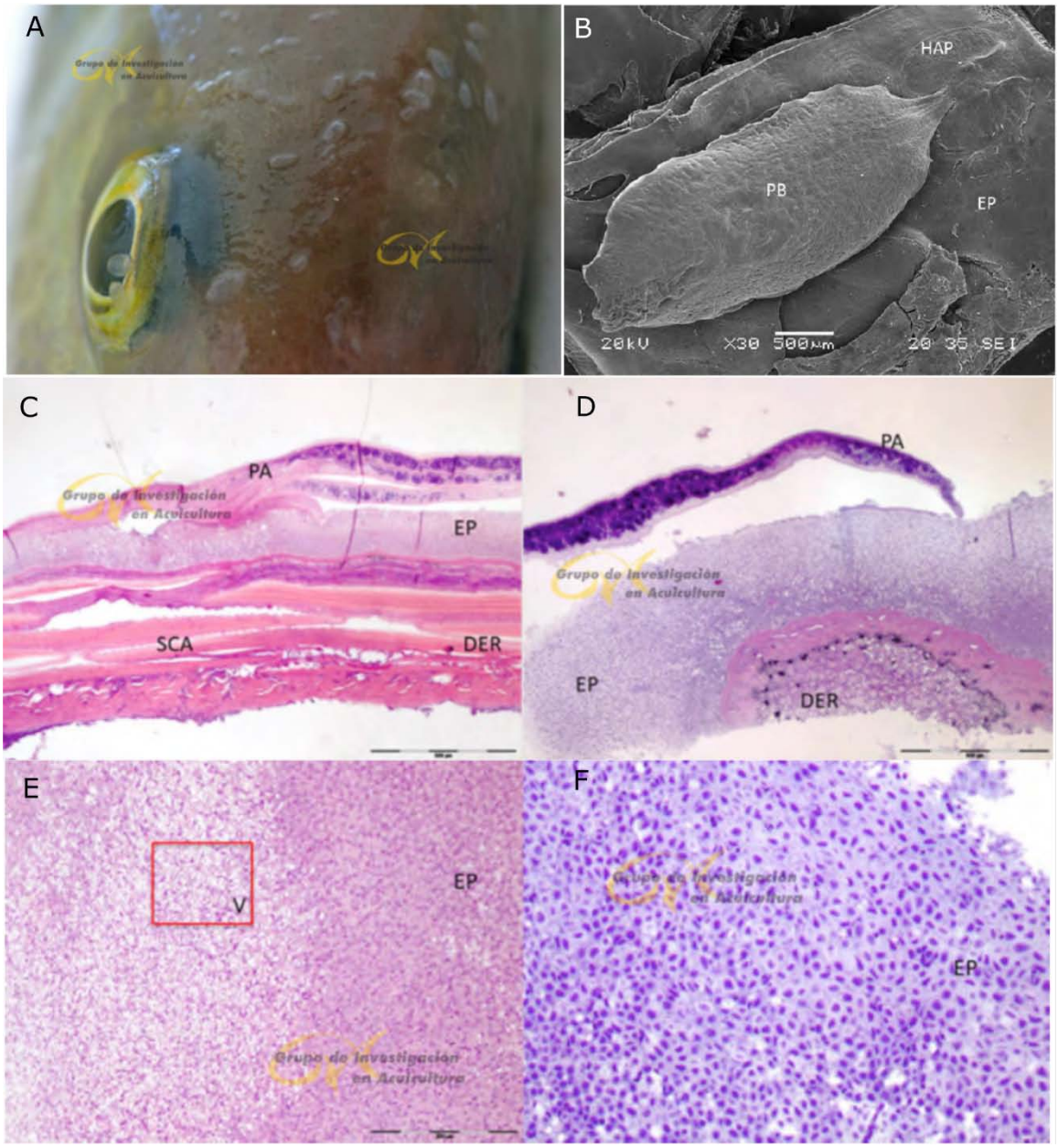


Figure 11. A) *Neobenedenia girellae* adults attached to the head of *Seriola dumerili*. **B)** Scanning Electron Microscopy (SEM) picture of *Neobenedenia girellae*. HAP: Haptor; PB: Parasite body; EP: Epidermis. **C, D)** Dorsal (left) and cranial (right) region. PA: parasite; EP: epidermis; SCA: scales; DER: Dermis. **E, F)** Vacuolization in the epidermis of *greater amberjack*. High level of hydropic degeneration that develops into spongiosis (left) *vs* epidermis of a non-parasitized animal (right). V: Vacuolization; MCLT: Mononuclear cells - lymphocytic type. All pictures from Dr. Daniel Montero, FCPCT.

The main characteristic for distinguishing between the genus *Neobenedenia* and *Benedenia* is size, with *Benedenia* being bigger than other parasites infecting amberjacks.

Secondly, the anterior structure is slightly concave or flat, starting with the horizontal top of their anterior suckers hooded. *Neobenedenia* on greater amberjack skin is shown in **Figures 10, 11A & 11B**.

The comparison between the dorsal and the cranial region epithelium of juvenile greater amberjack showed that dorsal region of parasitized fish presented a thinner epidermis than the cranial region. More goblet cells were observed in the epidermal layer in the dorsal region and the dermis layer in the dorsal region was thinner than in the cranial region (**Figure 11C & D**).

Parasite damage on greater amberjack skin: At the cellular level after parasite infection, a hydropic degeneration was observed around the parasite site of adhesion. Vacuoles presented a water imbalance and occupied almost all the cytoplasm, moving the nucleus and all the cellular organelles to the cell extremes. This hydropic vacuole degeneration could cover big areas and produce spongiosis with intercellular edema (**Figure 11E**). At a tissue level, a clear disruption of the epidermal layer was observed. The haptor with the attachment structures produce an overpressure in the region where the parasite is fixed, which results in disruption of the

typical structure of the tissue and the cells **(Figure 12).**

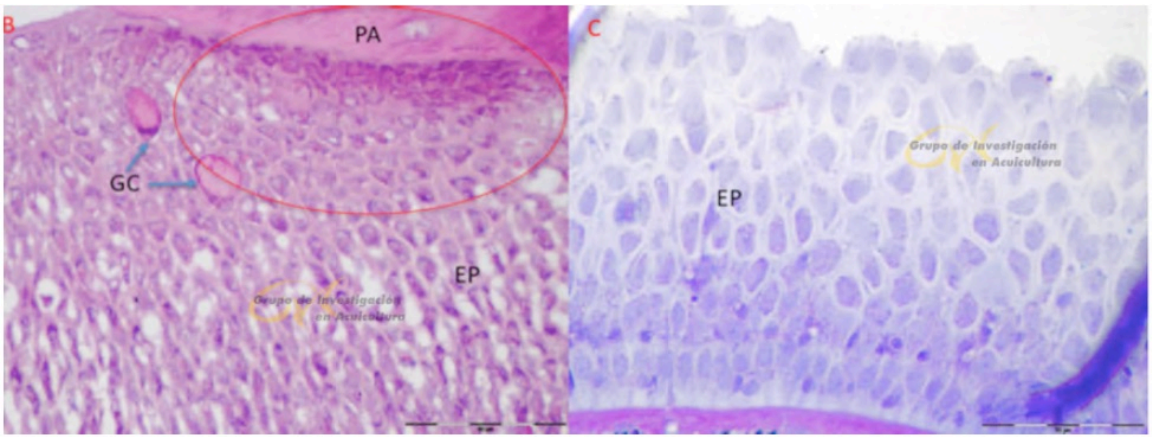


Figure 12. Disruption of the tissue and the cells (left) compared with a non parasitized animal (right). PA: parasite; EP: epidermis; GC: Goblet cells.

Mechanical damage produced by the attachment structures, the hooks (hamulus) and the anchors, was also observed (**Figure 13**). A disorganization of tissue can be seen.

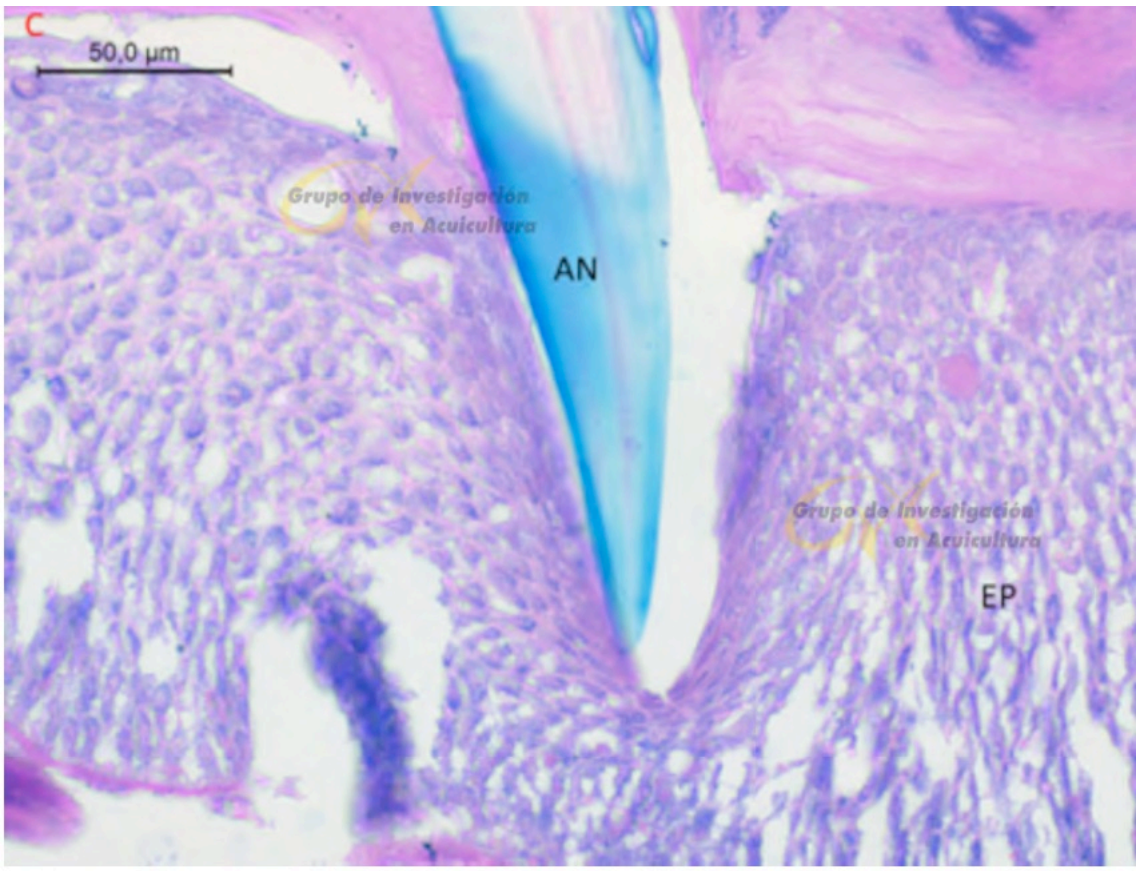


Figure 13. Mechanical damage produced at the epidermis by the attachment structures. AN: Anchor; EP: Epidermis.

The parasite infection also induced an increase in the number of goblet cells (**Figure 14**).

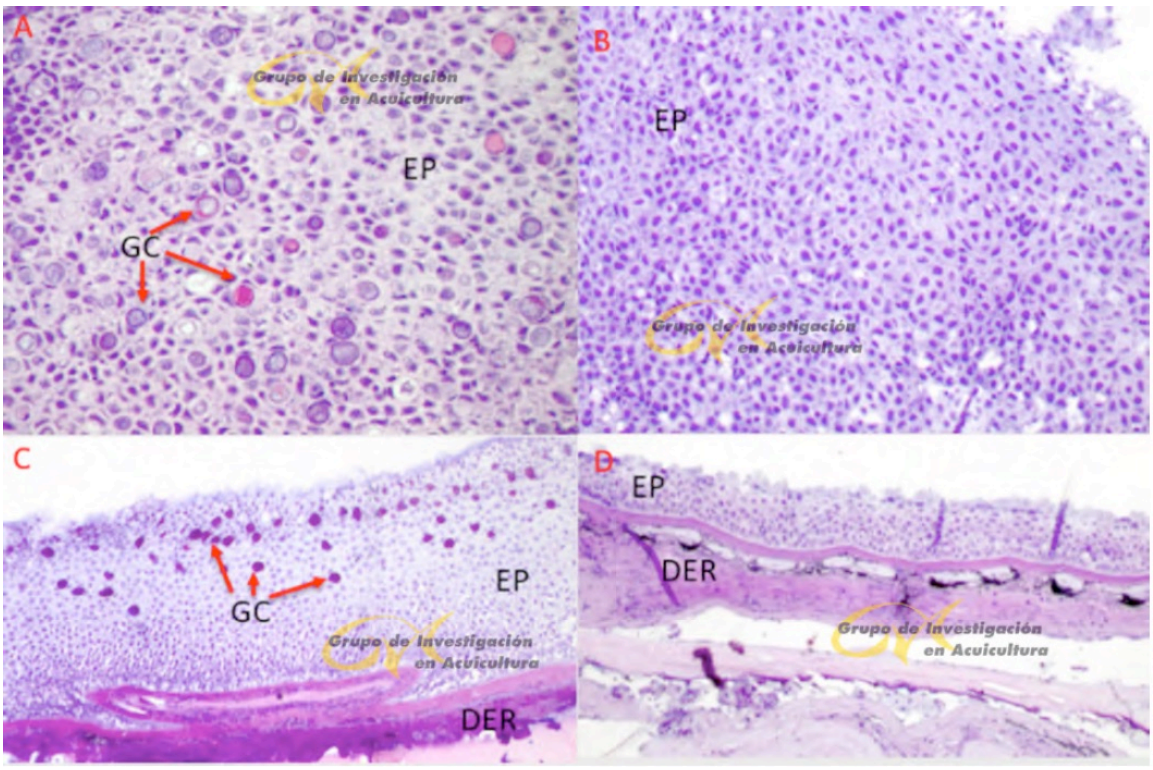


Figure 14. Longitudinal section of the epidermis layer of a parasitized (A) and non-parasitized (B) greater amberjack. Transverse section of a parasitized (C) and non-parasitized animal (D) with different amounts of goblet cells. GC: Goblet cell; EP: Epidermis; DER: dermis.

At the immunological level or immune defence barrier some modifications were clearly observed when the parasite got attached to the host. Mononuclear cell lymphocytic type mobilization was observed near the regions where the anchors and hooks were introduced into the host. This immune cell mobilisation is a focal extravasation of lymphocytes related with blood vessels, producing a perivascular dermatitis (**Figure 15**).

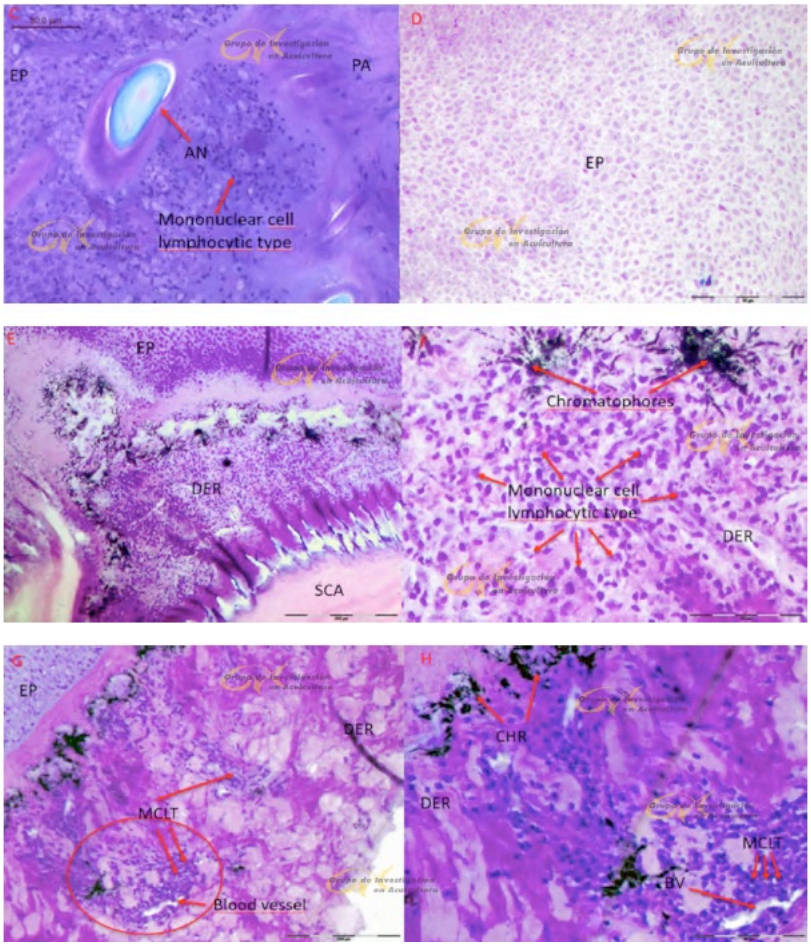
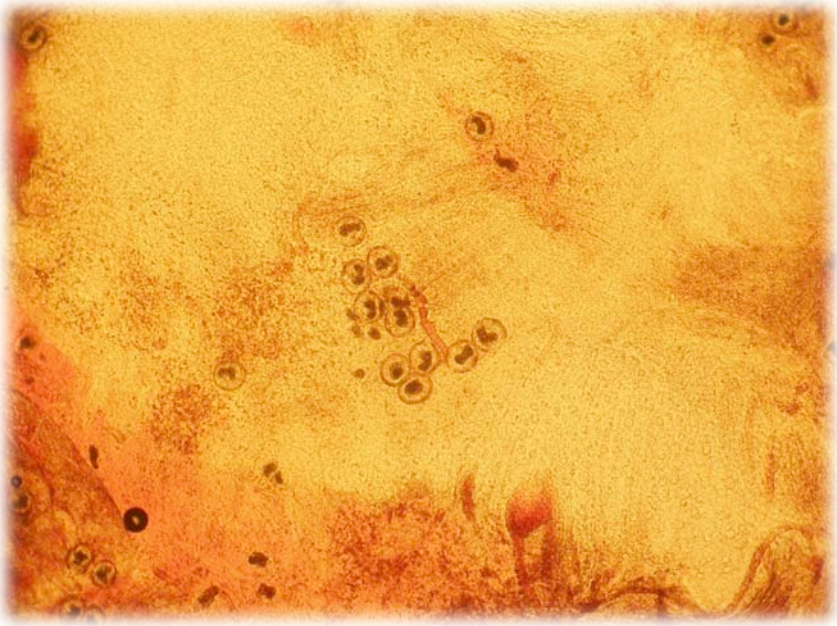


Figure 15. Mononuclear cell lymphocytic type infiltrations (MCLTI) around the anchors and hooks (C), compared with a non-parasitized animal (D). Massive MCLTI in the dermis layer (E,F), and focused extravasation related to blood vessels (G,H). MCLT: mononuclear cell lymphocytic type; CHR: Chromatophores; BV: Blood vessels.

Regarding the treatment of *Neobenedenia girellae*, promising results have been produced by the administration of mannan oligosaccharides (MOS and cMOS) as immunostimulants in the fish diet. These ingredients have shown to enhance mucus production which is a first line of defense against external parasites and to promote innate immune response of the fish. Administration of cMOS in healthy fish resulted in significant lower parasite load compared to control fish following challenge with *Neobenedenia girellae*. cMOS not only prevented parasite attachment, but also reduced the growth and development of the parasites concomitant with increased immune responses. A mobilization of fish defences to the skin mucus has been described as an effect of prebiotics, and could affect the correct development of parasites as they attempt to overcome the first physical and chemical barriers of the host.

5. PARADEONTACYLIX SP.

Pantelis Katharios (HCMR)



Paradeontaxylix sp.

The blood fluke, *Paradeontacylix* sp. has also been recorded in cultured greater amberjack (Montero et al., 2003). *Pradeontacylix* sp. is a digenean parasite of the family Sanguinicolidae. Two species have been reported to infect greater amberjack; *P. grandispinus* and *P. kampachi* (Montero et al., 2003). The parasite releases its eggs into the blood stream and are accumulated in the capillaries of the gills. When the oncomiracidia hatch, they break the gill epithelium and are released in the environment. The main pathological problem is the obstruction of the blood flow that may result in ischemic incidences and focal necrosis. The parasite has been associated with mortalities in Spain and Japan.

Figure 16. White spots on the gills are accumulations of *Paradeontacylix* sp. eggs and oncomiracidia.

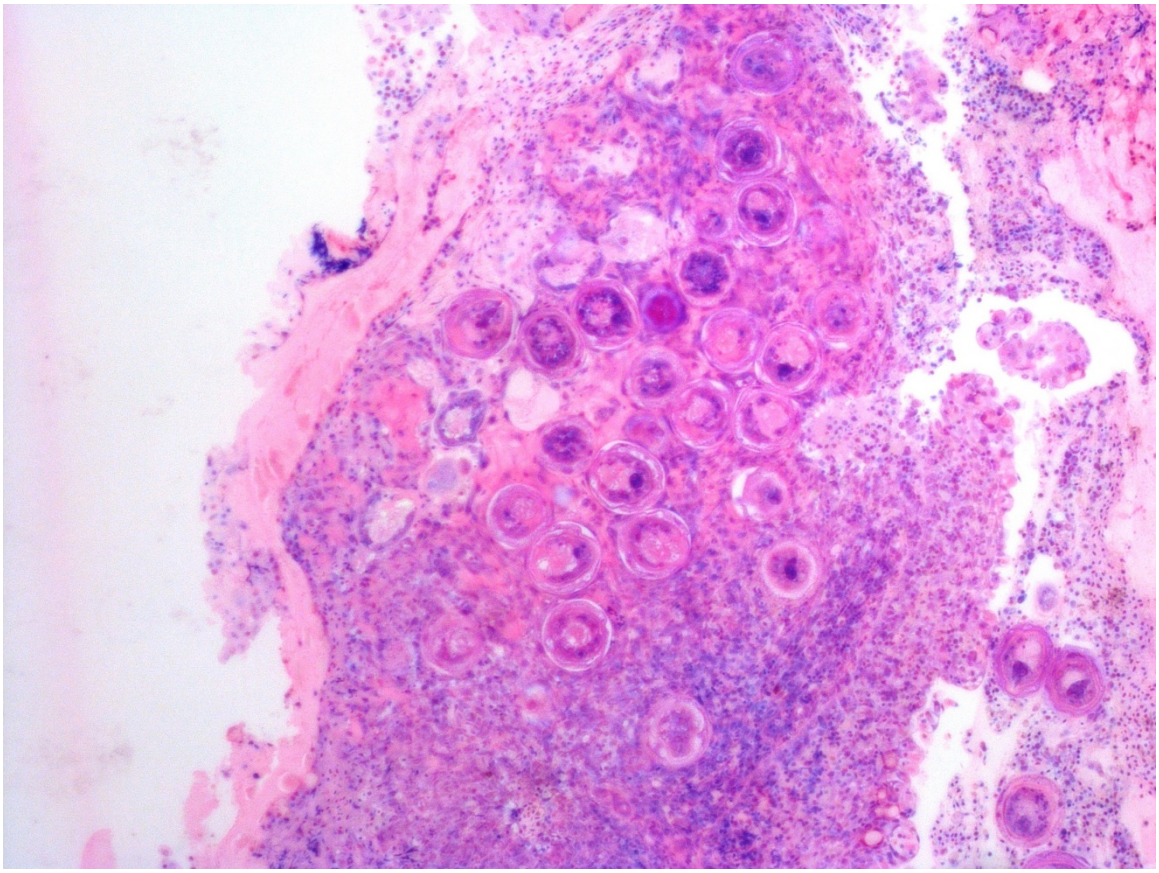


Figure 17. Histological section of greater amberjack gills showing the accumulation of *Paradeontacylix* sp. eggs.

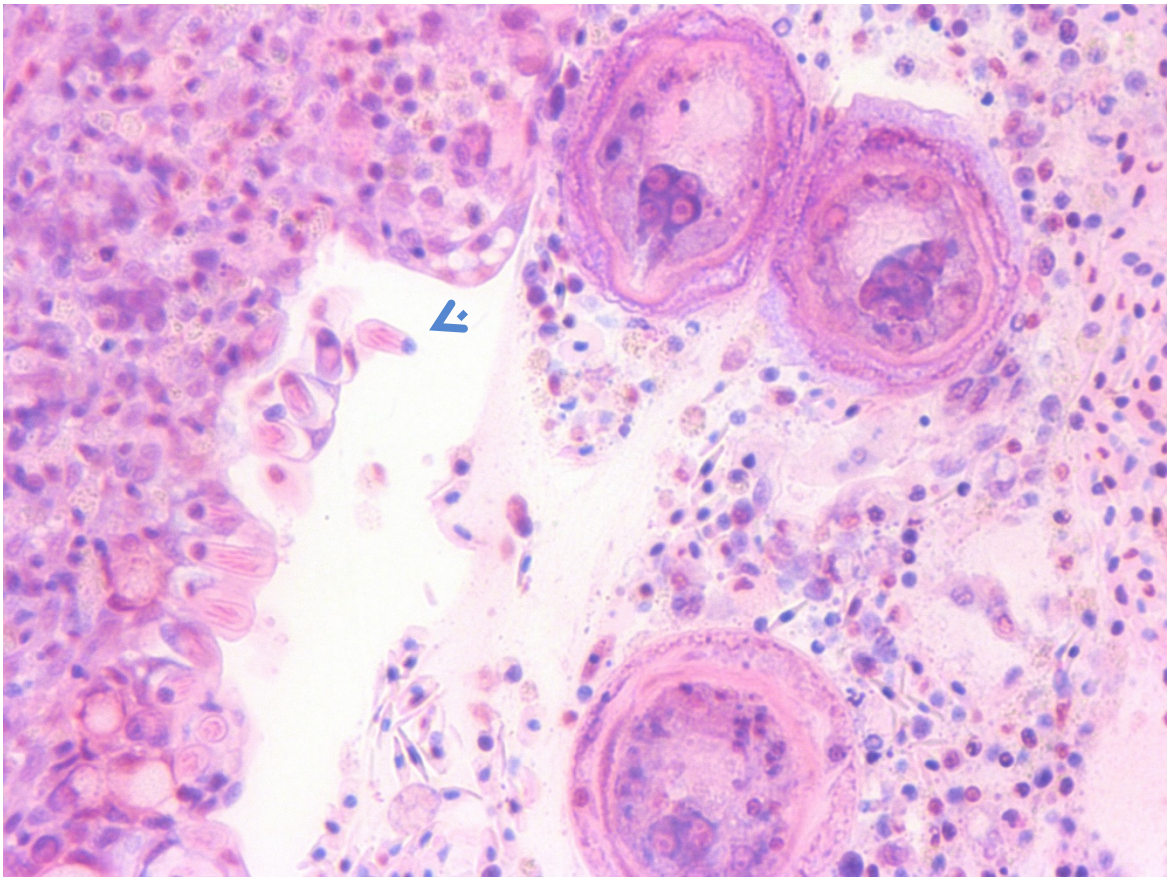
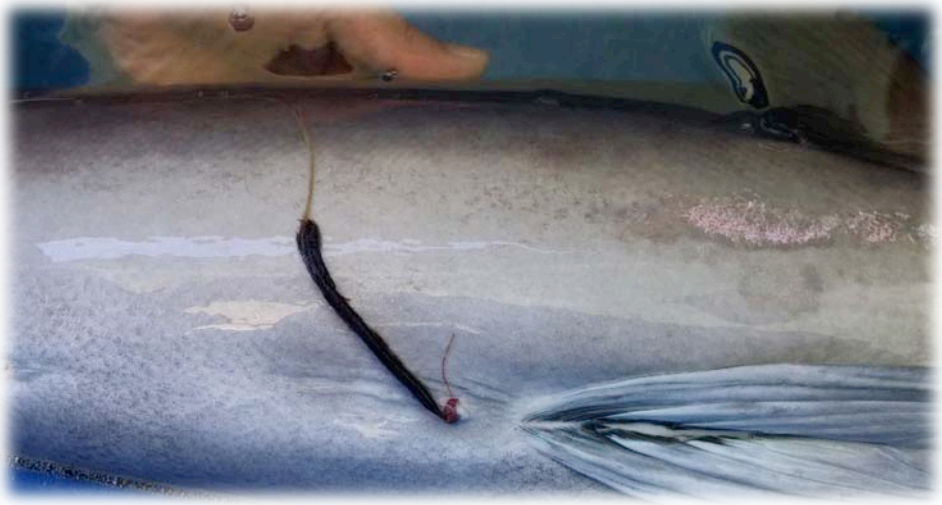


Figure 18. The parasite induces an inflammatory reaction of the host that includes the infiltration of rodlet cells.

5. PENNELLA SP.

Pantelis Katharios, Constantinos C. Mylonas and Yiannis Fakriadis (HCMR)



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Pennella sp.

The copepod parasite *Pennella* sp. is one of the largest copepod parasites of fish (Hogans, 1987; Öktener, 2009; Tuncer et al., 2010). It is embedded in the skin and musculature of the fish. The parasite is common in swordfish, *Xiphias gladius* and has been also reported from marine mammals. Very little is known about the pathology may cause to the host, however it is not considered a significant threat for cultured fish.

Figure 19. *Pennella* sp. embedded in the musculature of greater amberjack (Photo Dr. Constantinos C. Mylonas)

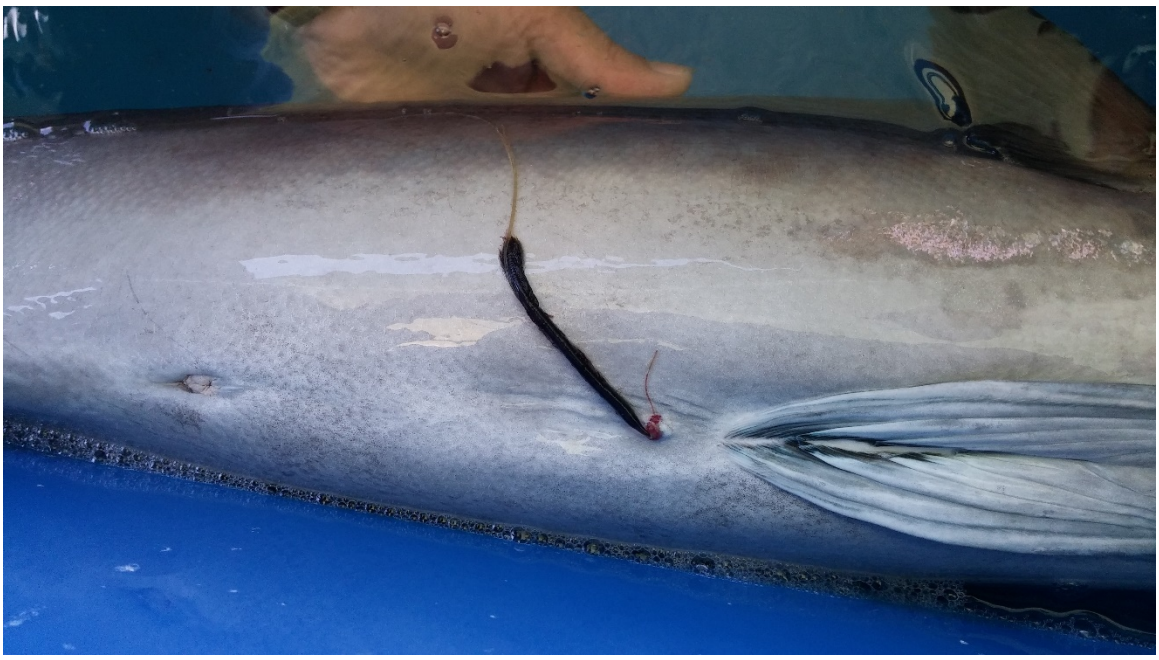

Figure 20. *Pennella* sp. embedded in the musculature of greater amberjack (Photo Dr. Constantinos C Mylonas)

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