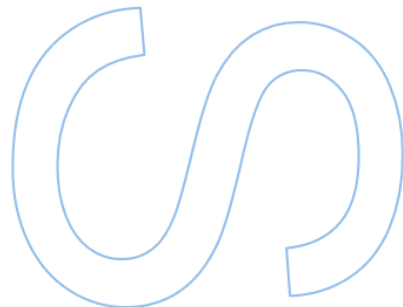
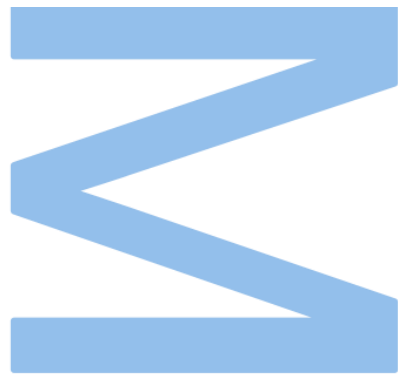


Solutions for Managing Ectoparasitic Infections in European Sea Bass (*Dicentrarchus labrax*)

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Abstract

Parasitic infestations are becoming increasingly common in aquaculture and European sea bass (*Dicentrarchus labrax*) is no exception. Farmers are already facing unstable market prices and production costs, and if they are faced with parasite infections on top of these pressures it could be detrimental to the farm sustainability. Copepod parasites such as *Caligus sp.* and *Lernanthropus kroyeri* are becoming more frequent, and although not always lethal, they can impair fish growth and welfare and further increase the production costs of farmers.

Piscicultura do Vale da Lama is a semi-intensive farm with 36 years of experience in rearing European sea bass and was facing a severe ectoparasitic infestation. As such, this internship had the goal to not only acquire experience in a commercial aquaculture setting but also to investigate ectoparasitic infections in cultured European sea bass. The study began with the observation of pathological symptoms in naturally infected fish, followed by the manual removal and maintenance of parasites under artificial conditions, enabling the study of their larval hatching and development. *In vitro* assays were performed on both adult and larval stages of *Caligus sp.* and *L. kroyeri* with several compounds. Formalin was the only one that showed consistent efficacy against both species and life stages. *In vivo* trials were conducted using a chemotherapeutant recommended by a veterinarian (referred to as Compound A). The results showed that this compound was effective in reducing parasitic load and conferred a protective effect that lasts for at least 7 weeks post-treatment, even when fish were exposed to infected cohabitants.

The results obtained in this work contribute not only to identifying practical solutions to deal with copepod infestations in European sea bass but also to the understanding of the biology of these parasites. These findings offer a basis for the development of more sustainable and targeted interventions against copepod ectoparasites in marine aquaculture systems.

Key words: Aquaculture, European sea bass (*Dicentrarchus labrax*), Parasitic Infections, Ectoparasites, Copepods, *Caligus sp.*, *Lernanthropus kroyeri*, Formalin

Resumo

Infestações de parasitas são cada vez mais comuns em aquaculturas e o Robalo (*Dicentrarchus labrax*) não é exceção. Produtores já enfrentam instabilidade no preço de mercado e de produção, e se tiverem de lidar com infeções de parasitas conjuntamente com estes desafios poderá ser detrimental para a sustentabilidade da aquacultura. Parasitas copépodes como *Caligus sp.* e *Lernanthropus kroyeri* estão a aparecer mais frequentemente, e mesmo que nem sempre sejam letais, estes podem importunar o crescimento e bem estar dos peixes e aumentar os custos de produção.

A Piscicultura do Vale da Lama é uma aquacultura semi-intensiva com 36 anos de experiência no cultivo de robalo e estava a enfrentar uma infestação severa de ectoparasitas. Como tal, este estágio teve o objetivo de não só obter experiência num ambiente de aquacultura comercial, mas também de investigar infestações de ectoparasitas em robalo criado em aquacultura. Este estudo começou com a observação de sintomas patológicos em peixes infetados naturalmente, seguido de remoção manual e manutenção de parasitas em condições artificiais, permitindo o estudo da eclosão e desenvolvimento larvar. Foram realizados testes *in vitro* em adultos e larvas de *Caligus sp.* e *L. kroyeri* com diversos compostos. Formol foi o único que demonstrou eficácia consistente contra as 2 espécies e estágios de vida. Foram também realizados testes *in vivo* com uma substância recomendada pelo veterinário (referido com Composto A). Os resultados mostraram que este composto foi efectivo a reduzir a carga parasitária e conferiu um efeito protetor que durou pelo menos 7 semanas após o tratamento, mesmo quando os peixes estavam expostos a outros ainda infetados.

Os resultados obtidos neste trabalho contribuem não só para a identificação de soluções práticas para lidar com infeções de copépodes em robalo, mas também para a melhor compreensão da biologia destes parasitas. Estes resultados oferecem uma base para o desenvolvimento de intervenções mais sustentáveis e específicas contra ectoparasitas copépodes em sistemas de aquacultura marinhos.

Palavras-chave: Aquacultura, Robalo (*Dicentrarchus labrax*), Infeções Parasitárias, Ectoparasitas, Copépodes, *Caligus sp.*, *Lernanthropus kroyeri*, Formalin

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1.Introduction

The human population continues to grow, and with it grows the pressure to scale food production at a global level. Traditional food production methods already exert enormous pressure on damaged ecosystems and biodiversity, such as land modifications perpetuated by agriculture or the overexploitation of fisheries. Concomitantly, climate change, an unstable geopolitical climate, and the recent COVID-19 pandemic have pushed the poorest section of society into further food scarcity. Around 735 million people face hunger problems globally, representing an increase of 122 million since the start of the 2019 pandemic. To aggravate the problem, over 3.1 billion people cannot afford a healthy diet, and at least 600 million individuals are estimated to be chronically undernourished in 2030. Consequentially, the necessity for sustainable and affordable food sources continues to rise (Blanchard et al., 2017; FAO, 2024a).

Aquatic foods with their great diversity of species and low environmental impact and carbon footprint have the capacity to mitigate world hunger in a less destructive way than traditional methods of food production. FAO has tried to take advantage of these characteristics to promote a transition into a more environmentally friendly food production industry, namely through the “Blue Transformation” vision, emphasizing its capacity to improve nutrition, strengthen food security, eradicate poverty, and achieve the 2030 Agenda for Sustainable Development. (FAO, 2024a) Aquatic foods are also a healthy source of animal protein and a source of several nutrients, such as iodine, selenium, iron, calcium, and omega-3 fatty acids, which are not commonly found in other foods. The health benefits offered by them include alleviation of symptoms of autoimmune disorders, protection from coronary heart diseases, and promotion of child’s brain development (Maulu et al., 2021; FAO, 2024a). Additionally, the growing demand for aquatic animals is increasing at a much faster rate than the world population, with the consumption rising 3% each year, while the human population increased by 1.6% per year from 1961 to 2021. This expansion is driven by several

factors, such as the ascension of a global middle class that wants to include more animal protein in their diets, changes in consumer preferences, and technological improvements in distribution and preservation (Blanchard et al., 2017; FAO, 2024a).

To meet the growing demand of aquatic foods, the production must be scaled up, and taking into consideration that fish stocks are already facing overwhelming pressure from overfishing (38% of fisheries stocks are already exploited to unsustainable levels), the necessity to favour aquaculture is evident, as it offers a way to upscale production without placing additional pressure on natural populations (FAO, 2024a; Roberts et al., 2024).

Aquaculture is defined by the monitored cultivation, feeding, and maintenance of aquatic organisms for conservational, recreational, or commercial purposes (Mizuta et al., 2021). The localisation of the production can be land-based (inshore), coastal (onshore) or in the open sea (offshore). Aquaculture systems can also be classified in terms of intensity, from extensive to intensive. Extensive aquaculture is characterised by a low to no level of human intervention, usually relying on the ecosystem to ensure food provisioning and water quality management. The technological level is low, as well as the stocking density, which usually leads to diminished production rates. Intensive aquaculture typically has a high level of technology, needing elevated inputs of energy and skilled workers, as they present a high degree of health, nutrition and water quality monitoring. The high feed rate and specialised artificial feeds optimised to guarantee low energy losses and low FCRs, combined with high stock densities, result in production rates much higher than its extensive counterpart. Semi-intensive explorations represent the middle ground, partly relying on the ecosystem but also providing supplementary feeding, aeration and fertilisation to increase output (Lucas et al., 2019; Odsson, 2020).

1.1 Global Aquaculture Production

In 2022 the aquatic animal's production has reached a new record, with over 185 million tonnes, representing a 4% increase from 2020. For the first time, cultivated animal production surpassed that from fisheries, accounting for 51% of the total (over 94 million tonnes, while captured animals amounted to 91 million tonnes). With the exception of 2018, when captures reached 96.5 million tonnes, the 2022 reared aquatic animals' production surpassed all annual captures since 1950. If accounting for algae too, the total adds up to 131 million, an increase of 8.1 million tonnes since 2020. (Figure 1) In 2022 the aquaculture farm to gate production value increased to 313 billion USD, representing a 34 billion increase from 2020. Aquatic animals generated the highest value, contributing 279 billion USD, seconded by algae with 17 billion USD and shells and pearls with 139 million USD (FAO, 2024a).

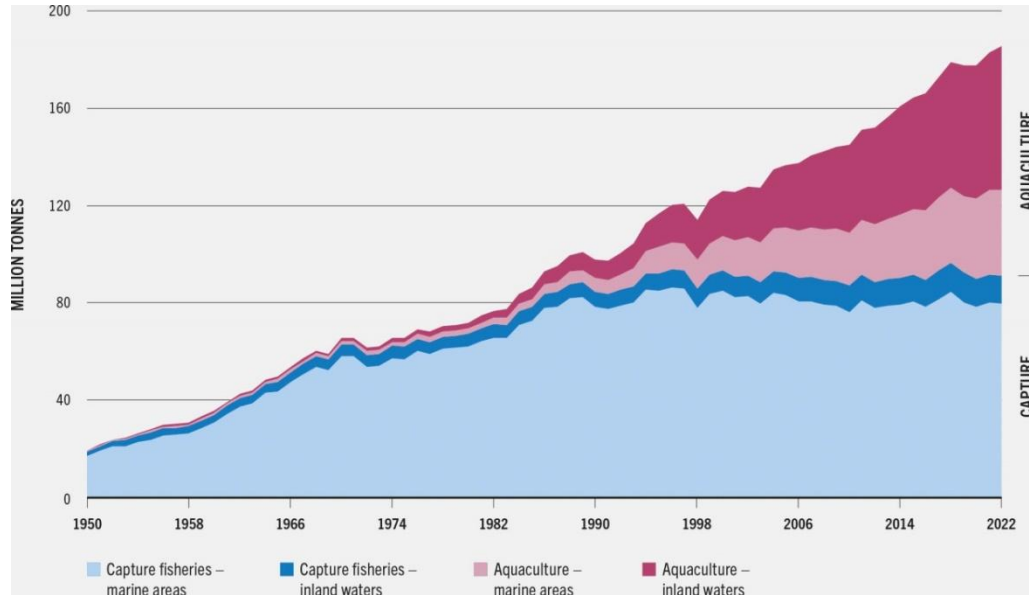


Fig. 1 - Global seafood production including fisheries and aquaculture (FAO, 2024a)

The aquaculture industry has been growing in a significant manner (expanding around 204% since 2000), although the expansion rate has been slowing down during the last 2 decades. During the 2000s the growth reached 6.1%, in the 2010s the growth diminished to 4.4% and finally reached a 3.7% increase during the first 3 years of the

2000s. Several factors influenced the diminishing growth, such as the reduction of the availability of adequate sites for production in traditional producing regions and a shift towards environmental protection policies in China (FAO, 2024a).

The leading contributors to global aquaculture of farmed animals in 2022 were Asian countries, with a total of 83 million tonnes. As expected, China was responsible for the majority of this output, rearing 53 million tonnes. Their production accounted for 63% of the Asian harvest and 56% of the global production. India followed with 10 million tonnes. Indonesia, Viet Nam and Bangladesh were also significant contributors, collectively contributing 826 thousand tonnes. The American countries harvested 5 million tonnes, with the most important contributors being Chile, with 1.5 million tonnes, Ecuador, producing 1 million tonnes, and Brazil, with 700 thousand tonnes. In Europe a total of 3.5 million tonnes were harvested. Norway was the leading country, producing a total of 1.6 million tonnes. The Russian Federation was the second largest producer in Europe, with 300 thousand tonnes. Africa and Oceania have suffered with diminishing harvest volumes in Nigeria, Egypt, Australia, and New Zealand, which caused a decrease in production in 2022. Aquaculture employs 22.1 million people around the globe, with the majority located in Asia (95%), followed by African countries (3%) and Latin America and the Caribbean (2%) (FAO, 2024a).

The majority of the total global production of aquatic animals comes from inland aquaculture, which accounts for 63% (59 million tonnes). The most commonly employed farming method was the cultivation of finfish in artificial earthen ponds. As such, finfish dominated with 90% of total inland production, followed by crustaceans, with 9%. Marine aquaculture accounted for 35 million tonnes of aquatic animals, with cage culture being responsible for 65% of marine finfish production. 36 million tonnes of algae were also produced, resulting in a combined output of 71 million tonnes produced by marine aquaculture in 2022 (FAO, 2024a).

The number of reared species continues to grow, with 731 statistical units reported in 2024 (which include species, hybrids and groups identified at the family, genus or higher levels), representing an 11% increase from 2022. Nevertheless, even with the growing number of cultivated species aquaculture production is still dominated by a small number of them. Carps were the most cultivated group in 2022, totalling 32

million tonnes of live weight and accounting for 52% of finfish production. Other important finfish species were catfishes with 6.6 million tonnes, cichlids with 6.5 million tonnes, and salmonids with 4.2 million tonnes. The penaeid shrimps were the most produced crustaceans, with 8 million tonnes, representing 62% of that group. Red swamp crayfish accounted for 4 million tonnes, 23% of global crustacean aquaculture. Oysters were the most important group of cultivated molluscs, totalling 7 million tonnes (38% of cultivated molluscs). In terms of algae, the red algae contributed 20 million tonnes, followed by brown algae with 16 million tonnes (together achieving 99% of total global algae production) (Figure 2) (FAO, 2024a).

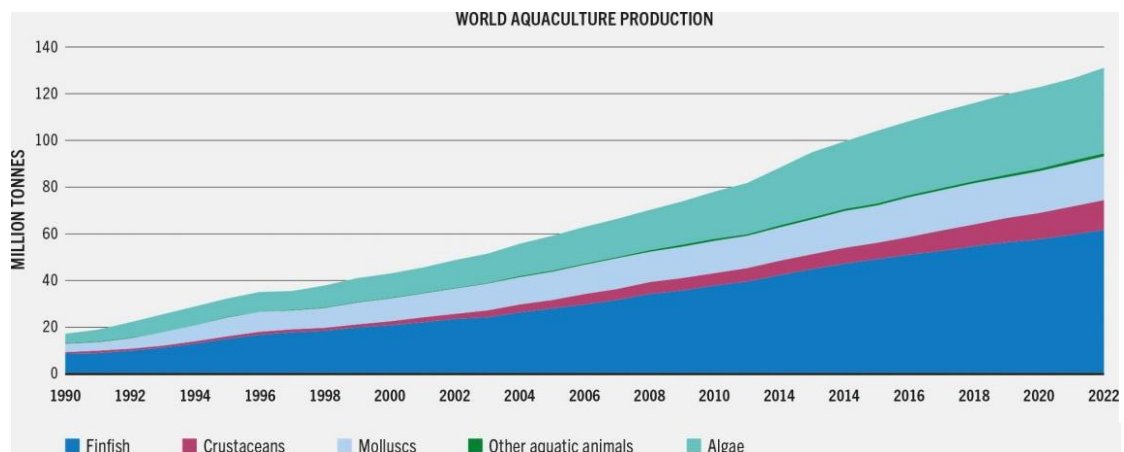


Fig. 2- World aquaculture production divided by explored groups, 1990-2022 (FAO, 2024a)

1.1.2 Aquaculture in Portugal

Portuguese culture and the sea are deeply connected, with the country being one of the bigger consumers of seafood in the world, ranked as the third largest global consumer, and being the top consumer within the European Union. As Portugal is considered a popular tourist destination, the demand during the summer is even higher, with consumption reaching 59.9 kg per capita in 2019 (Rocha et al., 2022; Murthy et al., 2023).

Although fishing has played a central role in the Portuguese economy, aquaculture has also been practised for centuries, mainly as a familiar activity. It originally involved the

entrapment of juveniles in transitional systems, such as salt pans or ponds. The most commonly farmed species included sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*), mullet (*Mugil sp.*), sole (*Solea vulgaris*), and eel (*Anguilla anguilla*) (Bernardino, 2000; Rocha et al., 2022). When this practice turned into a commercial activity, the main focus was on freshwater species, with rainbow trout (*Oncorhynchus mykiss*) as the main explored species. During the 1970s, mullets were the main cultivated group, accounting for 80% of reared fish. In the 1980s rainbow trout and bivalves became the main explored species. During the 1990s marine aquaculture initiated its development, initially focusing on European sea bass and gilthead sea bream. In recent years the turbot (*Scophthalmus maximus*) production has also been expanding (Rocha et al., 2022).

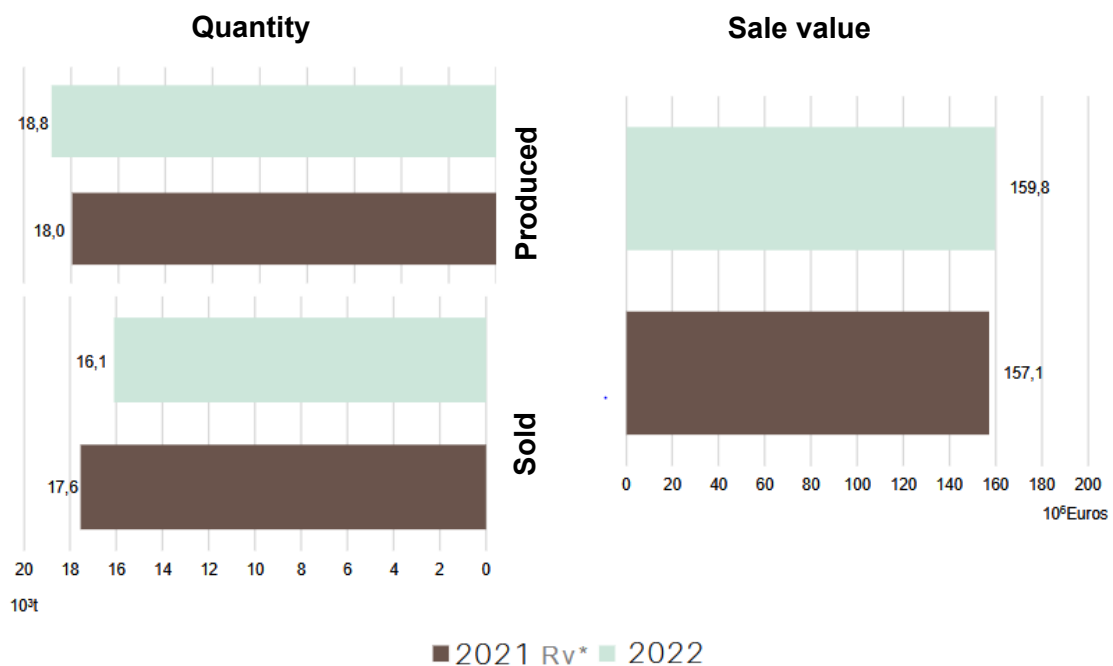


Fig. 3– Portuguese aquaculture total production and sales (INE, 2024)

Portuguese aquaculture total production reached 18882 tonnes in 2022, representing an increase of 4.8% since 2021. Despite experiencing an 8.2% reduction in sales, the total value was nearly 160 million euros, an increase of 1.7% since 2021 (Figure 4) (INE, 2024).

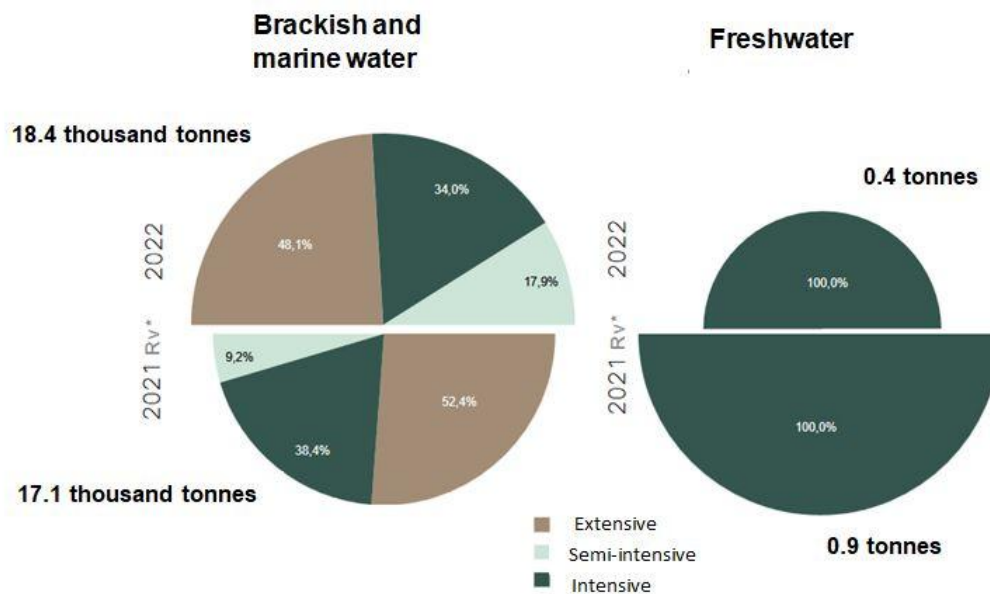


Fig. 4- Portuguese aquaculture production by water type and production system (INE, 2024)

Most of the Portuguese production comes from marine and brackish environments, accounting for 18804 tonnes in 2022, which represents 97.8% of total production. The majority comes from extensive systems (48.1%), while intensive production accounts for 34%. Freshwater explorations (strictly extensive) have faced a decrease in production of 54.3% since 2021, largely explained by a drought that impaired the water supply in many explorations, contributing only 414 tonnes (Figure 4) (INE, 2024).

The most cultivated group was fish, representing 46.1% of the 2022 production, with the majority (8527 tonnes) coming from brackish and marine explorations. The gilthead sea bream was the most cultivated fish, with 3352 tonnes, representing an 8.5% growth from 2021 to 2022, followed by turbot, accounting for 3074 tonnes, a decrease of 12.1% from 2021 to 2022, and European sea bass, with 1481 tonnes, an increase of 55.3% from 2021 to 2022. Inland explorations focused almost exclusively on trout (INE, 2024).

The production of European sea bass in Portugal is typically performed in semi-intensive systems. The ample amount of abandoned water reservoirs and salt pans in coastal areas and the environmental legislation that prohibits building concrete ponds has led to the widespread use of artificial earthen ponds, especially in the southern area (Pousão et al., 1995; Mosqueira et al., 2022). European sea bass is typically fed

pelleted feed, while the natural food sources are usually consumed by opportunistic species. Some of these species can have commercial value, and although not reared intentionally, they can also marginally contribute to the economic sustainability of the production (Pousão et al., 1995).

Mollusc production rose by 11.3% (10,151 tonnes), representing 53.9% of total production. Clams were the leading group, with 4346 tonnes (a 21.2% increase since 2021), followed by mussels with 3189 tonnes (a 4.8% increase), and finally oysters with 2465 tonnes (a 7.5% increase) (Figure 5) (INE, 2024).

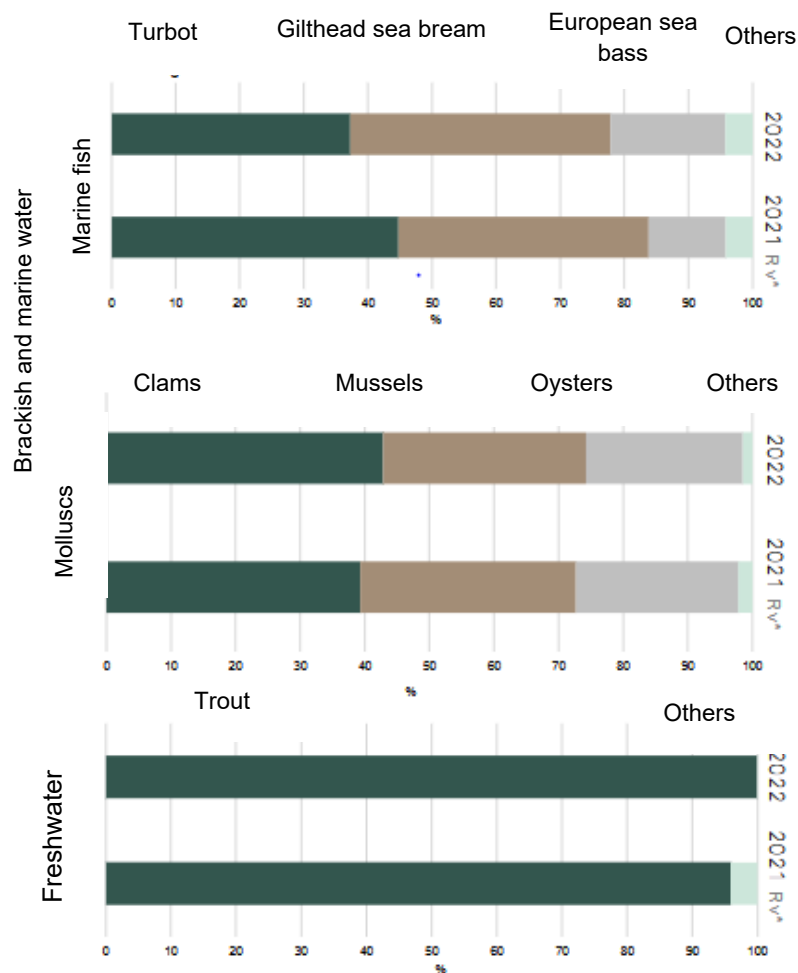


Fig. 5- Portuguese aquaculture production by water type and production system (INE, 2024)

The aquaculture sector can grow significantly in Portugal, as the country still imports most of the seafood it needs to meet demands, reaching 336,500 tonnes in 2018 (mainly finfish imports). The potential growth of the sector seems promising, especially when taking into consideration the vast Exclusive Economic Zone (EEZ) of Portugal, which covers approximately 1.7 million km², nearly 50% of the EU's EEZ, and reveals the possibility for offshore aquaculture to expand in the future GDP (Rocha et al., 2022; Murthy et al., 2023). However, Portuguese aquaculture accounts only for 0.5% of total European production in 2022 (FAO, 2024a). This presents an opportunity for scaling up national production, which would reduce the dependence on imports, promote the use of marine areas, alleviate the pressure on natural populations and positively contribute to national GDP (Rocha et al., 2022; Murthy et al., 2023).

1.2 European sea bass (*Dicentrarchus labrax*)

The European sea bass, *Dicentrarchus labrax* (Linnaeus, 1758) (Figure 6), is a fish belonging to the Teleostei class, order Eupercaria, family Moronidae, and genus *Dicentrarchus*. (WoRMS, 2025) Its body is rather elongate, featuring a terminal and moderately protractile mouth equipped with vomerine teeth. The species is characterised by a pair of asymmetrical dorsal fins, one with 8 to 10 spines and the other with 1 spine and 12 to 13 soft rays. The anal fin features 3 spines and 10 to 12 soft rays, while the caudal fin is slightly forked (Bagni, 2009; FAO, 2025a).

The scales are small, with the lateral line comprising 62 to 74 scales. The body colouration is typically silvery on the flanks, while the back presents a silvery grey to bluish colouration. Yellow tones may occasionally appear on the ventral region. In contrast to adults, juveniles can present dark spots on the dorsal region. A diffuse spot can be observed at the edge of the operculum (Bagni, 2009; FAO, 2025a).



Fig. 6- *Dicentrarchus labrax* illustration (adapted from fish-commercial-names.ec.europa.eu, 2025)

D. labrax can be found in the Eastern Atlantic, from Senegal to Norway, and it is also found in the Mediterranean and Black Sea (Figure 7) (Bagni, 2009; Pérez-Ruzafa & Marcos, 2014; FishBase, 2025). This species presents an oceanodromous behaviour, and although being a marine species, it spends the majority of its life in brackish environments (such as coastal lagoons and estuaries). Due to its euryhaline capabilities, it can tolerate a wide range of salinities (from as low as 3‰ to hyperaline waters) and has even been sporadically observed in rivers (Bagni, 2009; Pérez-Ruzafa & Marcos, 2014; Vandeputte et al., 2019).

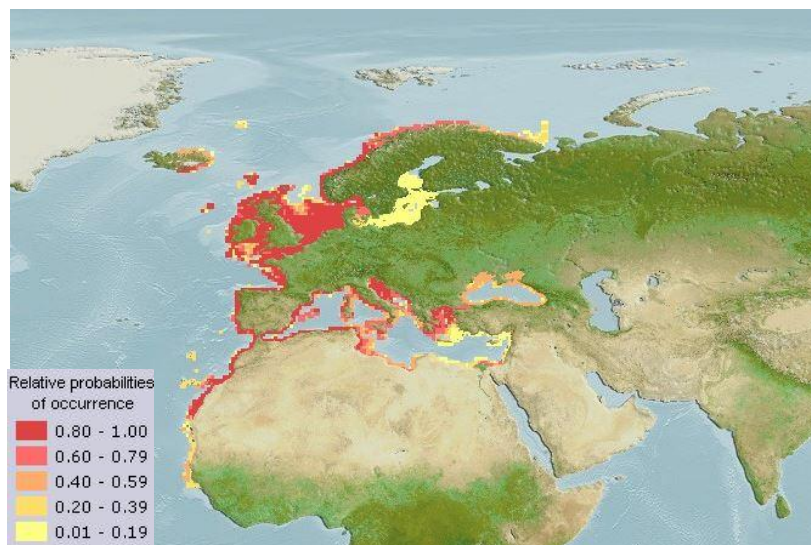


Fig. 7- *Dicentrarchus labrax* distribution (Fish Base, 2025)

It is also considered a eurythermic species, commonly found in waters ranging from 8°C to 24°C, though it also tolerates extreme temperatures, from 5°C in cold northern Mediterranean coastal lagoons to 32°C in Tunisian lagoons. It is commonly found in shallow waters (2-10 metres) but can also inhabit depths exceeding 100 metres (Bagni, 2009; Pérez-Ruzafa & Marcos, 2014; Vandeputte et al., 2019). They can reach over 70 cm in length and surpass 6 kg in body weight. In the wild, males reach maturity when

they measure 30 to 40 cm in length, while females mature once they reach 36 to 46 cm (FAO, 2025a).

The typical environmental variability of estuaries and coastal lagoons, particularly in temperature and salinity, can disrupt the energy budget balance of *D. labrax* and possibly be detrimental when resources should be invested in reproduction. As a matter of fact, Claireux & Lagardère (1999) noted that at temperatures below 10°C their metabolic performance becomes deficient, while Barnabé & Billard (1984) reported that although gametogenesis could be successfully carried out at salinities as low as 3‰, spawning will not occur. As a result, European sea bass tends to migrate to the open ocean in search of deeper areas as an adaptation mechanism, seeking temperatures above 9°C in the breeding season (Pérez-Ruzafa & Marcos, 2014; Vandeputte et al., 2019).

In the Mediterranean populations the annual reproductive season extends from December to March, while in the Atlantic population it spans from January to June. Females feature group-synchronous ovaries and a high reproductive rate, spawning approximately 200,000 small pelagic eggs per kg of body weight (Bagni, 2009; Vandeputte et al., 2012; Pérez-Ruzafa & Marcos, 2014). As males are neither protective of their eggs nor territorial, the majority of the reproductive energy investment is devoted to gamete production. Females display a reproductive effort significantly higher than the males, which converts into a higher ratio of digestive tract-to-body weight (up to 26% higher) and a larger body size (up to 20-30% larger than males). In wild populations a naturally skewed sex ratio towards females occurs, which becomes more predominant as size increases, growing from 52% of females in fish under 30 cm to 70% in larger individuals. This is mainly due to the male's shorter lifespan and the female's higher growth rate (Pérez-Ruzafa & Marcos, 2014).

The European sea bass presents a high level of adaptability and opportunistic behaviour, which makes it a top predator, reaching a trophic level of 3.5 ± 0.5 (FishBase, 2025). Their diet consists of a wide range of species, preying on whichever is abundant at the time. The juvenile's diet consists of isopods, copepodites, amphipods and Mysidacea, and to a minor extent, small fishes, decapods and bivalves. Sea bass over 20 cm mainly prey on molluscs, fish, shrimps and crabs. Teleosts make up up to 36%

of their diet, and crustaceans up to 48%, while they also consume seagrasses, seaweeds, polychaetes, snails and aquatic insects to a lesser extent (Figure 8) (Bagni, 2009; Pérez-Ruzafa & Marcos, 2014; FAO, 2025a). In a study conducted by Abdel-Hakim et al. (2010), it was noted that the ratio of crustacean to fish in their diet decreases simultaneously with growth, mainly due to larger individuals being better equipped to catch and ingest a larger variety of prey. Georgalas et al. (2007) reported that larvae are also capable of adjusting their swimming activity in response to prey density, as they intensify their feeding effort if prey is abundant.

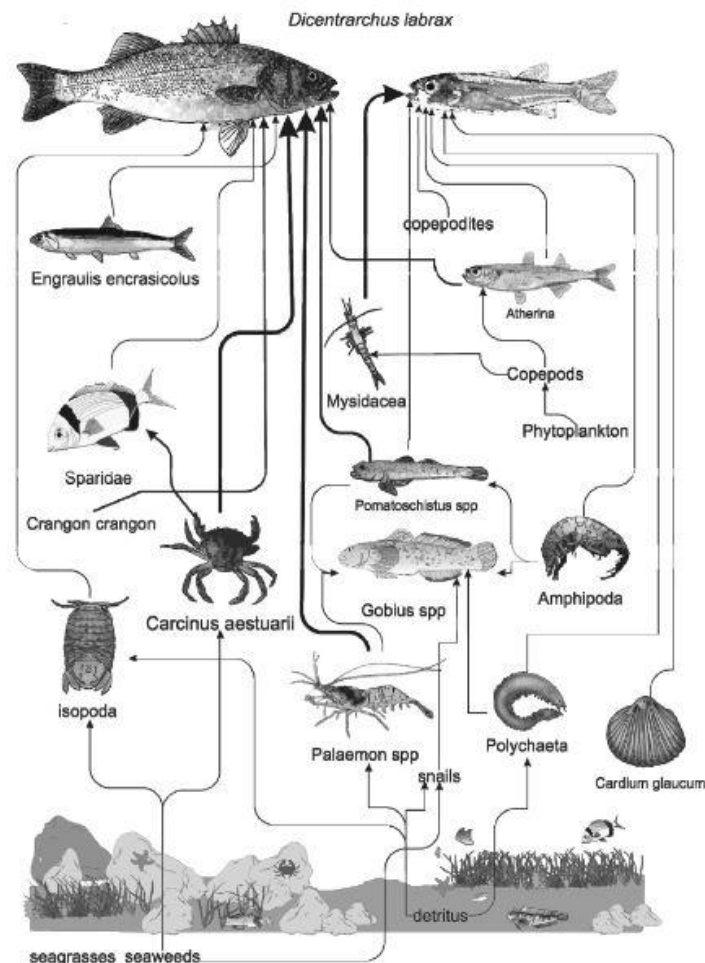


Fig. 8- *Dicentrarchus labrax* adults and juveniles main trophic interactions (Pérez-Ruzafa & Marcos, 2014)

1.2.1 European sea bass production

European sea bass global production has reached 292523 tonnes in 2023 (FishStatJ, 2025). It is a highly appreciated species in Europe, where, in 2016, the average annual per capita consumption reached 190 g. This fish is even more valuable for some Mediterranean countries, where the consumption exceeds 500 g per person, as in the case of Portugal, Spain, Italy and Cyprus (European Commission, 2019).

The majority of capture fisheries in Europe come from the northeastern Atlantic, where recreational fishing also plays an important role, accounting for 30 to 50% of Atlantic catches, while a smaller share of the catches comes from the Mediterranean Sea (European Commission, 2019; Vandeputte et al., 2019; European Commission, 2024). Seines, pelagic trawls, and hooks and lines are the most commonly employed fishing methods. However, from 2013 to 2022, catches within the EU have declined by 42%. The main reason was the regulations imposed on fishing as a way to alleviate the impacts of overfishing and diminishing reproduction rates in wild populations (European Commission, 2019; European Commission, 2024).

The majority of production has come from aquaculture since 1992. In 2023 the reared European sea bass accounted for 98% of total production (286.968 tonnes), while fisheries contributed only 5556 tonnes. The value of global production surpasses 1.87 billion USD in 2023 (FishStatJ, 2025). The majority of the world's production comes from the Mediterranean region, with Turkey as the largest contributor with 160802 tonnes, amounting to 1.05 billion USD. Within the EU, Greece is the leading producer, rearing 44201 tonnes in 2023, generating 308 million USD. Spain is the second largest producer in the EU, with 24413 tonnes in 2023, representing 216 million USD. Egypt is a significant contributor as well, harvesting 34077 tonnes in 2023, valued at 109 million USD (Vandeputte et al., 2019; FishStatJ, 2025).

The European sea bass domestication was a pioneer in European aquaculture, being the first non-salmonid marine species to be commercially explored. In the 1970s the process began by entrapping wild juveniles in semi-artificial lagoons. In the 1980s, the decline of natural resources combined with the increasing interest in acquiring wild fry resulted in the creation of the first small-scale intensive systems in Italy and France,

providing an alternative source of juveniles. The advancement of breeding and hatchery techniques and the achieving of full life cycle control made it possible to initiate the first breeding programmes using hatchery-born broodstock in the 1990s, thus marking the beginning of the domestication process at the genetic level (Chatain & Chavanne, 2009; Vandeputte et al., 2019). Nevertheless, this process is still at an early stage, as in 2016 the longest domesticated *D. labrax* strains were merely eight generations apart from their wild ancestors (Chavanne et al., 2016; Vandeputte et al., 2019).

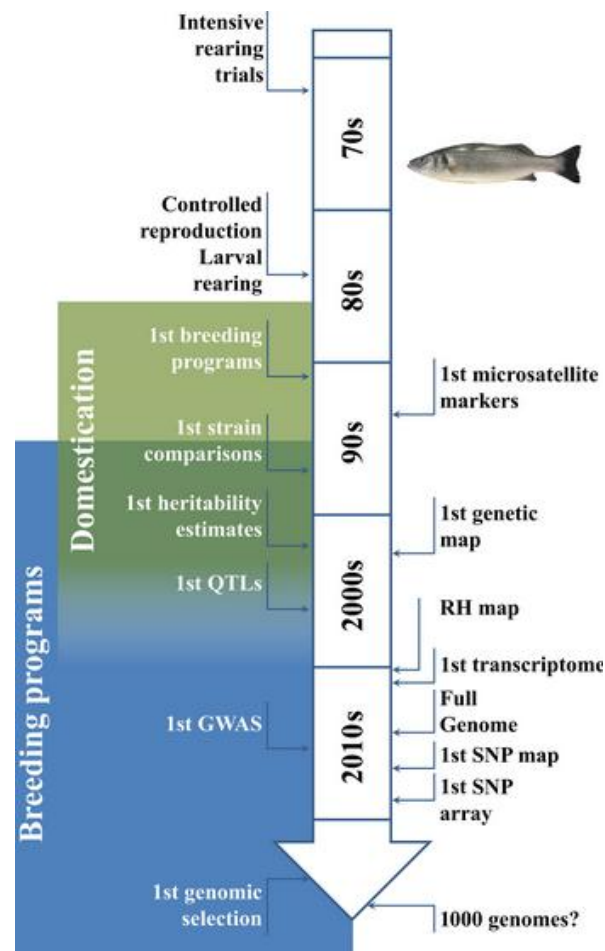


Fig. 9– European sea bass domestication process
(Vandeputte et al., 2019)

As domestication progressed, and with the facilitated access to fry, the industry expanded rapidly during the late 1990s. In the early 2000s, the fast and uncontrolled

growth led to an abrupt decline in global prices for European sea bass, hitting the lowest point in 2001. During periods of excessive production, prices even dropped below production costs, resulting in a major crisis that forced a reorganisation of the sector. Since then, although production and profitability have experienced cyclical fluctuations, the industry has continued to expand at a slower pace (Llorente et al., 2020).

The production cycle includes the following stages:

- **Reproduction:** Breeders are maintained in broodstock units, originating from wild populations or from farms. While males reach an optimal reproductive age at 2 to 4 years, females only attain optimal reproductive performance from 5 to 8 years. The production cycle initiates in hatcheries, including broodstock maturation and ovulation induction by hormonal treatments or photoperiod manipulation (Moretti et al., 1999; Bagni, 2005).
- **Spawning:** When the spawning season begins, broodstock are relocated to adequate spawning tanks that enable the evaluation of performance. This also guarantees that the breeders' needs are meticulously met, particularly by providing a diet rich in polyunsaturated fatty acids, lipids, proteins, vitamins and other micronutrients, essential to ensure a higher yield of viable eggs and larvae. Males are chosen when they release sperm, either through stripping or manually. Females usually undergo ovarian biopsy, utilising a catheter to extract oocytes from the ovary. When oocytes are in the late-vitellogenic stage and have diameters larger than 650 µm, the females are considered ready. Sex ratios are usually kept at 2 males to 1 female.
- **Egg management:** Fertilised eggs float in full seawater while the non-viable eggs sink, allowing for the use of egg collectors that harvest floating viable eggs (Figure 10). Although incubation can be performed directly in the tanks, it's preferred to be carried out in specialised incubation tanks. These are normally circular with a conical bottom (Figure 11), promoting water flow while facilitating the separation of post-hatch debris and non-viable eggs. The larvae are checked for viability one day post-hatch, assessing body shape, fin integrity,

external parasites, internal organ disposition, pigmentation and erratic swimming behaviour. Deviant individuals are removed, and only the viable larvae are transferred to the rearing facilities (Moretti et al., 1999).



Fig. 10– Overflow collector (Moretti et al., 1999)



Fig. 11– Fiberglass cylindroconical tank used for eggs

- Larval rearing (Figure 12): After hatching, the larvae possess a large yolk sac that contains nutrient reserves, which will serve as an energy source during the first 3 to 4 days (letters A to E), as they lack functional eyes and mouths. After this phase the larvae's mouth opens, the eyes become active and exhibit a dark colouration, and they begin to swim actively, which allows them to stay in a horizontal position (letter E). The digestive tract is now ready to assimilate food, thus signalling the initiation of the post-larval stage (Moretti et al., 1999). At this stage live feed is supplied, including rotifers, and later brine shrimp nauplii (*Artemia sp.*). The young fish undergo a metamorphosis after 40 to 50 days and are now referred to as fingerlings or juveniles. At this phase the juveniles are ready for weaning, the transitioning from live to artificial feed. Usually this

process is undertaken in a dedicated part of the hatchery, where the fingerlings grow to 2-3 grams (Moretti et al., 1999; Saka et al., 2007).

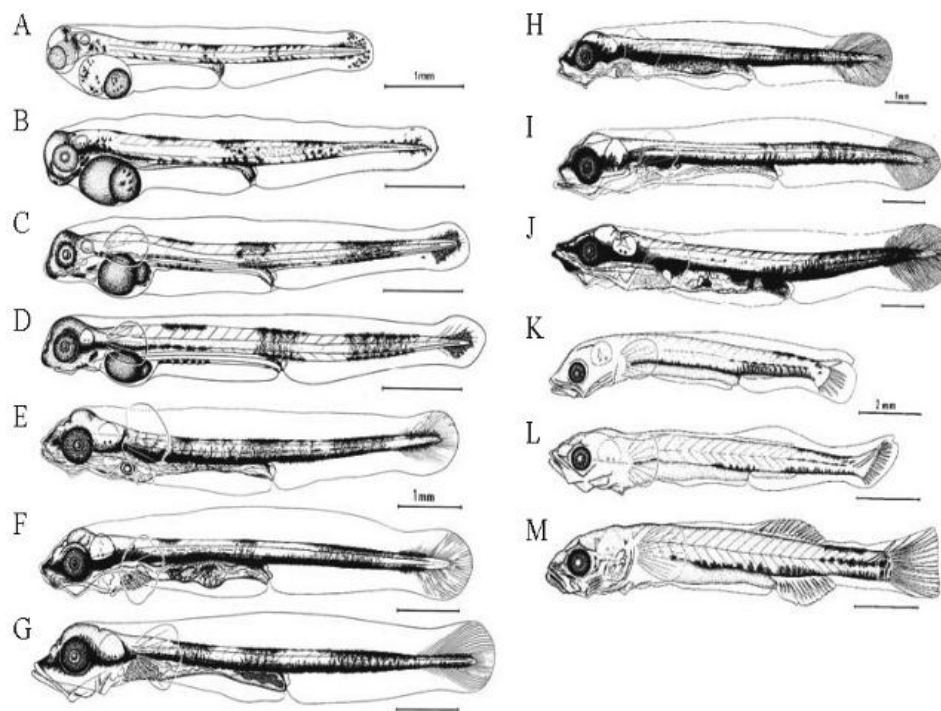


Fig. 12– European sea bass larval development (Gysbert et al., 2014)

- **Grow-Out Stage:** When the fingerlings' weight reaches 1.5 to 2.5 grams, they are moved to on-growing systems, where they will attain a market size of 400 to 450 grams during the following 18 to 24 months. To minimise cannibalism and reduce growth disparities, it's advised to perform at least 2 to 3 grading procedures throughout the production cycle. Sea cage explorations are responsible for the larger portion of production, with seawater lagoons and ponds also contributing to a smaller degree (Bagni, 2005).

Due to its high trophic level, *D. labrax* requires a dietary inclusion rate of at least 50% protein and an energy content of 21 kJ g⁻¹ dry matter (Aires Oliva-Teles, 2000). This level of protein inclusion translates into high farming expenses, with feed constituting the largest cost factor, sometimes even surpassing 50% of the farming budget (Nhhala et al., 2022). The following major expenses are usually

juvenile costs and labour expenditures (Figure 13). The share of “Other operating costs” mentioned in Figure 13 covers a wide range of expenditures, including health management, site services, office based professionals, equipment rental and servicing. The financial performance of European sea bass productions in the EU is also negatively impacted by the high prices of energy, feed and labour (Nielsen et al., 2025).

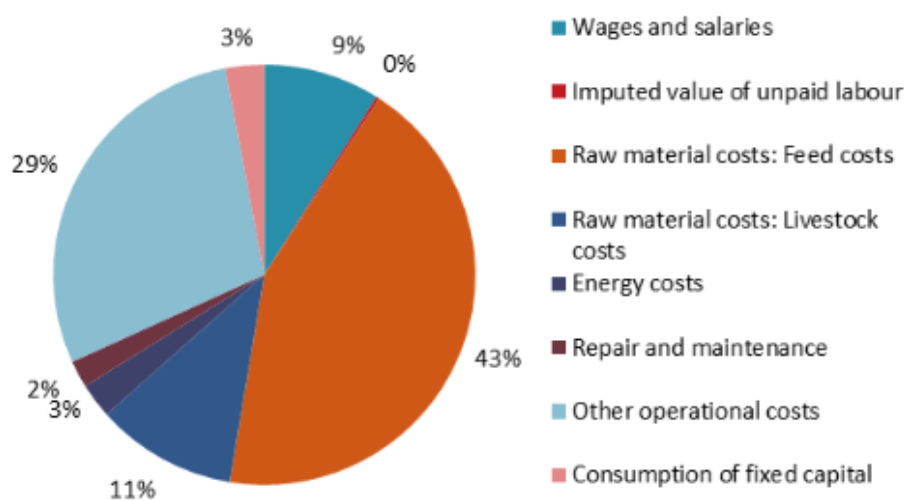


Fig. 13– Cost breakdown of European sea bass production (Nielsen et al., 2025)

In a study conducted by Sánchez et al. (2022b), it was estimated that a 450-gram sea bass would require €4.06/kg to be reared in medium- to large-sized explorations, 4.67€/kg to be produced in a small-sized farm, and 5.99€/kg to be produced in a micro-sized production. Taking into consideration that the market price in Spain was fluctuating around 4.95 to 7.3€/kg for medium-sized fish from September to December of 2024 and around 4.65 and 5.5€/kg for small-sized fish from July to August of 2024, it is obvious that not only is the profit margin extremely narrow, but it is also dependent on market fluctuations, particularly in small and micro-sized farms (FAO, 2024b; FAO, 2025b). In fact, European sea bass sea-cage aquaculture in the EU experienced a decline in profitability in 2022, reaching a record low (Figure 14), primarily due to the rising operational costs. To a lesser extent, the energy crisis afflicting the continent, the increasing inflation, and the 2019 pandemic also contributed to the decline in profitability (Nielsen et al., 2025).

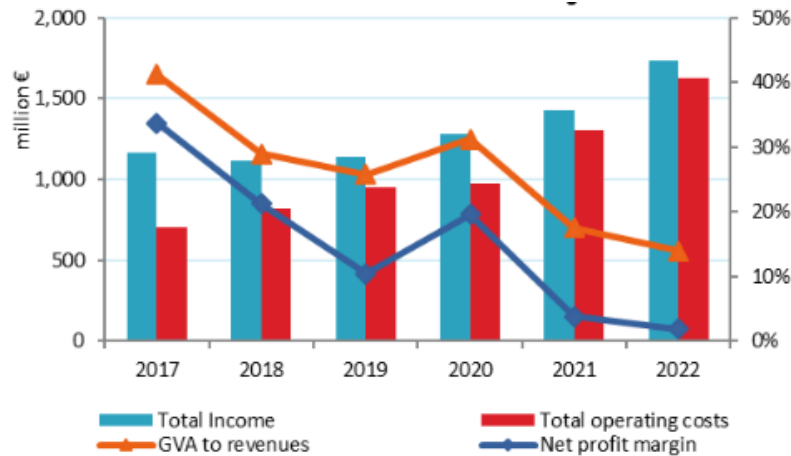


Fig. 14– European sea bass performance economic indicators (European Commission, 2025)

On the contrary, *D. labrax* farms in Turkey continue to present a higher level of profitability when compared with their EU counterparts. In fact, Arikan & Aral (2019) reported an average production cost of 4.57 USD/kg, with a net profit margin of 19%. Likewise, Peker & Ertkein (2012) noted that rearing European sea bass in earthen ponds costs around 4.91 USD/kg, with a net profit margin of 20.7%, whereas production in sea cages costs approximately 4.97 USD/kg, with a profitability of 18.9%. Multiple factors explain the greater economic sustainability of farms in Turkey, including the reduced operational expenses (particularly labour), the lower regulation level compared to EU countries, the subsidies conceded to Turkish farmers, and the Turkish lira depreciation. These factors contributed to the market dominance by Turkish sea bass producers, which also facilitated a 58% increase in production from 2013 to 2023 (Bjørndal et al., 2019; FishStatJ, 2025).

European sea bass is the third most produced fish species in Portuguese aquaculture, with 1703 tonnes reared in 2023. Nonetheless, the contribution to global production remains vestigial (0.6%) (INE, 2024; FishStatJ, 2025). However, Portugal is one of the top global consumers of sea bass, with 8397 tonnes consumed in 2021, corresponding to 800 grams per capita. To meet domestic demand, a total of 7003 tonnes were imported during that year (European Commission, 2024). Moreover, the water quality in Portugal is ideal for the expansion of marine aquaculture, with pH, nitrogen compounds, salinity, and oxygen levels all within optimal ranges. The water temperature is also favourable for the establishment of offshore aquaculture, which

highlights the sector's potential to not only meet the population demand but also become a significant contributor to global production (Mosqueira et al., 2022).

1.3 Main disease and parasites affecting aquaculture systems

Multiple factors have slowed the aquaculture industry expansion, such as poor environmental management, limitations in access to space and capital, and technical constraints (Raux & Agúndez, 2021). Nevertheless, diseases affecting aquaculture systems are the main bottleneck to the development of the sector (Stentiford et al., 2017). The destructive potential of infectious diseases is evident, as in the case of shrimp aquaculture, where losses exceeding 40% of global production have led to the collapse of production in multiple Asian countries (Bondad-Reantaso et al., 2005; Stentiford et al., 2017). The likelihood and severity of disease outbreaks are increased by the properties of the aquatic environment, such as the absence of physical barriers and the high diversity of aquatic pathogens. In addition, certain practices within the aquaculture industry also contribute to the spread of these infections, for example, the increasing number of cultivated species, poor biosecurity measures, and interactions between wild and cultured populations (Bondad-Reantaso et al., 2005; Stentiford et al., 2017; Tlusty, 2020).

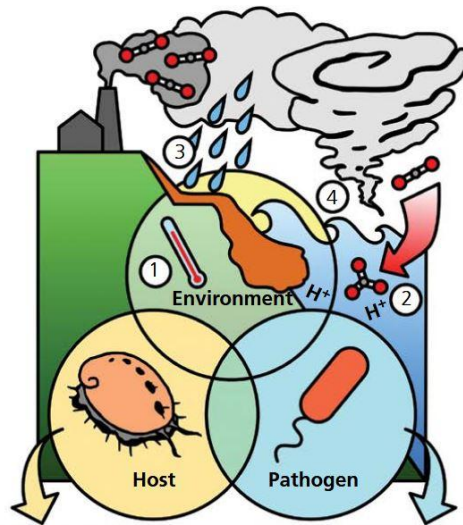
To intensify production, producers resort to major environmental alterations, with many favouring the spread of virulent pathogens by altering their replication and transmission rates. The high densities typical of aquaculture systems are a major contributing factor, as they not only facilitate disease transmission but also induce stress in fish (Boyd, 2017). Moreover, the high host density is favourable to the persistence of highly virulent pathogens, which in the wild would kill hosts too rapidly to sustain an outbreak. On the contrary, the abundance of hosts in aquaculture allows these pathogens to spread and persist (Kennedy et al., 2016).

With increases in stock density, the necessity for a greater feed input also grows, which can lead to water quality degradation caused by the accumulation of excretions and

uneaten feed. The decomposition process increases nitrogenous compounds and carbon dioxide to dangerous levels, while also reducing the level of dissolved oxygen. Exposure to stressors causes a suppression of the adaptive and innate immune response, turning fish more vulnerable to infectious diseases. (Datta, 2012; Boyd, 2017)

To further improve production, farmers resort to selective breeding, favouring traits that lead to a greater output, such as disease resistance, faster growth and better feed conversion. Nevertheless, in addition to the founder effect and inbreeding of broodstock, it can also cause the genetic diversity to be limited. This allows the pathogens to specialise and increase virulence in a genetically uniform population, which can be detrimental for farm sustainability (Kennedy et al., 2016).

The alterations provoked by climate change may worsen the disease problem, as they have the capacity to change both pathogen and host behaviour (Figure 15). For example, rising water temperatures can accelerate the life cycle of some pathogens (Nguyen, 2024). Higher water temperatures are also responsible for increased stress levels and compromised immune response in fish (Boyd, 2017). The combination of these alterations, along with changes in the distribution of disease vectors, predators and competitors have the potential to further worsen the impacts, the full consequences of which are not yet entirely understood (Nguyen, 2024).



Changes in host	Climate factor	Changes in pathogen
• Increased susceptibility • Invasions • Range shifts	① 	• Increased activity • Change in virulence • New emergence • Invasions • Range shifts
• Increased susceptibility • Change in larval survival/recruitment • Changes in diversity	② 	• Change in virulence • Change in abundance
• Increased susceptibility • Nutrient stress • Salinity stress • Contaminant stress	③ 	• New emergence • Change in virulence • Invasions • Range shifts • Change in abundance
• Increased susceptibility • Physical injury	④ 	• New emergence • Invasions • Range shifts • Mixing/resuspension of particles

Fig. 15- Climate change impacts on marine host-pathogen-environment relationships (Burge & Hershberger, 2020)

The economic damage caused by infectious diseases in aquaculture amounts to several billion USD annually. These are not limited to the direct losses, such as treatment expenditures and mortalities, but also indirectly through diminished productivity and growth. Diseases can also delay the maturation process, extending production cycles and hindering profitability. Farmers are usually advised to monitor for signs of reduced growth, as this is often the first sign not only of declining water quality

but also of a potential disease outbreak (Behringer et al., 2020). This is directly related to the fact that disturbances to the normal functioning of the animal will disrupt their energy balance, obligating the fish to expend a higher fraction of their energy to maintain homeostasis, while a lesser portion is dedicated to growth. This also impairs the immune response and also increases the susceptibility to infections (Boyd, 2017). Producers also face a decrease in marketability when fish are visibly affected by pathogens, as the consumer will avoid animals with visual indicators of disease. To aggravate the problem, some pathologies also decrease the fillet value through post-mortem degradation. If possible, farmers resort to selling the fish at lower prices to the consumers or to processing industries as a way to alleviate losses (Behringer et al., 2020).

1.3.1 Main diseases affecting European sea bass aquaculture

European sea bass aquaculture development has also been hindered by the bottleneck caused by infectious pathogens. Considering the serious sustainability challenges faced by EU sea bass farmers due to limitations in profitability, the impact of a disease outbreak could be devastating. The economic viability of farmers is severely affected, as reduced performance, treatment expenditures and disease related mortalities can escalate production costs, which can reach an increase of 2.41€/kg to 8.01€/kg during a disease outbreak (Sánchez et al., 2022a). The adoption of sea cages has allowed a production expansion; however, their environmental characteristics have indirectly facilitated the spread of some pathogens, as the artificial conditions within these cages create a favourable environment for them. Fish effluents, uncontrolled water and the cage surfaces, which are favourable to biofouling growth (providing an ideal attachment and settlement location for some parasites), can accelerate the spread of diseases. Additionally, contact with wild fish acting as potential intermediate, reservoir or natural hosts, as well as the introduction of fish from different regions, can facilitate the entry of new pathogens into farms or surrounding ecosystems (Sitjà-Bobadilla et al., 2014). Vibriosis, Viral Encephalopathy and Retinopathy/Viral Nervous Necrosis (VER-VNN) and tenacibaculosis are the most destructive diseases affecting European sea bass aquaculture in the Mediterranean region (Figure 16) (Muniesa et al., 2020).

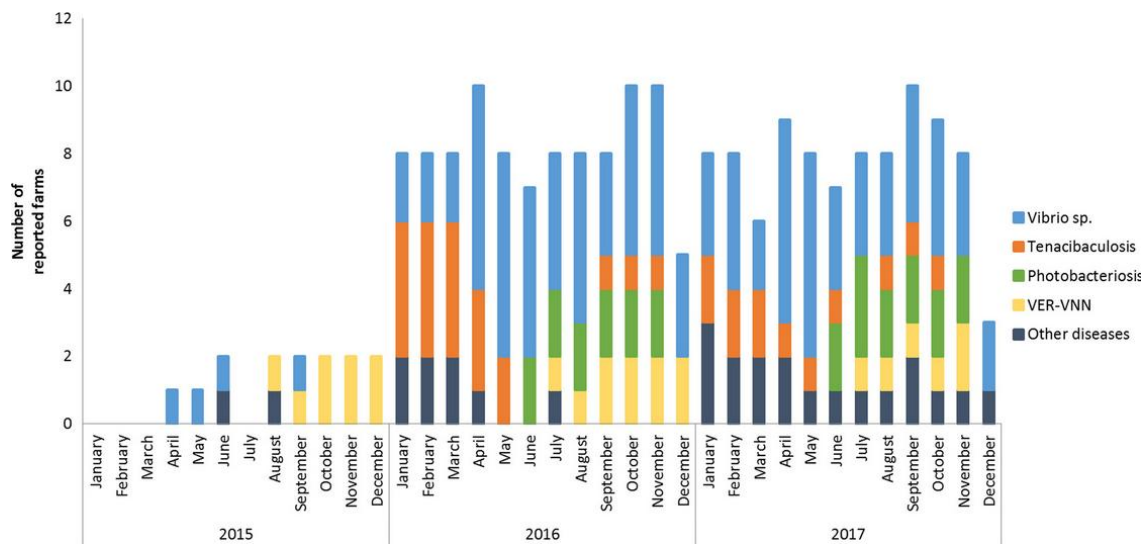


Fig. 16– Most common diseases of European sea bass reported by 27 farms, from 2015 to 2017 (Muniesa et al., 2020)

Tenacibaculum maritimum is the main causing agent of tenacibaculosis, one of the most detrimental diseases for Mediterranean *D. labrax* aquaculture (Muniesa et al., 2020; Zrnčić et al., 2020). Infected fish present tail rot, skin ulcers, ulcerative conditions with mouth haemorrhages and erosion, frayed fins, and atypical anorexia that manifests quickly in contained fish (Sitjà-Bobadilla et al., 2014; Zrnčić et al., 2020). The severity of this disease is increased at salinities of 30‰ to 35‰, and when temperatures surpass 15°C. Fish exhibit a higher degree of susceptibility at earlier life stages. As this pathogen can be autochthonous to the skin microbiome, it needs a fish mucous reservoir for long-term survival in the water column. Although this bacterium can be transmitted through food, it primarily infects the host through external surfaces, particularly the gills (Sitjà-Bobadilla et al., 2014).

Vibriosis, a disease caused by bacteria of the genus *Vibrio*, was also considered as one of the most problematic pathologies for the Mediterranean region, with sea bass as the most impacted species. *Vibrio anguillarum* is the most commonly encountered species and is the causative agent of a deadly pathology affecting over 50 species (Sitjà-Bobadilla et al., 2014). The symptoms include red spots on the lateral areas and at the base of the fins, corneal opacity, dark skin lesions, exophthalmia, lethargy and

haemorrhagic generalised septicaemia (Figure 17). Environmental stressors cause rises in mortality rates, such as water quality, elevated temperatures and high population density (Sitjà-Bobadilla et al., 2014; Zrnčić et al., 2020). This pathogen is transmitted through the skin, usually infecting its host through pre-existent injuries (Sitjà-Bobadilla et al., 2014). Ragab et al. (2021) corroborated this mechanism, reporting that a *Vibrio alginolyticus* outbreak, which entered the host through wounds caused by *Amiloodinium ocellatum*, has caused mass mortalities of 1 gram fry in an Egyptian hatchery, affecting 70% of the population.



Fig. 17– Symptoms of vibriosis: a) Hemorrhages on the mouth, operculum fin base and fins; b) Exophthalmos; c) hemorrhages on the liver and intestines (Zrnčić et al., 2020)

Viral Encephalopathy and Retinopathy/Viral Nervous Necrosis (VER/VNN) is the main viral pathology affecting Mediterranean aquaculture, and sea bass are no exception (Vendramin et al., 2016; Muniesa et al., 2020). Larvae and fingerlings are the most

affected stages. This virus is transmitted horizontally through water, but in some species it can also be transmitted vertically through the eggs. This disease does not cause gross pathology on the body or gills, although indirect lesions such as ulcers and erosion may appear on the head and eyes as a result of erratic swimming, which is the only visible symptom. Histologically it's possible to observe vacuolisation of the retina and central nervous system and severe extended necrosis (Figure 18). Infected European sea bass larvae and juveniles can reach mortality levels of 50 to 100%, with susceptibility diminishing with age, as was confirmed by Sitar et al. (2021), where a farm in Slovenia reported 50% mortality in infected fish, with weekly deaths exceeding 11000 in 300 gram fish (Sitjà-Bobadilla et al., 2014; Zrnčić et al., 2020).



Fig. 18 – VER/VNN symptoms: a) Dead fish at the bottom of the tank, while live fish show erratic swimming behaviour and a characteristic "sickle" position; b) Hyperinsufflation of swim bladder; c) Several head lesions. Left fish: mild congestion; Middle fish: erosion of head skin and congestion; Right fish: necrosis of the nostrils d) Deceased sea bass with hyperaemia of the brain (Zrnčić et al., 2020)

1.4 Parasitic diseases in aquaculture

Diseases caused by viruses and bacteria have the greatest devastating capacity and cause enormous economic losses. Nevertheless, parasitic infections are being recognised as an emerging threat. The parasite related literature is growing, which reflects an increasing number of parasite epidemics affecting both farmed and wild fish (Shivam et al., 2021). Reduction in productivity, diminished marketability of the fish and mortalities associated with parasites cause tremendous economic losses, with at least 1.05 to 9.58 billion USD lost annually (Shinn et al., 2015; Paladini et al., 2017; Shivam et al., 2021; Madsen & Stauffer, 2024). Additionally, the feeding and fixating habits of ectoparasites can cause mechanical damage, which can facilitate the spread of secondary infections and consequentially exacerbate the losses (Paladini et al., 2017; Abd-Elrahman et al., 2023).

Parasites are naturally present in aquatic ecosystems and, in most cases, have little to no harmful effect on the host's growth and survival (Barber et al., 2000; Timi & Buchmann, 2023; Madsen & Stauffer, 2024). Nevertheless, parasites have a high reproductive capacity and will overwhelm culture systems if the conditions are met. Additionally, some species can be zoonotic and cause dangerous illnesses, representing a threat to the consumer (Abd-Elrahman et al., 2023; Madsen & Stauffer, 2024). Parasites that infect internal tissues or organs are classified as endoparasites, while those infecting the surface of the gills and skin are called ectoparasites (Barber et al., 2000; Abd-Elrahman et al., 2023; Abbas et al., 2024; Madsen & Stauffer, 2024). Parasites can also complete their life cycle within a single host, exhibiting a direct life cycle, or they may require several hosts, having an indirect life cycle (Barber et al., 2000). Aquaculture systems are dominated by parasites with simple or direct life cycles, which is corroborated by the quantity of infections mentioned in literature involving monogeneans, protistans and parasitic crustaceans (Paladini et al., 2017).

1.4.1 Major parasitic threats affecting European sea bass in aquaculture systems

The parasite taxa present in any environment are determined by the geographical location, reared species, the type of aquaculture systems and feed (Paladini et al., 2017; Madsen & Stauffer, 2024). When non-native species are introduced, they may not only carry new parasite species, but they also can be posteriorly infected by native parasites. Additionally, they may transmit these parasites to wild animals, which represent an additional threat to this issue (Madsen & Stauffer, 2024).

As one of the main cultivated species in Mediterranean aquaculture, European sea bass is an important source of income for the local farmers. Mass mortalities related to parasitic infections are not yet usually observed, but the impacts of their occurrence still impair the productivity and sustainability of the producers. The main parasitic groups impacting reared European seabass include protozoans, monogeneans, isopods and copepods (Antonelli & Marchand, 2012).

Protozoans are unicellular beings that have the capacity to move independently, in contrast with animal, plant and fungal cells (Madsen & Stauffer, 2024). Some examples of parasitic protozoans are flagellates, amoebae and ciliates. These parasites have a direct life cycle and characteristically reproduce by binary fission. They also transmit themselves through contact, allowing for a rapid proliferation, especially under stressful conditions (Paladini et al., 2017; Madsen & Stauffer, 2024). Therefore, when an outbreak occurs, it is essential to perform environmental manipulations to alleviate the impact and mortality level (Paladini et al., 2017).

Amyloodinium ocellatum (Figure 19) is an example of a dangerous protozoan. This ectoparasitic dinoflagellate is the causative agent of velvet disease, or Amyloodiniosis, one of the most harmful pathologies of warm water marine aquaculture. It is usually found on the skin, buccal cavity and gills of numerous brackish and marine water fish. This obligate ectoparasite starts its life cycle as a trophont attached to fish, succeeded by the tomont, which multiplies itself, and lastly the dinospore, a free-living stage that infects new fish (Ragab et al., 2022). European sea bass infected with the velvet disease show signs of emaciation, distress and may be anorexic. They also present

excessive mucous production, darkened and friable skin, and flashing behaviour. In histological analysis it is possible to see severe hyperplasia in the gill epithelium, with the secondary lamellae frequently fused or destroyed. Mild hyperplasia and desquamation of the covering epithelium are also common to observe (Seoud et al., 2017). Moreover, the feeding and attaching activity may cause lesions that may serve as entry points for secondary infections (Ragab et al., 2022). In a study conducted by Ragab et al. (2022), it was noted that injuries caused by *A. ocellatum* enabled a *Vibrio alginolyticus* secondary infection, which resulted in mortality of 70% of fry from an Egyptian hatchery. In another study by Seoud et al. (2017), an *A. ocellatum* outbreak in several Egyptian farms has caused fish to exhibit flashing behaviour, fast gill movements, signs of distress, anorexia and a tendency to lie at the bottom. Moreover, they had a dark colouration, fragile skin and excessive mucous production. The peak of infection was registered in spring, reaching 90% of prevalence, although the mass mortalities only occurred in July. Formalin, hydrogen peroxide and copper sulphate baths are among the most commonly used intervention methods (Seoud et al., 2017; Rigos et al., 2023).

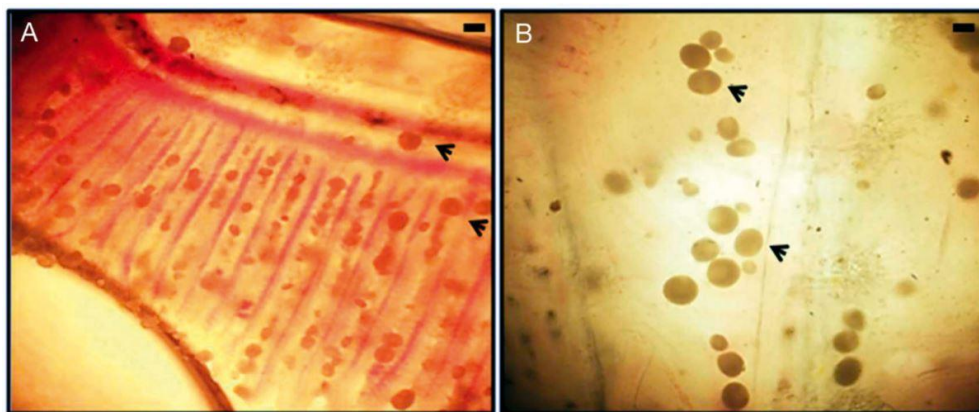


Fig. 19– Smears from European sea bass (A) gill and (B) skin showing trophonts of *Amyloodinium ocellatum* (arrowheads). Scale bars = 300 μ m (Bessat & Fadel, 2018)

Monogeneans are parasitic flatworms, belonging to the phylum Platyhelminthes. They are usually found on the skin, buccal cavity and gills of fish, although a small number of species are endoparasites. These parasites are characterised by the opisthaptor, an attaching organ equipped with hooks and other structures, whose arrangement and number vary and are essential to identify species. They have high host specificity,

typically infecting only a single host during their life cycle. While no monogenean is known to be zoonotic, the impacts on reared fish can be devastating (Paladini et al., 2017; Madsen & Stauffer, 2024). For example, *Diplectanum aequans* (Figure 20) is a monogenean parasite found on the gills of *D. labrax*, with annual mortality rates of 5 to 10% reported in juveniles in Italy (Dezfuli et al., 2007; Aly et al., 2024). Contaminated fish appear emaciated, with discoloured gills and skin, lethargic and with a tendency to swim near the surface. Excessive mucous production can also be observed on the gills that may also appear congested and pale. Histologically, it is possible to observe the destruction of the secondary lamellae due to the opisthaptor penetrating into the basement membrane and connective tissue of the primary lamellae. Intra-tissue haemorrhages can also be observed in the kidney, liver, intestine and spleen, due to degenerative lesions (Aly et al., 2024). Yardimci & Pekmezci (2012) reported an outbreak of a co-infection of *Lernanthropus kroyeri* and *D. aequans* in *D. labrax*, with both parasites attaining 100% prevalence. The combined gill damage caused by both parasites has caused a daily mortality rate of 1.5% of total production. Trichlorfon and rafoxanide administered via feed or bath are some of the most commonly utilised chemotherapeutants (Toksen et al., 2010; Toksen et al., 2012; Rigos et al., 2023).



Fig. 20– Whole body of *Diplectanum aequans*. Scale bar= 500 μm (Aydin & Pekmezci, 2023)

Copepod parasites are a threat to several reared species, including *D. labrax*. *Lernanthropus kroyeri* is a crustacean parasite that is extremely problematic for European sea bass, commonly found attached to the gill filaments. Their most destructive activity is their feeding habits, as they feed on the mucous, blood and

epithelial tissue of the host. Their most prominent pathological features are hypertrophy, branchial circulation occlusion, and damage due to pressure feeding. In histological examinations it is possible to observe lesions due to compression by the parasite (compression atrophy of the hemibranch adductor muscle and lumen reduction), exposed blood vessels, destruction of the secondary lamellae (necrosis, erosion, desquamation), exposure of the primary lamellae cartilage due to erosion, mucous cell proliferation and lamellar fusion (Manera & Dezfali, 2003; Toksen, 2007; Toksen et al., 2010). Toksen (2007) conducted a study in which an outbreak with 100% prevalence (29 to 77 parasites per fish) in a marine farm resulted in daily mortality rates of about 3% of total production. The most common intervention method against copepod parasites is the use of emamectin benzoate through feed (Toksen et al., 2010; Rigos et al., 2023).

Ceratothoa oestroides (Figure 21) is an isopod parasite that infects a wide number of fish and represents a threat to the sustainability of *D. labrax* aquaculture, particularly in the northeastern Mediterranean. They usually attach themselves to the buccal cavity in pairs, where they later sexually differentiate and reproduce, releasing hundreds of free-swimming manca. Younger fish are more affected, with mortalities occurring due to anaemia and emaciation. Adults usually have lesions in the buccal area and, in severe cases, granulomatous lesions and severe ulcers that can cause blindness. In histological analysis, it is possible to observe a decrease in the number of mucous cells, hyperplasia of the squamous epithelium of the tongue and inflammatory cell infiltration. Mortality is not common in adults, although a growth reduction of around 20% impairs the sustainability of farmers (Piazzon et al., 2021). In a study realised by Mladineo (2002), it was noted that a *C. oestroides* infection with a prevalence of 27% had reduced growth by around 67% and resulted in the death of 10% of the stock. Mechanical obstruction of water flow impairing respiration, anorexia and emaciation due to anaemia were the causes of death. Diflubenzuron (chitin synthesis inhibitor) and deltamethrin baths are among the most used treatment methods (Rigos et al., 2023).



Fig. 21– Adult female *Ceratothoa oestroides* settled in the buccal cavity of European sea bass (adapted from Wikipedia)

1.4.2 Control of parasite diseases in aquaculture systems

Parasitic infections are responsible for substantial economic impacts, with mortalities in hatcheries that can reach 20% of world production and up to 10% of global grow-out production. Nevertheless, any attempt to quantify economic losses is restricted by the deficient data on impaired growth, therapeutic costs, total mortalities and other associated expenditures (Rigos et al., 2023). Farmers adopt different approaches to mitigate the impacts, but considering the tremendous adaptive capacity of parasites, no single strategy is expected to be effective on its own (Buchmann, 2022).

Using chemotherapeutants is normally the first employed method to control a parasitic outbreak, usually derived from historical use in agriculture or veterinary (Shinn & Bron, 2012; Paladini et al., 2017; Buchmann, 2022; Rigos et al., 2023). The development of compounds intended exclusively for aquaculture is rare; nevertheless, considerable effort and investment have been directed towards developing effective chemicals to treat copepod and monogenean infections. The lack of new compounds is a

consequence of the limited returns from aquaculture-exclusive chemotherapeutants, especially when compared to those developed for human and mammalian health (Paladini et al., 2017). Additionally, regulations on antiparasitic chemicals due to environmental and health concerns, together with the increasing resistance of parasites to available drugs, restrict the efficacious treatment options (Shinn & Bron, 2012; Rigos et al., 2023).

One of the most employed methods is bath treatments, commonly used to treat ectoparasitic infections. In land based farms it is simple to apply, as the chemotherapeutants can be directly administered in the rearing tank or in a distinct tank or container (Rigos et al., 2023). On the contrary, applying bath treatments in cage aquaculture is more challenging, often involving complex logistics and requiring a substantial volume of the compound, translating into high costs and potential environmental contamination (Shinn & Bronn, 2012; Rigos et al., 2023). Additionally, calculating the adequate dose can be difficult, especially when strong water currents are present, which can lead to under- or overdosing (Shinn & Bron, 2012). Formalin is one of the most used chemicals in bath treatments, being the most used chemotherapeutant to control ectoparasitic infections in Mediterranean aquaculture. It is also commonly used to control the proliferation of protozoans in hatcheries (Buchamm, 2022; Rigos et al., 2023).

Administering drugs in feed is simpler, quicker, and more cost-effective when compared with bath treatments. As such, in-feed treatments are favoured when the choice is available (Buchmann, 2022; Rigos et al., 2023). This method is usually employed to control endoparasites, although some can also be effective in controlling ectoparasitic infections. Nevertheless, the efficacy of the chemical can be reduced due to reduced palatability. The deposition of chemotherapeutants in non-target tissues, along with the potential delays in manufacturing medicated feed in feed mills, are also issues worth considering when selecting the most adequate treatment (Rigos et al., 2023). One of the most commonly employed in-feed treatments is praziquantel, a promising anthelmintic drug with proven efficacy in controlling ecto- and endoparasites in humans. Its use in aquaculture is usually directed at Platyhelminthes, although it can also be used to control several other parasites. The effectiveness can be hindered by palatability problems, although the inclusion of masking agents can alleviate the

problem. It can also be supplied in bath treatment (Buchmann, 2022; Rigos et al., 2023).

Physical measures can also be used to control or to prevent parasitic infections, mostly through the installation of structures that restrict the entrance of alien species to the system. For example, by installing egg traps in which the parasite attaches the egg cluster that can posteriorly be removed, mechanical filters that remove free-living stages of certain parasites, or nets and bird scarers to avoid their entry into ponds, thus limiting the deposition of certain parasite life stages by defecation (Paladini et al., 2017; Buchmann, 2022). Ectoparasites can also be removed through mechanical means, and although stressful to the fish, it is an effective control method that is used to remove sea lice from salmon (*Salmo salar*) (Buchmann, 2022).

Ectoparasitic infections can also be controlled through biological methods. For example, in salmon aquaculture, where cleaner fish, namely lumpfish (*Cyclopterus lumpus*) and ballan wrasse (*Labrus bergylla*) (Figure 22), are employed to control sea lice infestations (Paladini et al., 2017; Brooker et al., 2018; Buchmann, 2022). These fish present opportunistic feeding habits, which facilitate a symbiotic relationship with infected salmon. Nevertheless, to ensure their effectiveness, it is crucial to promptly remove mortalities and control biofouling, as these factors may impair the cleaner fish's delousing process by providing alternative food sources (Brooker et al., 2018). Additionally, the enormous adaptive capacity of sea lice has led to the selection of lice with reduced pigmentation, making them less visible to the cleaner fish and consequentially decreasing the effectiveness of this approach (Buchmann, 2022). The use of blue mussels (*Mytilus edulis*) for water filtration and the capture of free-living parasite larval stages is another example of biological control (Buchmann, 2022).



Fig. 22– Cleaner fish *Cyclopterus lumpus* (left) and *Labrus bergylta* (right) (adapted from biodiversity4all)

Immunological control can also be employed to prevent, or at least mitigate, the potentially devastating impacts of a parasitic infection, usually through the incorporation of immunostimulants in feed (Paladini et al., 2017; Farooqi & Qureshi, 2018; Buchmann, 2022). Their origin can be extracts of animals or plants, bacterial, chemical, cytokines or polysaccharides. By interacting with and activating immune cells, immunostimulants enhance the fish's immune response. Although having a short-term response due to the absence of a memory component in the immune response, it remains an effective strategy for enhancing disease resistance in fish (Farooqi & Qureshi, 2018).

Parasites have an enormous adaptive capacity, which can turn effective treatments obsolete in the span of a few years (Buchmann, 2022). As such, it is crucial to apply integrated control strategies, with careful planning and consideration of the most effective method to apply, and employ several different control measures (Shinn & Bronn, 2012; Buchmann, 2022).

It is essential to identify the specific life stage of the parasite causing the infection, guaranteeing that the adequate treatment is administered, so as not to worsen the development of the pathology. It is also crucial to select an adequate method, ensuring that the chosen strategy, timing, and dosage, avoiding under-dosing which can contribute to resistance development, as well as overdosing, which may be toxic to both the fish and the environment (Shinn & Bronn, 2012; Paladini et al., 2017; Buchmann, 2022). Alternating between multiple treatments is likewise fundamental, as it can delay resistance development (Shinn & Bronn, 2012; Buchmann, 2022).

1.5 Sea Lice

Sea lice are parasitic copepods that feed on the blood, mucous and skin of their hosts. They are capable of impairing the welfare of their hosts, especially when present in large numbers. These parasites are responsible for huge economic losses, particularly in salmonid aquaculture. They have a high reproductive capacity, releasing a massive amount of free-swimming larvae, which allows not only the fast spread among cultured fish but also poses a threat to environmental sustainability by infecting wild fish (Parsons et al., 2025).

The impacts of a sea lice outbreak include diminished fecundity and growth, ulcerations, osmoregulatory failure, skin erosion and other lesions associated with their feeding and attaching habits. These effects lead to increased susceptibility to secondary infections, anaemia, osmotic imbalance, and to elevated stress levels in fish (Boxaspen, 2002; Zhang et al., 2023). Mortalities directly caused by sea lice alone are uncommon, usually occurring exclusively in extreme cases. Nevertheless, the associated secondary health impacts may lead to higher mortality rates. Moreover, the reduced feed conversion and stunted growth severely affect the economic sustainability of farmers. In salmon, outbreaks can lead to a growth reduction of 200 grams per fish, potentially resulting in losses of up to 13% of the farm's total revenue (Abolofia & Wilen, 2017).

Antiparasitic chemicals are the primary strategy employed to control a sea lice outbreak, the majority of which were initially applied in Norwegian aquaculture to control sea lice infestations in Atlantic salmon (*Salmo salar*) (Rigos et al., 2023; Parsons et al., 2025). In the EU, especially in Mediterranean aquaculture, parasitic copepods, such as *Lernanthropus kroyeri*, are responsible for significant damage, particularly in *D. labrax* aquaculture. Since almost no EU country produces salmonids, farmers face a scarcity of licensed compounds. Consequentially, they must rely on the few approved drugs or on the use of “off-label” chemicals, always under the responsibility of the prescriber (Rigos et al., 2023).

Chemotherapeutants to combat sea lice can be administered through medicated feed or bath treatments, directly in the rearing tank or cage, or using well-boats. Some of the most used compounds are the disinfectant hydrogen peroxide, the organophosphate azamethiphos, the synthetic pyrethroid insecticide deltamethrin and the neonicotinoid insecticide imidacloprid, which can be administered via bath. The ivermectin emamectin benzoate and the benzoylphenyl urea insecticides diflubenzuron and teflubenzuron, are applied as in-feed treatments (Rigos et al., 2023; Parsons et al., 2025).

Farmers can also apply non-chemical strategies. For example, thermal treatment surged in Norwegian aquaculture in 2016 and in 2019 already accounted for 60% of employed control methods for adult sea lice. The efficiency is high, and the treatment usually involves the exposure of fish to up to 36°C water for 30 seconds. Another strategy involves the use of mechanical methods, where fish pass through automated systems where the sea lice are removed through the use of turbulence, brushes or pressurised water. As sea lice are sensitive to low salinities, freshwater baths can also be employed. Biological control is also widely employed in Norway, with almost 60 million cleaner fish (namely *Cyclopterus lumpus* and several Labridae family members) currently employed in aquaculture systems globally (Coates et al., 2021).

Preventive measures are also applied as a way to not only avoid outbreaks but also to prevent the need for drugs, which can increase farming expenses and may be dangerous for the environment. While none of these methods target attached sea lice, they can greatly reduce the rate of new infestations. These strategies can either affect the survival and attachment rates of copepodids or reduce the probability that infective free-living stages encounter a host. Methods aimed at reducing the survival of early parasite stages include selective breeding of fish for enhanced resistance, functional feeds that stimulate skin immune response, and vaccines, although only one partially effective vaccine is available for sea lice in Chile. Strategies designed to prevent encounters between parasite and host include the promotion of deep swimming through deep feeding and lights, incapacitation of sea lice through direct current electricity, ultrasonic cavitation or short-wavelength light, and employing traps, filters, or barriers such as skirts that surround the superficial layer of the cage where copepodids tend to concentrate (Boxaspen, 2006).

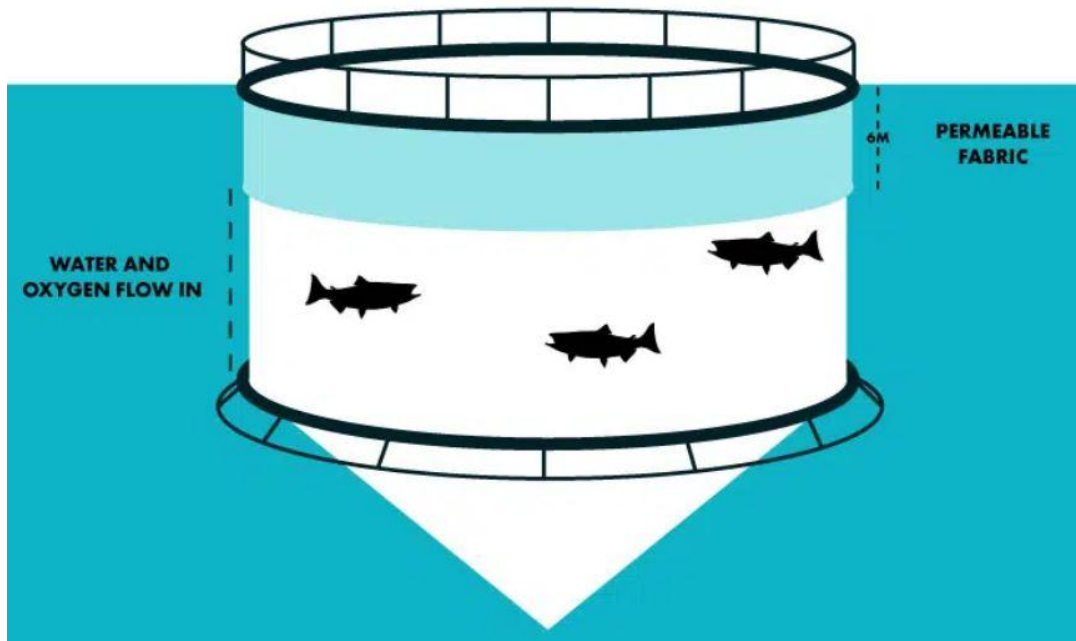


Fig. 23- Scheme of a skirt used in cage aquaculture to avoid encounters between fish and copepod parasites (adapted from industrial.cunninghamcovers.co.uk)

The reduced number of effective drugs, particularly outside of Norway, has pushed farmers to employ the same chemotherapeutants numerous times, which consequentially increased selection pressure, thus facilitating resistance development in sea lice (Figure 24) (Aaen et al., 2015; Coates et al., 2020). This problem was further aggravated by inadequate treatment procedures, such as poorly adjusted protocols and suboptimal drug dispersal (Aaen et al., 2015; Rigos et al., 2023). For example, azamethiphos has seen its efficacy drop to levels as low as 7% in some farms, just two years after its introduction (Coates et al., 2020). The problem of resistance is not exclusive to chemical approaches, as successive employment of any method can impose a strong selection. For example, employing thermal control strategies can lead to the selection of phenotypes with higher thermal tolerance, or the use of barriers possibly leading to the selection of individuals with deeper swimming preferences. Nevertheless, while chemical resistance can be developed exclusively through mutation, non-chemical resistance requires complex behavioural, physiological and anatomical changes (Barret et al., 2020; Coates et al., 2020). Farmers are advised to employ different strategies simultaneously to avoid directional selection, while also

implementing periodic delousing procedures to prevent genetic proliferation and to selectively breed fish with a greater genetic resistance to sea lice as a way to mitigate the development of resistance (Aen et al., 2015; Coates et al., 2020). Additionally, when planning a farm, location is crucial to ensure that sufficient distance separates prior existing farms, so as to not only prevent the spread of resistant strains between farms but also to reduce reinfestation rates (Barret et al., 2020).

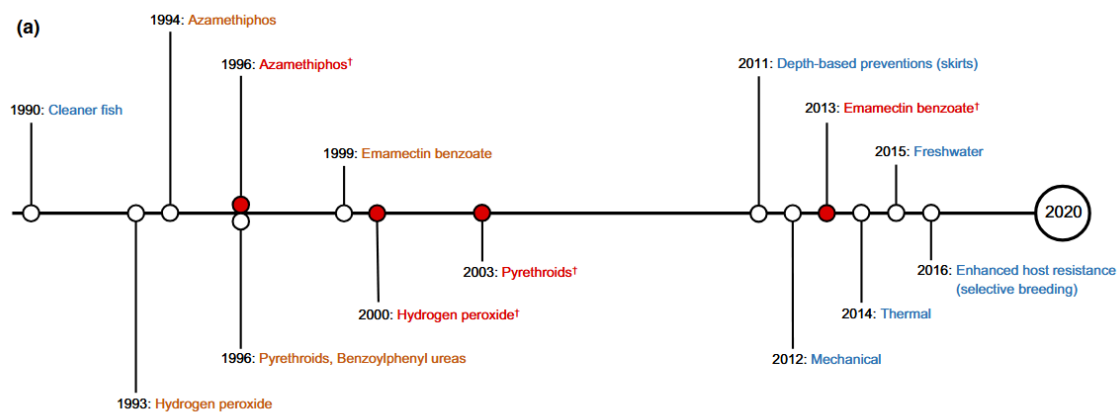


Fig. 24— Year of introduction of non-chemical (blue) and chemical (orange) louse controlling strategies, and first report of resistance (red) (Coates et al., 2020)

1.5.1 *Lernanthropus kroyeri*

Lernanthropus kroyeri (Figure 25) is a copepod ectoparasite commonly found infecting European sea bass (*Dicentrarchus labrax*) in aquaculture systems (Manera & Dezfuli, 2003; Toksen et al., 2008). The *Lernanthropus* genus is the most widely distributed and the most numerous in species in its family, Lernanthropidae (Toksen et al., 2008). Like other species of this genus, it attaches to the gill filament of its host through the piercing action of its second antennae, reinforced by their third pair of legs (Manera & Dezfuli, 2003; Toksen et al., 2008; Zayed et al., 2023). Its distribution reaches several areas of the globe, being reported in the Mediterranean Sea, Adriatic Sea, North Sea,

the coast of Africa and numerous Asian countries (Toksen et al., 2008; Vinoth et al., 2010).



Fig. 25- Male (up) and female (down) of *Lernanthropus kroyeri* (photo by author, 2025)

1.5.1.1 Taxonomy

Kingdom: Animalia

Phylum: Arthropoda

Subphylum: Crustacea

Superclass: Multicrustacea

Class: Copepoda

Infraclass: Neocopepoda

Superorder: Podoplea

Order: Siphonostomatoida

Family: *Lernanthropidae*

Genus: *Lernanthropus*

Species: *Lernanthropus kroyeri* (WORMS, 2025)

1.5.1.2 Occurrence in wild and reared species

Although some species of its genus are strictly host specific, *L. kroyeri* can infect a wide range of hosts. Nevertheless, this copepod is exceptionally well adapted to parasitising the gills of sea bass. In Vellar estuary, India, Vinoth et al. (2010) conducted a study where it was noted that this parasite was infecting the gills of Asian sea bass (*Lates calcarifer*), showing a prevalence of 20% with a mean intensity of 20. It was also noted that *L. kroyeri* inflicted serious gill damage to these fish, and it was considered as a potential fatal threat for the host species (Khidr et al., 2014).

In the Suez Canal in Egypt, Qoorani et al. (2020) found that this copepod was infecting both wild spotted sea bass (*Dicentrarchus punctatus*) and *D. labrax*. The former presented a prevalence rate of 67%, while the latter had a 57% prevalence. The prevalence in *D. punctatus* was lower in spring (57.89%) and higher in summer (70.97%), while in *D. labrax* it was lower in spring (51.85%) and higher in winter (88.24%). Zayed et al. (2023) conducted a study on the Alexandria coast, Egypt, where it was noted that wild European sea bass had a prevalence of this parasite of around 29%, with a mean intensity of 3.8. The highest prevalence was recorded in the summer.

A study realised by Manera et al. (2003) in Greece reported an infection of this copepod on 2-year-old farmed European sea bass, with prevalence levels of around 35% and a mean intensity of 10.86. The intensity of the outbreak was more severe between February and June, mainly due to the recruitment of this parasite to the host population. Antonelli et al. (2012) conducted another study in Corsica, France, where an outbreak occurred during all years with a peak of severity in summer. The prevalence was between 30 and 90%, and the mean intensity ranged from 0.7 to 9.3. In another study by Toksen (2007), an outbreak of *L. kroyeri* was also reported in a

Turkish European sea bass farm. The infection was severe, with a prevalence of 100% and a mean intensity of 50.3, leading to a daily mortality rate of 3% of total production.

The abundance of this copepod on the Adriatic Sea fish farms shows no significant seasonal differences. Nevertheless, females, which represent the majority of the population during the year, show seasonal dynamic patterns. Non-gravid females dominate during colder seasons, while gravid females represent the majority of the population during warmer months. Consequentially, immature parasites are present in a much lower percentage during colder months (Čolak et al., 2021).

Although the prevalence varied between wild and cultured fish, the intensity has a tendency to be higher in reared fish. This is due to typical environmental modifications perpetuated by aquaculture systems, where elevated densities and practices that often deteriorate water quality tend to favour the spreading of parasitic outbreaks (Madsen & Stauffer, 2024). Moreover, the absence of an intermediate host and their enormous reproductive capacity facilitate the quick proliferation and establishment in aquaculture systems. The highest prevalence levels were always reported in summer, which indicates that this copepod presents an annual life cycle and is well adapted to higher temperatures. Additionally, the transition of temperature from winter to summer may also affect the immune response of fish, which may translate into higher infection rates (Antonelli et al., 2012).

1.5.1.3 Life cycle

The developmental cycle of *Lernanthropus kroyeri* consists of two free-swimming naupliar phases, followed by the infective copepodite, four attached copepodite stages in which the parasite fixes to the gill filaments of the host, then a pre-adult stage and finally the adult. Subtle sexual differences can already be noticed from the very first copepodite phase, although they are difficult to detect (Cabral et al., 1984).

In the nauplius I stage (Figure 26), the organism shows a rounded body shape with a pair of stabilising at the posterior end. The antennule carries two short smooth setae

together with two feathered apical ones in its terminal segment. The antenna is biramous, with a two-segmented endopod; the apical part bears two long setae, a smaller apical seta, and one median smooth seta. The mandible also appears biramous. At this stage, body size is usually in the range of 160–180 μm (Cabral et al., 1984)

In the nauplius II phase (Figure 26), the parasite's body becomes more elongated and oval compared with the earlier form. The antennule now bears three short lateral setae together with one short apical seta, while both the antenna and mandible remain similar to those described for nauplius I. Body length at this stage ranges between 240 and 320 μm (Cabral et al., 1984).

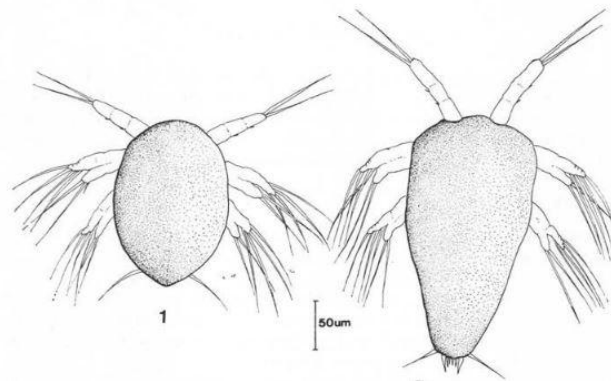


Fig. 26 - Nauplius I (left) and Nauplius II of *L. kroyeri*

When the infective copepodite stage is reached, the body continues to elongate and the cephalothorax appears oval in shape. At this point, the antenna becomes disproportionately large in relation to the body and is the main organ of attachment to the host gill filament. It ends with a delicate hook supported by two strong spines. The mandible is armed with eight equal teeth. This is the last planktonic stage, and individuals typically measure 320–380 μm (Cabral et al., 1984).

In the fixed copepodite stages (Figure 27), important changes occur to allow a sedentary existence. These are most obvious in the first and second pairs of thoracic legs, where the setae decrease in both size and number. Sexual dimorphism can be

noted in the third thoracic legs, which take on a lamellar form. In females the inner edge is smooth, whereas in males a shallow and continuous depression can be seen. At this stage, body size varies between 650 and 850 μm (Cabral et al., 1984).

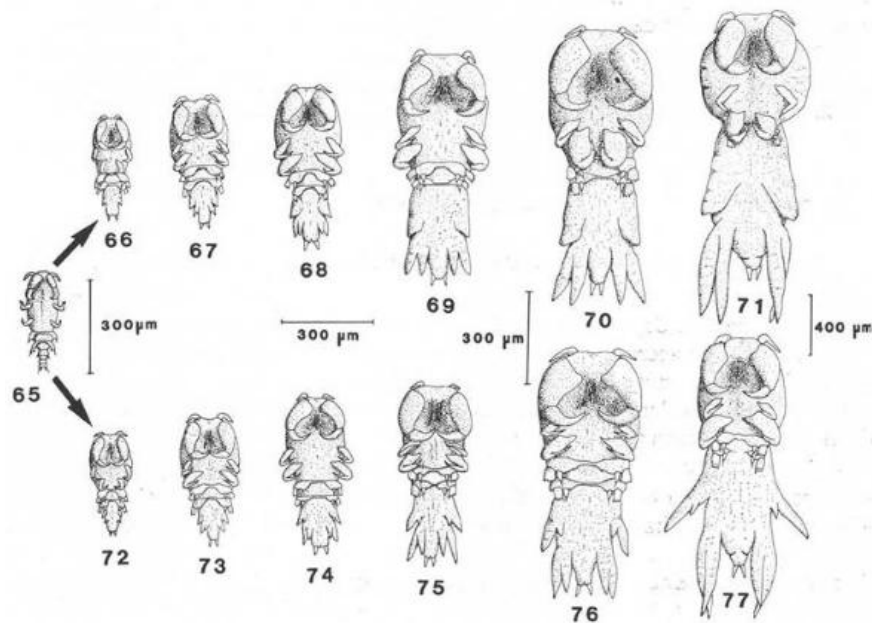


Fig. 27– Infective copepodid (left) and subsequent life stages of female (up) and male (down) of *L. kroyeri*

The pre-adult stage is marked by the formation of genital slits, which appear less developed in males. Males also lack both the third and fifth thoracic legs. Sizes vary, with males ranging from 820 to 1200 μm , while females reach 850–1390 μm (Cabral et al., 1984).

In adults, young females do not initially show the complete adult form, as the dorsal shield and cephalothorax are still in development and the third thoracic legs have not yet taken their characteristic curve. These features are only completed once the parasite shifts from the external to the internal surface of the gill filaments, where the third legs and lateral lobes assume their definitive form. By contrast, immature males already show most adult features. Male appendages differ from those of females: the antenna has a more elongated hook and the second thoracic leg is strongly modified.

Adult females average about 1300 µm in length, while males are slightly smaller, around 1100 µm (Cabral et al., 1984).

The adult cephalothorax bears five pairs of cephalic appendages (mandibles, maxillae, maxillules, antennae, and antennules), together with three pairs of thoracic appendages (two pairs of legs plus the maxillipeds). The trunk carries the caudal rami, the genito-abdominal complex, and either two or three pairs of legs depending on sex. Adult males measure between 1.5 and 2.4 mm, while females grow larger, between 3.3 and 4.5 mm. (Toksen et al., 2008; Antonelli et al., 2012).

Males: the second leg is shorter than that of the female and is structurally different, with the endopod bearing several spines. The third leg is biramous, lanceolate, and fused at its base, whereas the fifth leg is absent. Uropods are narrow at the base and end with two terminal and two dorsally placed setules. These specialisations relate to the reproductive role of males, enabling them to grasp females, locate the genital complex, and manipulate spermatophores (Zaid et al., 2022).

Females: the second leg is biramous with a cylindrical endopod, while the third leg is large, uniramous, non-segmented, slightly folded along its axis, curved, and elongated. The fifth leg is reduced to a vestige. The abdomen carries dorsolateral genital openings, and the uropods, which have two proximal and terminal setae dorsally, are located at the posterior end. The anus is posterodorsally positioned. Females are easily recognised by the presence of two conspicuous egg sacs, visible to the naked eye (Zaid et al., 2022).

1.5.1.4 Fish infection and Pathological features

When the infective copepodid attaches itself to the gill filament, it causes occlusion of the branchial circulation and hypertrophy. Due to its feeding habits, it also causes the destruction of the surrounding tissue. As larger fish have a wider gill surface, they are more susceptible to being infected by *L. kroyeri*, as they can provide more food and more suitable attachment sites. Moreover, fish length and *L. kroyeri* egg string length

appear to be positively correlated, indicating a higher reproductive potential for this parasite in older fish (Toksem, 2007; Antonelli et al., 2012; Čolak et al., 2021).

Infected fish often appear emaciated, with a reduced condition factor. They show lethargic behaviour, a tendency to swim near the surface, respiratory distress, and dark-coloured skin (Manera & Dezfuli, 2003; Toksen et al., 2008; Zaid et al., 2022; Lattos et al., 2024).

Visible symptoms include excessive mucous production and haemorrhagic areas in several parts of the body. The gills may have congested and anaemic areas, which confer a marbled appearance, and the filaments may be haemorrhagic, stunted, disorganised or bent. The cephalic extremity of the parasite induces compression of the branchial afferent artery, which may result in lumen reduction, as well as in the hemibranch adductor muscle, causing compression atrophy (Manera & Dezfuli, 2003; Toksen et al., 2008; Zaid et al., 2022; Lattos et al., 2024).

In histological analysis it may be possible to observe necrosis, desquamation, erosion, and fusion of the secondary lamellae. Hypertrophy and hyperplasia of the second lamellar epithelium are also possible to observe, and in extreme cases it can be partially or totally destroyed, leaving the primary filaments denuded. It is also possible to note erosion of the gill's raker covering epithelium and of the primary lamellae. Due to erosion the blood vessels and lamellae cartilage may also be observed. Other possible histopathological symptoms include necrosis of the muscle, hyalinisation, vacuolar degeneration, and telangiectasis of the arches and filaments of the gills' blood vessels (Manera & Dezfuli, 2003; Toksen et al., 2007; Zaid et al., 2022; Lattos et al., 2024).

1.5.1.5 Treatment strategies

Emamectin benzoate, an ivermectin, is the main control method employed to control an *L. kroyeri* outbreak. It is administered orally through feed. In a study conducted by Toksen et al. (2006), it was noted that applying this chemotherapeutant at a

concentration of 100 µg kg⁻¹ was efficient against all life stages of *L. kroyeri*. The treatment resulted in a reduction of 74% of parasitic load, with no larvae detected after 14 days.

Another treatment option is trichlorfon, the organophosphate acetylcholinesterase inhibitor. Toksen et al. (2010) realised a study where European sea bass infected with *L. kroyeri* were treated with trichlorfon-coated pellets at a concentration of 50 mg kg⁻¹ for 7 days. This compound was shown to be efficient, with a reduction in *L. kroyeri* abundance of 97.1%.

Teflubenzuron is a chitin synthesis inhibitor that was also tested for the control of *L. kroyeri* infections. As this type of chemotherapeutant only affects moulting stages, it is not effective for killing adult parasites. Nevertheless, it is expected that this drug impairs larval stages, which may lead to a gradual reduction, or at least stabilisation, of the number of adult parasites (Toksen et al., 2009).

1.5.2 *Caligus* sp.

Belonging to the genus *Caligus* (Figure 28), these ectoparasitic copepods are predominantly associated with marine environments, but some species can be found in fresh and brackish water, with their distribution reaching the entire world (Hemmingsen et al., 2020; Tavares-Dias & Oliveira, 2023). Its genus is the most numerous in species, with their members usually feeding on the blood, mucous and skin of their hosts. Most of the species in this genus are host-specific, although some have the capacity to infect a wide range of hosts (Tavares-Dias & Oliveira, 2023).



Fig. 28– *Caligus* sp. (Natural history of Orange county website, 2025)

1.5.2.1 Taxonomy

Kingdom: Animalia

Phylum: Arthropoda

Subphylum: Crustacea

Superclass: Multicrustacea

Class: Copepoda

Infraclass: Neocopepoda

Superorder: Podoplea

Order: Siphonostomatoida family: *Caligidae*

Genus: *Caligus* (WORMS, 2025)

1.5.2.2 Occurrence in wild species and reared species

The diverse species in this genus display distinct habits. The adults have the capacity to swim freely, which enhances their infective potential in contrast with other copepod parasites that rely on the copepodid free-living stage to find a suitable host. Therefore, these parasites not only thrive in the characteristically high rearing densities of

aquaculture systems, but they also represent a bigger threat to wild animals, especially where there is abundant fish fauna (Heuch et al., 2007).

An example of a generalist species in this genus is *Caligus elongatus*, which is known to infect a wide range of pelagic and benthic species in the North Sea. Heuch et al. (2007) conducted a study on the Norwegian coast where it was found that 21 out of 27 caught species were infected with this parasite. The most infected species was the lumpfish *Cyclopterus lumpus*, with a prevalence of 84% when captured in the North Sea and 61% when caught on the coast, with a mean intensity of 8 and 4, respectively. The Gadidae family also acts as an important reservoir of this copepod, as 5 out of the 15 species with the highest prevalence belong to this family, although the mean intensity was lower than in lumpfish. The highest prevalence was noted in September, coinciding with the beginning of the colder season.

Culurgioni et al. (2010) conducted a study in a Mediterranean lagoon in St Gilla, Italy, where it was noted that wild sea bass were infected with *Caligus minimus* with a prevalence of 20% and a mean intensity of 4.2. In Chile, Sepúlveda et al. (2004) noted that wild *E. maclovinus* were infected with *C. rogercresseyi*, presenting a prevalence of 73% and a mean intensity of 2.66. Initially this fish acted as a vector of this parasite to farmed salmonids (which infection had a prevalence of 70% and a mean intensity of 5.8) in the Puerto Montt channels and fjords. However, in the fall, *E. maclovinus* migrates to rivers and estuaries where the infection subsides, and later, when they return to the sea in spring, it is the salmonids that serve as a reinfection source.

In reared fish, Yalim et al. (2015) noted an infestation of *C. minimus* in Beymelek Lagoon, Turkey, where infected European sea bass showed a 30% prevalence and a mean intensity of 6.22. In a Greek lagoon, Ragias et al. (2004) reported an outbreak of *C. pageti*, *C. apodus*, *C. mugils*, and *C. minimus* in reared *D. labrax*. The most abundant species was *C. minimus*, which presented an intensity of 21.14, with the peak of the infection noted in winter.

In the Suez Canal, in Egypt, Abdelsalam et al. (2025) conducted a study on reared grey mullet (*Mugil cephalus*) and European sea bass infected with *C. clemensis*. For grey mullet the peak of prevalence and intensity was in spring (80% and 20,

respectively), which is in agreement with Abouelhassan et al. (2022), who reported that reared Mugilid fish had an infection peak in spring (17.3%). For sea bass, the peak prevalence and intensity were noted in winter (75% and 17.5, respectively), corroborating the study of Heuch et al. (2003), who noted that the peak of infestation was in January for reared *D. labrax*. This is mainly due to increased adult longevity associated with lower temperatures and the elevated numbers of mobile lice.

Although the prevalence of infection was variable between reared and wild fish, the intensity was always higher in cultured fish, mainly due to the elevated densities and degraded water quality, which favour the spread of parasitic infections (Madsen & Stauffer, 2024). The peak of the infestations was invariably registered in winter for both reared and wild fish. This is due to several factors, including that members of the Caligidae family have longer lifespans at lower temperatures, and they continue to produce eggs through winter. Control methods also have reduced efficacy during colder months (Abdelsalam et al., 2025). The fish immune system is also compromised at lower temperatures, which may allow the infection to spread faster (Abram et al., 2017). Moreover, wild European sea bass tend to migrate to wintering basins with weak currents during colder months, reducing their swimming activity and facilitating the transfer of parasites between hosts (Ragias et al., 2004).

1.5.2.3 Life cycle

Members of the genus *Caligus* usually pass through eight main stages during their development: two nauplii, one infective copepodid, four chalimus phases and finally the adult form. Once the nauplii hatch they still look somewhat similar to the small cylindrical egg they came from, although their body quickly becomes more elongated and a bit oval. At this point they have three pairs of swimming appendages (antennae, antennules and mandibles), which they use to move in the water. This stage is light-attracted and the size normally does not exceed about 500 µm (Nguyen et al., 2019; Hemmingsen et al., 2020).

The infective copepodid stage that follows is shaped in a more hydrodynamic way, better suited for active swimming compared with the earlier form. In this phase they already show ten pairs of appendages: the antennules, antennae, mandibles, maxillules, maxillae, maxillipeds, postantennary processes and also three pairs of thoracic legs. Like the nauplii, copepodids are also drawn to light, and they tend to remain under 1000 µm long (Nguyen et al., 2019; Hemmingsen et al., 2020).

When they come across a suitable host, attachment first occurs with the help of the antennae and maxillae, and afterwards a frontal filament is extruded. This filament pierces the skin surface and fixes into the basement membrane close to the scales, which allows the parasite to hold on while it moults and changes into the first chalimus stage (Nguyen et al., 2019; Hemmingsen et al., 2020)."

Chalimus stages are characterised by a shift in attachment strategy. The antennae, once the main anchoring organs, regress, and the larva remains fixed to the host by the frontal filament emerging from the cephalic region. Because the filament is short, the parasite's movement is limited to a confined area. Although chalimus I–III differ in segmentation, leg morphology, and size, they share several common traits: an overall elliptical form, an unsegmented prosome, and a metasome with only weak segmentation. From chalimus III onwards, sex can be distinguished based on appendage morphology and the number of abdominal segments. Chalimus IV displays dorsoventral flattening, a larger cephalothorax, and the development of a ventral structure known as the sternal furca (Nguyen et al., 2019; Hemmingsen et al., 2020).

Young and mature adults are morphologically similar, aside from the relative size of the genital complex. Juveniles remain attached for a short time, but while both sexes mature simultaneously, males detach earlier. According to Nguyen et al. (2019), dead males were often observed at the bottom of experimental containers after mating, suggesting a post-copulatory mortality pattern.

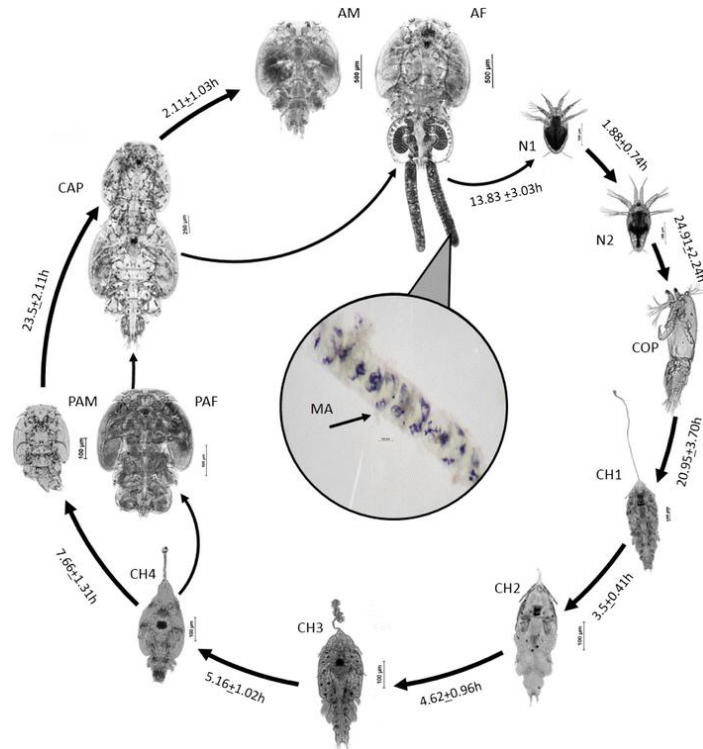


Fig. 29– *Caligus minimus* life cycle at 30°C (Nguyen et al., 2019)

The adult body is divided into four tagmata: the cephalothorax, the abdomen, the genital complex, and the somite carrying the fourth leg. The cephalothorax itself consists of the cephalon and the somites that bear the maxilliped and the first three pairs of legs. On its ventral side are found the antennules, antennae, maxillipeds, maxillules, maxillae, postantennary processes, and three thoracic leg pairs (Hemmingsen et al., 2020).

A convex carapace covers the cephalothorax and bears a valve-like flap, which closes when suction is produced, thereby improving adhesion by preventing water from passing between the carapace and the host. Two anterolateral lunules, shaped like small circular cups, provide additional attachment capacity, while the sternal furca functions as both a brace and a lever, enabling the cephalothorax to be raised, reducing pressure, and enhancing suction. The combined action of maxillipeds and antennae reinforces attachment. These features give the parasite the ability to resist strong currents, cling to smooth surfaces, and still retain mobility (Hemmingsen et al., 2020).

The oral region is modified into a tubular feeding cone composed of the labrum and labium, which overlap to form a siphon. Within it are a pair of mandibles. During feeding, the cone is pressed against the skin, exposing the strigil armed with sharp teeth, which scrape and lacerate epidermal tissues. The mandibles then collect the tissue fragments and pass them to the buccal cavity (Hemmingsen et al., 2020).

The clearest external marker of sexual dimorphism in adults is the female reproductive system. Mature females display a genital complex containing an oviduct channel and bear long, rod-like egg sacs with both developing and mature eggs, whereas males lack this structure (Hemmingsen et al., 2020).

1.5.2.4 Pathological features

The damage caused by movement and attachment of *Caligus* causes negligible damage to their host, with their feeding activity being solely responsible for the majority of the damage. To feed, they excavate a hole through the epidermis and into the basement membrane, which is lined with necrotic cells, in some cases causing hyperplasia of the surrounding excavated area (Hemmingsen et al., 2020).

Infected fish tend to increase their jumping activity and to rub against solid substrate, which results in stroke injuries. The fish also tend to swim near to the surface and near the water inlet, where it is possible to observe them gasping for atmospheric air and with accelerated bulging of the opercula. They also show a greater susceptibility to secondary infections, sluggish movements and reduced feeding activity (Hemmingsen et al., 2020; Tavares-Dias & Oliveira, 2023).

The visible symptoms include eye damage, lesions on the head and around the buccal cavity, skin irritation, frayed fins, excessive mucous production, opaque skin, ulceration, severe erosion, emaciation and a marked inflammatory response near the attachment site (C. Johnson et al., 2004; Hemmingsen et al., 2020).

1.5.2.5 Treatment strategies

The use of drugs administered through bath or feed is the main control method for *Caligus* outbreaks. Although costly, as in Chile the cost of *S. salar* production increases around 1.45 USD/kg when treatment is needed, it compensates the farmers with higher production levels (Tavares-Dias & Oliveira, 2023).

Emamectin benzoate, an ivermectin, administered through feed is the most commonly employed control method. In a study conducted by Hamre et al. (2011) in Norway, it was reported that an infestation of *C. curtus* in Atlantic cod (*Gadus morhua* L.) was controlled by administering emamectin benzoate in feed at a concentration of 50 µg kg⁻¹ during 7 days. The treated fish were free of *Caligus* even after 5 weeks, while the control fish had a mean intensity of 19.3 parasites per fish, which proves the efficacy of this drug.

Azamethiphos, an organophosphate, is also widely used to control *Caligus* infections through bath treatment, representing almost 80% of all sea lice treatments in Chile in 2018. In a study realised by Jimenèz et al. (2018), data was collected from salmon farms in Chile that were treating *Caligus* infections with azamethiphos. It was noted that this compound is extremely efficient at controlling adult parasites, reducing their numbers by 95%. Nevertheless, the efficacy for juvenile stages was lower, at 55%, with more than 95% of the farms reporting to find juveniles in treated pens.

Other employed compounds include the disinfectant hydrogen peroxide and the insecticides cypermethrin and deltamethrin, although their efficacy is lower than azamethiphos and emamectin benzoate (Tavares-Dias & Oliveira, 2023). For example, the survival of copepodids is hindered by applying hydrogen peroxide baths, but the main effect in adults is immobilisation. The adult parasites usually stay vulnerable for a maximum of 2 hours, after which they can re-infect the host, with females presenting a re-infestation ranging from 26.7% to 33.3% and the males from 57.9% to 66.6% (Marín et al., 2017).

2. Internship description

2.1 Fish farm description

Piscicultura do Vale da Lama (Figure 30) it's a farm located in the Ria do Alvor, in the Faro district. It was established in 1989, and initially specialized in the cultivation of European sea bass and gilthead sea bream. Today, the farm employs 10 people.

It is located about 800 metres from the sea, in an area free from organic pollution, not only due to the absence of contamination sources but also thanks to the presence of surrounding salt marshes and bivalves, which provide natural depuration of the environment. Although located near the Mediterranean, the area presents an Atlantic profile. Water temperatures range from 14°C and 24°C, with an average annual temperature of 18°C.



Fig. 30 – Earthen ponds from Piscicultura do Vale da Lama (aqualvor.pt, 2025)

The water supplying the tanks is seawater. However, during the last year, heavy rainfall led to a decrease in salinity levels. During periods of intense rain muddy freshwater flowed into the tanks, resulting in fish refusing to feed.

The farm has 9 earthen ponds: 6 are currently used for Gilthead sea bream production, 2 for European sea bass, and 1 is currently empty. It also has 2 pre-fattening indoor cement tanks. The outer ponds have an average depth of 2.5 metres, range between 0.4 and 0.7 hectares in size, and contain approximately 10 000 to 12 000 m³ of water. Each pond has a water inlet, drawing from the Ria do Alvor, and a water outlet, connecting to a draining ditch that flows parallel to the ponds.

To prevent escapes, a 10 x 10 mm mesh net is used when fingerlings are in the ponds. As the fish grow, the net is replaced with a 2 x 2 cm mesh and later with wider grids. Each pond has 4 automatic feeders, which are adjusted throughout the day in function of the level of feed acceptability. There is an electric control panel for every 2 tanks, managing feeders, pumps and probes. The farm also has 2 generators, guaranteeing the system is always operational even in the event of a power failure.

The production cycle initiates in the pre-fattening tanks, using fingerlings prevented from external hatcheries, weighing between 2g and 15 g. If an earthen pond is available, the fingerlings may be transferred directly to the outer tanks. Once the fish attain 30g to 60g, they are moved to an earthen pond. Around 100,000 individuals are transferred (mean density of 3 kg/m³), where they grow to a commercial size of 400g, with the cycle lasting 2 years. In some cases, sea bass batches are reared until 800g to 1000g in an extended production cycle of at least 3 years.

2.2 Copepod infestations in European sea bass

Parasitic copepods are a becoming threat not only to salmonid aquaculture but also to global aquaculture sustainability. *Lernanthropus kroyeri* is the most problematic crustacean parasite for reared *D. labrax*, as they attach to the gill filaments and cause serious pathological reactions. Their feeding habits also contribute to the destructive impact, as they feed on the blood, epithelial tissue and mucous of the host (Manera & Dezfuli, 2003; Toksen, 2007; Toksen et al., 2010).

Although the impacts of *Caligus sp.* on European sea bass are less severe, they can cause significant damage when present in high numbers. Their ability to swim freely even in the adult stages increases their infective potential, which presents additional risks to reared fish and the nearby wild populations (Heuch et al., 2007). The crescent number of parasitic infestations affects the expansion of aquaculture not only in Portugal but also globally. Even when they are not directly lethal to their hosts, they can cause significant damage by reducing growth rates, decreasing commercial value and increasing the risk of secondary infections (Timi & Buchmann, 2023).

Aquaculture companies face the challenge that most available antiparasitic chemotherapeutants have been developed for combating sea lice in salmonid producing countries. As a result, few of these compounds are accessible or approved for use in other parts of Europe. This issue is particularly problematic in the Mediterranean area, where salmonids are not produced, difficulting the licensing and use of these chemicals (Rigos et al., 2023).

The location of the host fish farm near Ria do Alvor offers some advantages but also exposes them to a higher risk of ectoparasitic infections. Fluctuations of the properties of aquatic environments, along with rising water temperatures driven by climate change, are known to increase parasite abundance. As these alterations also affect the fish immune response the number of parasitic infestations is expected to continue rising.

As a consequence, unusually high ectoparasitic loads were observed in European sea bass reared in the farm, with the prevalence of both *Caligus sp.* and *L. kroyeri* reaching 100%, which were hypothesised to be exacerbated by several factors:

- Use of untreated water from Ria do Alvor, which, although usually free of pollutants, can introduce larval mobile stages of parasites from external sources
- Proximity to other European sea bass aquaculture facilities that can act as a reservoir of parasites and increase the chances of transmission between farms
- Low water circulation in the earthen ponds, which can promote the accumulation of free-living parasitic stages and increase the chance of infecting and reinfesting the fish

These combined pressures, along with high host density characteristic of a semi intensive fish farm created favourable conditions for the establishment of ectoparasitic populations. Upon the start of the internship an infestation conditioned the European sea bass production, highlighting the need for investigation and intervention as soon as possible.

2.3 Objectives

The primary objective was to of this internship was to obtain practical knowledge and first-hand experience of the day-to-day management and health monitoring practices in a commercial aquaculture setting. To achieve this goal the intern was involved in the daily routines of the aquaculture. These activities included monitoring the number of ectoparasites in routine health check-ups, sampling ponds for the health and growth assessment, and daily observations of fish feeding behaviour and welfare.

During the time of my internship, the farm was facing an outbreak of ectoparasites, particularly *Caligus sp.* and *Lernanthropus kroyeri* in European sea bass. As such, the main objective of my work became the study and testing of several control strategies for ectoparasitic infestations. I was then integrated into the Fish Health and Welfare team, where I helped in the monitoring and management of parasitic infections and other diseases.

To achieve the main goal, specific objectives were determined. Those include the assessment of the infestation levels, the evaluation of the clinical and histopathological effects of high ectoparasitic loads on fish health, the investigation of control measures, particularly the adequate chemotherapeutant, and finally the evaluation of the efficacy of the chosen compound through a trial.

Ultimately, all tasks provided a foundation for achieving the overarching goal of improving the farm's long-term health by proposing preventive strategies, conducting environmental risk assessments, and evaluating treatment efficacy for ectoparasitic infections.

3. Experimental trial

3.1 Material and methods

3.1.1 Observation and monitoring of ectoparasites on the gills of European sea bass and its pathological effects

To achieve the internship's goal, a daily assessment of the general condition of reared *D. labrax* in the ponds was conducted. Although not quantifiable, the observation of visible symptoms in the ponds contributed to a better understanding of the impacts of a ectoparasitic infection, while providing insight into the progression of the clinical signs over time.

One hundred and six European sea bass were examined, with a mean weight of 307.4 ± 48.8 g and a mean length of 29.9 ± 2.2 cm. A total of 80 live individuals were examined under anaesthesia. The parasitic load was assessed by anaesthetising the fish in a 100 L container with clove oil at a concentration of 0.02 mg L^{-1} , guaranteeing that the container was equipped with an air pump to ensure oxygenation and ensuring fish welfare throughout the process.

The remaining 26 individuals were sampled during routine health check-ups, where 2 to 3 fish were randomly selected from each pond using a standard fishing rod. After being caught, the fish were euthanised in ice, taking care to avoid direct contact with it, which could alter parasite behaviour or damage the tissues needed for examination. Each fish was then measured and weighed, providing basic data to check for any link between size and parasite load or health status. A quick visual check followed, looking for external signs of lesions or deformities, which can be indicators of a disease, or render the fish unsuitable for sale.

Following this initial assessment, the parasitic load was analysed. As *Caligus sp.* and *L. kroyeri* are large and visible to the naked eye the counting was performed without the use of magnification tools. That allowed a rapid assessment of the parasite

presence and load, which is essential, specifically when handling live fish. A section of the gills was then carefully removed from the same location (second gill arch) and of similar size in each fish, ensuring that parasite counts were consistent and comparable. The gill sample was then placed on a glass slide with a drop of seawater from the rearing ponds to prevent desiccation and to aid in the microscopic assessment for ectoparasite presence on the gills. In live fish, 2 to 3 gill sections were removed from each 10 individuals, to minimize stress. In contrast, in sacrificed fish all individuals had a section of the gills removed. The samples (n=52) were examined under a microscope, using the second objective lens (10x), allowing the detection of small or nearly transparent parasites, that can easily be overlooked.

Subsequently, a full necropsy was performed where the fish's overall condition was evaluated. Particular attention was given to specific indicators of health and performance, such as: stomach and intestinal content, to evaluate recent feeding activity, body fat reserves, to assess their nutritional status, liver condition, as a general health indicator, spleen size and appearance, as an early sign of disease, gonadal development, to check the reproductive status, and any internal abnormalities or signs of bacterial infection

All the collected data was then compiled into a report and stored in the farm's internal records, thus creating a reference system that can be consulted in the future to correlate symptoms and parasitic loads with specific health or environmental conditions. Length, weight and the parasite intensity and abundance are presented as mean and standard deviation of the mean.

3.1.2 Observation of *Caligus* sp. and *L. kroyeri* life cycle

To better understand the life cycle of *Caligus* sp. and *L. kroyeri*, were maintained under artificial conditions, allowing for the observation of their reproductive and early development stages. Parasites were carefully removed from euthanised fish from routine health check-ups or freshly dead fish collected from the ponds. *Caligus* sp.

individuals were delicately removed using fine tweezers, without the use of magnifying instruments. The relatively large size and their preferred location in the buccal and opercular cavities made them accessible to the naked eye and allow an efficient collection.

In contrast, for *L. kroyeri*, a more delicate method was required. The gill arches were first removed from the fish and placed in a Petri dish with pond water, as to guarantee parasite viability. Using a laboratory magnifying lens and fine tweezers, parasites were carefully removed from the gill arches. This method was necessary due to the fragile nature of *L. kroyeri* adults. Additionally, due to the small size of juvenile stages and the lack of egg strings on males, extraction with the naked eye would easily overlook a significant percentage of these parasites.

The collected parasites were then transferred to small 2 L tanks, equipped with air pumps. This allowed for short-term maintenance of live parasites under controlled conditions, allowing for close observation. To observe the parasites they were collected using a Pasteur pipette and transferred to a glass slide for microscopic examination. The mean temperature was 14.8°C.

3.1.3 *In vitro* trials with antiparasitic compounds

Individuals of *L. kroyeri* and *Caligus* were carefully removed from freshly dead or moribund European sea bass, collected directly from the production ponds using a hand net. The parasite were collected using fine tweezers and were directly transferred to 2 L containers filled with pond water and equipped with air pumps, ensuring oxygenation and viability.

Per trial 6 individual Petri dishes were prepared with 50 ml of pond water, 3 with the desired compound, and other 3 control dishes without any added treatment. 5 live *Caligus* or *L. kroyeri* individuals were transferred to each plate using fine tweezers. After a short acclimatization period of 2 to 3 minutes one of the following substances were added to each treatment group to reach the desired concentration: copper

sulphate (CuSO₄), bleach (NaClO), praziquantel, oxyvirin, or formalin. The tested concentrations were recommended by the hosting farm, taking in consideration what would be realistically applicable in a commercial setting, while balancing economic efficiency, ecological impacts, and fish welfare.

Parasite viability was assessed at several points in time:

- After 1 hour, simulating the conditions of a short-duration bath treatment in dedicated containers
- After 24 hours, simulating the application of the compound in a rearing pond
- And after 48, to observe the impact on the development and hatching of larval stages

After the 24 hour assessment, the parasites were carefully transferred to new Petri dishes containing pond water, to simulate post-treatment conditions in tanks. The assessment conducted after 48 hours aimed to observe the effects on hatching, motility and development of the larvae after being exposed to the compounds.

The results were classified into 3 categories: unaffected, when all or nearly all larvae hatched and showed normal movement; moderately affected, when approximately half of the larvae were immobile; and severely affected, when no larvae hatched or exhibited movement. Parasites were considered dead if no movement was observed following a gentle touch with a needle. All observations were made under a stereomicroscope.

3.1.4 *In Vivo* trial for the control of *Caligus* sp. and *L. kroyeri*

A trial was conducted in February-March using twenty European sea bass (307±49 g) from Vale da Lama fish farm. Fish were distributed by two 500 L tanks while maintaining the same water quality conditions as in the original stock system. Fish were acclimated to the experimental tanks for one week and were fed the control diet for 2 weeks. Parasitic load was then assessed by anaesthetising all individuals in a 100-L container with clove oil (0.02 mg L⁻¹), using continuous aeration to maintain oxygen levels and safeguard welfare during handling.

One tank was then used as control, while the other was submitted to a medicated feed that included an antiparasitic compound. The experiment was conducted without replicates due to the limited availability of tanks. Salinity (35 ppm) and temperature (14-15 C) were stable throughout the trial period.

The medicated feed was prepared by mixing an antiparasitic compound (A) with a commercial control diet, using a concrete mixer, with 2% fish oil, which served as a binding agent. The compound A was added at the veterinarian-recommended dose and thoroughly mixed to ensure even distribution. The feed was then left to dry at room temperature before use, as to minimise leaching of the chemotherapeutant to the water.

After the acclimatization period, fish from one of the tanks continued to be fed the control diet, while the other tank was fed the medicated feed to satiation for a period of 2 weeks. During the trial, fish were anaesthetised for the manual counting of parasite load on days 0, 7 and 14, allowing tracking parasite load progression in a non-lethal manner. In each sampling a section of the gills was removed from 3 randomly selected fish from each tank, specifically from the second gill arch and of similar size to ensure comparability. These were then microscopically examined to evaluate overall gill condition and detect lesions associated with parasitism or treatment.

3.1.5 Assessment of the duration of protective effect of the antiparasitic compound

After the *in vivo* trial described in 3.1.4 section, the duration of the compound A's protective effect against parasites was evaluated. Three parasite-free fish previously fed the medicated diet were transferred into the control tank, where 10 resident fish were all infected with *Caligus sp.* and *L. kroyeri*. This experiment was conducted between March and May and had a duration of 7 weeks. All fish were fed a commercial control diet. Due to the limited availability of tanks the experiment was carried out without replicates. Despite the constraint it did not hinder the intended objective of evaluating the protection conferred by the treatment under artificial parasitic pressure.

Parasite load was assessed weekly in all fish of the tank as described in the previous section. Particular attention was given to the newly introduced individuals to monitor the potential reestablishment of the infestation.

3.2 Formulas used in data analysis

The following formulas were used for the statistical analysis:

Mean, where $\bar{x}$ = mean, n = number of observations, and x_i =individual value:

$$\bar{x} = \frac{\sum_{i=1}^n x_i}{n}$$

Standard deviation (used for entire populations), where σ =population standard deviation, x_i =individual value, μ =population mean, and n =total number of values in population

$$\sigma = \sqrt{\frac{\sum_{i=1}^n (x_i - \mu)^2}{n}}$$

Standard deviation (used for samples), where S =sample standard deviation, x_i =individual value, $\bar{x}$ =population mean, and n =number of observations

$$S = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n - 1}}$$

Intensity of parasitic infection:

$$Intensity = \frac{Total\ number\ of\ parasites}{Number\ of\ infected\ hosts}$$

Abundance of parasitic infection:

$$Abundance = \frac{\text{Total number of parasites}}{\text{Total number of hosts examined}}$$

Efficacy of anti-parasitic compounds:

$$Efficacy (\%) = \frac{\text{Mean initial number of parasites} - \text{Mean final number of parasites}}{\text{Mean initial number of parasites}} \times 100$$

3.3 Statistical analysis

Independent t-tests were used to compare mean parasite load between control and treated groups at each sampling point during the trial evaluating the effectiveness of compound A against *Caligus sp.* and *L. kroyeri*. The significance threshold for rejecting the null hypothesis was set at $p < 0.05$. All statistical analysis were performed using Microsoft Excel 2010.

3.4 Results

3.4.1 Monitoring of microscopic ectoparasites on the gills of European sea bass

Although not the primary focus of this work, observing and monitoring ectoparasites on the gills was a valuable component of the internship, providing knowledge and experience in disease surveillance and early detection in a commercial aquaculture.

The parasite load was classified using a semi-quantitative scale from I to V, depending on the level of the infestation. While levels I and II indicate low infestation levels that cause little to no harm, levels IV and V represent high parasitic loads, causing considerable damage to the host. Once these levels are reached, the need for therapeutic intervention was discussed with the Fish Health and Welfare team, usually involving the use of chemotherapeutants or immunostimulants incorporated in feed. During this internship, no microscopic ectoparasite reached dangerous levels. The most common microscopic ectoparasites found, their prevalence, mean abundance and intensity are summarised in Table 1.

Table 1- Prevalence, intensity and abundance of microscopic ectoparasites on the gills of *D. labrax* (N=52)

	Prevalence %	Mean intensity	Mean abundance
Monogenea	80	4,52	3,65
<i>Trichodina</i> sp.	42	I*	I*
<i>Gnathia</i> sp.	6	1	0,07

*Mean intensity and abundance of *Trichodina* is exclusively classified through a semi-quantitative scale due to their high parasitic load

3.4.1.1 Monogenea

Among the observed microscopic ectoparasites on the gills of *D. labrax*, monogeneans appeared most frequently (Figure 31). These flat and transparent parasites were found attached to the gill filaments and could be identified by the presence of the haptor, which is their posterior attachment organ. While taxonomic identification was not the objective in this study, the host species (European sea bass) and the geographic location of Piscicultura do Vale da Lama (Ria do Alvor, southern Portugal) suggest that the observed monogenean belongs to the species *Diplectanum aequans*. This parasite is one of the most common gill parasites found in European sea bass reared on the Mediterranean and Atlantic coasts (Saraiva et al., 2015; Ramos et al., 2023).

Monogenean infections can lead to diseases like diplectanosis when the parasitic load is high. The symptoms include haemorrhagic haemorrhages, excessive mucous production, fused lamellae, hyperplasia of the epithelium, haemorrhages and inflammation near the attachment site, with leucocyte infiltration associated with the presence of eosinophilic cells in the lesions. In extreme parasite loads the fish can appear anorectic, lethargic and show asphyxia symptoms (Toksen et al., 2010; Ogut & Uzun, 2012; Ozer et al., 2019). The treatment is usually applied through baths, using antihelminthic drugs, such as rafoxanid, or organophosphates, such as trichlorfon or azamethiphos (Toksen et al., 2012; Toksen et al., 2013).

Throughout the internship, monogenean infestations did not reach levels that required treatment. Only 3 out of 45 fish showed counts with more than 10 individuals, all of which were heavily co-infected with *Caligus sp.* and *L. kroyeri*, displaying a poor overall condition. This suggested that monogeneans were not the primary concern in these cases.



Fig. 31– Monogenean near to *L. kroyeri* egg sacks on the gills of European sea bass (photo by author, 2025)

3.4.1.2 *Trichodina* sp.

Trichodina sp. was the second most common microscopic parasite found on the gills of *D. labrax*. These ciliate parasites are protozoans that are found on the gills, skin or fins of most fish but are rarely present in sufficient numbers to cause damage to the host. They are round or bell-shaped and attach to the host through the adhesive disc, which features a tooth-like structure with specific patterns for each species (Plumb, 1991).

Their feeding habits, which consist of mucous and detritus, can cause irritation of the epithelium. This can lead to hyperplasia of the epithelial cells and fusion of the gill filaments. When the parasitic load is extremely high, it can cause deep ulcerative lesions that allow the spread of infectious diseases. The most common intervention methods are formalin baths and freshwater baths for marine species. Flushing may also be used to reduce infestation levels (Smith & Schwarz, 2019).

In Piscicultura do Vale da Lama, *Trichodina* sp. infestation levels are measured through a semi-quantitative scale from I to V. Only 3 of 45 examined fish showed infestation levels classified with IV or V, and all of them were co-infected with *Caligus* sp. and *L. kroyeri* in high numbers. As such, no treatment was applied.

3.4.1.3 *Gnathia* sp.

Gnathia sp. (Figure 32) is a parasitic isopod belonging to the Gnathiidae family. They have 2 distinct life stages: a larval stage which is ectoparasitic and a free-living adult stage that inhabits mud-sand bottom habitats. Their host specificity is low, as they are known to parasitise several elasmobranchs and teleosts. The main drivers of *Gnathia* sp. infestations are environmental, nutritional, biological and immunological (Genc, 2007; Hispano et al., 2013).

This parasite attaches itself mainly to the gills but can also be found on the eyes, nostrils, fins, lips and buccal cavity of their hosts, where they feed on the blood and tissue fluids. Although rarely causing damage to the host, they occasionally cause severe tissue damage and alterations in blood parameters and can serve as a vector for secondary parasitic infections. The organophosphate trichlorfon can be used to control infestations caused by *Gnathia* sp. (Genc, 2007; Hispano et al., 2013).

This parasite only appears sporadically in the Piscicultura do Vale da Lama systems. During the internship only 3 individuals were found on distinct fish. They only appear in low numbers, and as such, they do not pose a threat to reared *D. Labrax*.



Fig. 32 - *Gnathia* sp. and monogeneans on the gills of European sea bass (photo by author, 2025)

3.4.2 Observation and monitoring of *Caligus sp.* and *L. kroyeri* on European sea bass, and its pathological effects

All the analysed fish were infected with *Caligus sp.* and *L. kroyeri*. The mean intensity and abundance are shown in Table 2. The highest parasitic load was observed in individuals with an overall reduced condition, with the highest number of *Caligus sp.* in a fish being 69 and of *L. kroyeri* 23. As the other monitored parasites were present in negligible numbers, and no other disease was recorded, pathological signals and mortalities were assumed to be solely associated with parasitic copepod infections. The most common pathological symptoms observed were surface swimming, dark colouration, excessive mucous production, reduced feeding activity, tendency to swim in groups and near to the surface, grouping near the water inlet, flashing, emaciation and lethargy.

Table 4 - Prevalence and mean abundance and intensity of *Caligus sp.* and *L. kroyeri* infestation in *D. labrax*

	Number analyzed fish	of Prevalence (%)	Mean intensity	Mean abundance
<i>Caligus</i>	106	100	11,3±10,1	11,3±10,1
<i>L. kroyeri</i>	106	100	9,7±5,0	9,7±5,0

All values are presented as mean and standard deviation from the mean

During health check-ups, recurring clinical signs were also noted (Table 3). Although these lesions are not necessarily directly caused by copepod parasite infections, they may have resulted from secondary infections or be associated with a compromised condition. Histological analysis was not conducted due to the lack of appropriate facilities and instruments at the farm. Additionally, as a commercial aquaculture, the main focus of this work was not to fully understand the pathological effects but rather to identify practical and sustainable solutions for their control.

Table 7 - Frequency of pathological symptoms associated with *Caligus sp.* and *L. kroyeri* infections observed during routine health check-ups

	Anaemic liver	Absence of feed	Anaemic gills	Air bubbles	Tenacibaculosis
Frequency (%)	62	38	27	12	35

3.4.3 Observation of *Caligus sp.* and *L. kroyeri* life cycle

In *L. kroyeri* it was possible to observe the eggs being released from the egg strings, freshly eclosed larvae (Figure 33), and the nauplius I (Figure 34) and II stages (Figure 35). As the latter is the last mobile stage, the absence of a suitable host prevented that life cycle progression. In the case of *Caligus sp.*, it was possible to observe eggs, naupliar stages (Figure 36 and 37) and the first copepodid stage (Figure 38 and 39), which is the infective stage, and it's still able to swim freely.

It was not possible to observe *Caligus sp.* nauplius I due to their rapid development time into nauplius II (Nguyen et al. (2019) conducted a study on *Caligus minimus* life cycle at 30°C where it was noted that it only took 1.88 ± 0.74 hours to complete this process. Additionally, in the present study *Caligus sp.* larvae were observed to hatch and progress through the early developmental stages in a faster rate than *L. kroyeri* larvae.



Fig. 33- Freshly eclosed *L. kroyeri* larvae (photo by author, 2025)



Fig. 34- Nauplius I of *L. kroyeri* (photo by author, 2025)



Fig. 35- Nauplius II of *L. kroyeri*. (photo by author, 2025)



Fig. 36- Nauplius II of *Caligus* sp. (photo by author, 2025)



Fig. 37 - Nauplius II of *Caligus* sp. (photo by author, 2025)



Fig. 38- Infective copepodid of *Caligus* sp. (photo by author, 2025)



Fig. 39- Infective copepodid of *Caligus* sp. (photo by author, 2025)

The findings from this work were fundamental for the *in vitro* test of antiparasitic compounds, as it was noted that the parasites could survive for up to 1 week under artificial conditions. The larvae were also able to hatch and continue their life cycle, which was essential for the hosting farm's objective of testing the effects of the chemotherapeutants on early life stages.

3.4.4 *In vitro* trials with antiparasitic compounds

The evaluation of copper (CuSO₄) at a concentration of 1 ppm, and bleach (NHClO) at a concentration of 5 ppm, was only conducted to assess their antiparasitic effect *in vitro*, as both compounds are highly toxic to fish and harmful to the environment. The results were not promising, with both treatments showing little to no effect on the parasites after 24 hours of exposure. Copper exhibited slightly better activity than bleach, with *L. kroyeri* larvae showing no survivability. However, the compound only had a partial effect on *Caligus sp.* and *L. kroyeri* larvae, making it impractical for real world use. Higher concentrations were not tested due to the toxicity of these compounds, which limits its safe application, particularly in extensive and semi-intensive systems connected to natural water bodies. Mean temperature was 14°C for the copper trial, and 15°C for the bleach trial. Results are presented in table 4.

The trials with Ox-Virin also yielded insufficient results. Exposure to a 20 ppm concentration produced no effects on any life stage of the parasites. Although the 70 ppm concentration showed better results, full mortality was observed only in *Caligus sp.* larvae. Higher concentrations were not tested due to the high cost of the compound, which makes large scale use impractical. Mean temperature was 15°C. The results are presented in table 4.

The praziquantel trial was not conducted on *Caligus sp.*, as the compound became available only during the final month of the internship, by which the parasite's prevalence had significantly declined, making it impossible to obtain a sufficient number of individuals. Among all the tested compounds praziquantel produced the poorest results. Even at concentrations as high as 13.5 mg L⁻¹ no effect was observed

on *L. kroyeri*. Higher doses were not tested due to the impracticality of using large volumes of the compound, which would be economically unfeasible. Mean temperature was 15°C. The results are presented in table 4.

The formalin trials yielded the most promising results among all compounds tested. At a concentration of 20 ppm, formalin showed partial effectiveness, causing total mortality in adult *Caligus*, and 60% mortality in *L. kroyeri* adults. The 150 ppm concentration proved highly effective, resulting in total mortality of both *Caligus sp.* and *L. kroyeri*, and impairing larval hatching within just 1 hour. However, the high costs associated with this concentration and potential environmental risks raise concerns about its practical application. The 50 ppm concentration was tested as a more environmentally friendly and economically viable option, This dose achieved 73% mortality of *L. kroyeri* and 100% of *Caligus sp.* within 1 hour. After 24 hours complete mortality of both parasites was achieved, with no larval survival at the 48 hours assessment. Given that formalin is already familiar to the farm's personnel and procedures are in place for its safe handling, it was recognized and adopted as a potential method for the future control of ectoparasitic infection in the hosting farm. Mean temperature was 16°C. The results are presented in Table 4.

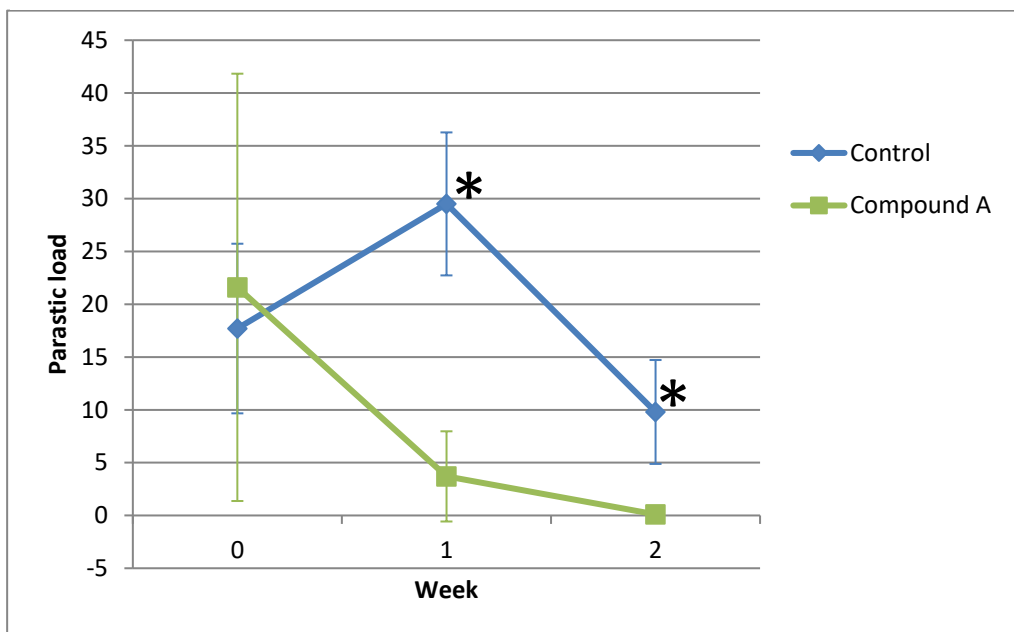
Table 9 - Survival rate of *Caligus sp.* and *L. kroyeri* adults after *in vitro* exposure to several compounds for 1 hour and 24 hours, and the effect on larvae after 48 hours

	<i>Caligus sp.</i>			<i>L. kroyeri</i>		
	1 hour	24 hours	Larvae	1 hour	24 hours	Larvae
CuSO₄ 1ppm	87%	73%	Moderately Affected	100%	67%	Severely Affected
Bleach 5 ppm	100%	93%	Unaffected	100%	87%	Unaffected
Praziquantel 2 mg L⁻¹	—	—	—	100%	100%	Unaffected
Praziquantel 7 mg L⁻¹	—	—	—	100%	100%	Unaffected
Praziquantel 13.5 mg L⁻¹	—	—	—	100%	100%	Unaffected
Oxy-Virin 20 ppm	100%	100%	Unaffected	100%	100%	Unaffected
Oxy-Virin 70 ppm	93%	73%	Severely Affected	93%	80%	Partially Affected
Formalin 20 ppm	73%	0%	Partially Affected	100%	40%	Unaffected
Formalin 150 ppm	0%	0%	Severely Affected	0%	0%	Severely Affected
Formalin 50 ppm	0%	0%	Severely Affected	27%	0%	Severely Affected

3.4.5 *In Vivo* trial for the control of *Caligus sp.* and *L. kroyeri*

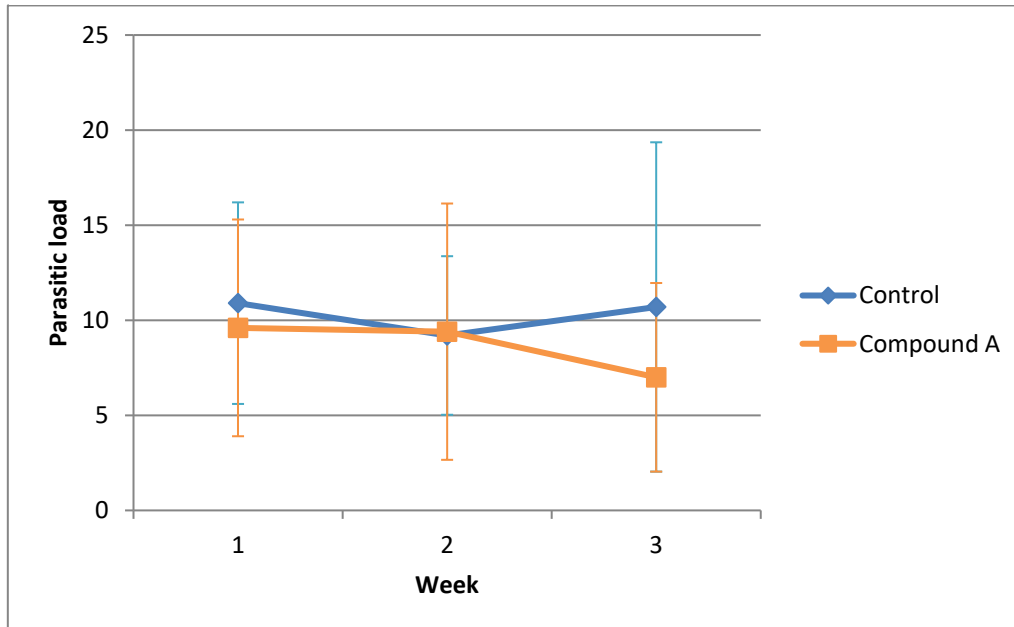
During this trial, no abnormal behaviour was noted. The feeding rate was 0.5% of the total biomass, taking in consideration the feed manufacturer indications and the water temperature at the time of trial. As the fish showed reduced appetite, the quantity was adjusted based on the group with the lowest feeding rate, ensuring consistency between treatment groups. Utmost care was taken as to not waste feed and to promptly remove any uneaten pellets, to avoid leaching the substance onto the water that could harm the fish or contaminate water sources. Mean water temperature was 14.8°C, and salinity was 35 ppt. The results are presented in Figure 40 and Figure 41.

Fig. 40– Effect of Compound A on the parasitic load of *Caligus sp.* on *D. labrax* over 14 days



* Indicates that significant statistical differences were found between groups at each sampling point (P<0.05)

Fig. 41– Effect of Compound A on the parasitic load of *L. kroyeri* on *D. labrax* over 14 days



No significant statistical differences were found between groups at each sampling point ($P < 0.05$)

Compound A demonstrated exceptional efficacy in controlling *Caligus sp.* infestations, reducing parasite numbers to zero in nearly all treated fish within 2 weeks. Although its effect on *L. kroyeri* was modest (a 27% reduction), and no statistical significant differences were found between treated and non-treated fish, it was still considered effective, especially taking in consideration that previous control methods tested by the hosting farm showed no effective results. Notably, *L. kroyeri* load continued to decline in treated fish even after the trial period. At the start of the resistance-duration assessment, *L. kroyeri* counts had dropped to zero per fish. These findings confirm that compound A is highly effective in controlling infections caused by these parasites.

3.4.6 Assessment of the duration of protective effect of the antiparasitic compound

During the trial no abnormal behaviour was noted. The mean temperature was 15°C and salinity 35‰. No signs of reinfestation of the non-infected fish were observed throughout the 7 weeks. Although *Caligus sp.* numbers dropped to 0 in all infected fish, the *L. kroyeri* population continued to increase until week 6 (Table 5). Juvenile's stages of the parasite were observed in infected fish, suggesting that the parasite maintained its life cycle and reproduction during the trial. In contrast, the fish previously treated with compound A showed 0 parasites throughout the 7 weeks, indicating that the treatment provided sustained resistance against both parasites for at least 7 weeks following administration.

Table 10– Duration of the protective effect of compound A in treated *D. labrax* exposed to infected fish

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
<i>Caligus sp.</i>							
on control fish	0.3±0.5	0.1±0.35	0.1±0.3	0.1±0.3	0	0	0
<i>L. kroyeri</i>							
on control fish	8.0±3.1	8.9±6.4	9.6±6.3	10.3±5.0	10.6±5.9	12.6±8.4	10.0±5.7
Parasitic load of treated fish	0	0	0	0	0	0	0

All values are presented as mean and standard deviation from the mean

4. Discussion

The main purpose of this internship was successfully accomplished, which was to acquire experience of the daily routines of a commercial aquaculture, as well as in management of ectoparasitic infections in European sea bass. To obtain that first-hand knowledge I had the opportunity to take part in a broad range of tasks, including tank sampling, participation in fishing activities, routine health and welfare assessments, and maintenance and repair of fundamental farm equipment.

I was also able to develop practical skills related to fish health management. I learned to identify different ectoparasites and to manually count parasitic loads in live fish, including the precautions to minimize stress during handling. I was able to study the complex life cycles of copepod parasites and their most vulnerable stages, aiding in choosing the most effective intervention time.

I gained experience through the maintenance of parasites in artificial conditions, the observation of early life and the designing of experimental setups to test compound efficiency on different life stages. Additionally, I manufactured medicated feed for treatment trials, conducted full necropsies to evaluate fish condition and contributed to the communication of findings to the farm's fish health and welfare team, supporting and collaborating in the planning of effective intervention strategies.

I also had the opportunity to expand my knowledge in other areas by contributing to projects not related to parasitology. These include sea grass and kelp restoration projects, where I gained the experience in the propagation of these species for conservation efforts. I also had the chance to aid in a gilthead sea bream colouration trial, which involved dietary supplementation with algae. My aid in sampling and in monitoring the evolution of pigmentation provided valuable insight into nutrition related trials. Overall, all the tasks where I was involved gave me valuable knowledge that will serve as a solid base for my future professional development in aquaculture and fish health management.

In the absence of specific licensed treatments for copepod ectoparasitic infections in European sea bass, producers are often left without management options. This study

explored alternative solutions, taking in consideration their practical, economic and environmental viability. The *in vitro* trials were essential in assessing the effects of the tested compounds, both in adults and early life stages. However, it was not possible to replicate these trials *in vivo* to evaluate treatment efficacy and potential effects of fish health. This limitation was due to the restricted number of available tanks and the limited duration of the internship.

This is particularly relevant in the case of *L. kroyeri*, as its attachment within the gill arches may offer it a degree of protection against treatments administered in the water. According to the hosting farm's prior experience, *L. kroyeri* had shown higher resistance to treatments in comparison with *Caligus sp.*. This trend was not strongly reflected in the *in vitro* results, where *L. kroyeri* appeared more susceptible to bleach and copper sulphate, but more resistant to Ox-Virin and formalin. This suggest that the additional resistance to treatments observed *in vivo* may not be the result of chemical resistance, but from its attachment location, which may shield it from direct exposure to the compounds.

Formalin demonstrated the highest efficacy among the tested compounds to control infections caused by both parasites. As one of the most commonly used treatments against ectoparasitic infestations in aquaculture, and the most employed antiparasitic method in Mediterranean aquaculture (Rigos et al., 2023), its effectiveness was further validated in this work. Danayadon & Krachaiwong (1996), reported similar results for *Caligus sp*, where formalin was used at a concentration of 250 ppm for 1 hour per day, for 3 consecutive days killed 100 % of this parasite in *Lates calcarifer*. On the contrary, Roth et al. (1993) reported that formalin was the first compound to be used to treat sea lice infestations in Norway, at a concentration of 400 mg L⁻¹ with little efficacy and with associated mortalities.

In this study formalin showed significant impact not only on adult parasites but also on early life stages. This confers the advantage of allowing for simultaneous control of established infections on fish, as well as the free living nauplius and copepodids, thereby limiting reinfection and transmission. Additionally, this compound has a relatively low environmental impact. After its application it releases formaldehyde, which breaks into carbon dioxide and water through oxidation in the water. Due to its

high solubility it does not bioaccumulate in aquatic organisms. Furthermore, it degrades rapidly by indirect photodegradation, and biodegradation by bacteria.

The results obtained from the *in vivo* trials with the antiparasitic compound were promising. *Caligus* sp. levels dropped to 0 within 2 weeks, while *L. kroyeri* load was reduced by approximately 30% in the same period, eventually reaching 0 in the subsequent weeks. The absence of juvenile stages in the treated fish suggests that the compound may have been effective against early life stages, although this hypothesis needs further investigation, as this study did not assess the presence of free-swimming stages in the water column.

Feeding activity was compromised during the trial, but it did not interfere with the results. Interestingly, the control group showed less appetite than the treated group, suggesting that reduced feeding activity was related to the stress and compromised health from the high parasitic loads and from the transition from the pond to tank conditions, not from reduced palatability of the feed. The resistance trial confirmed that the compound remained effective for at least 7 weeks post-treatment. These observations suggest it could be a useful method for managing ectoparasitic infections in European sea bass, but a dedicated trial is still warranted to confirm its effectiveness and safety to the fish.

The reduced feeding activity observed in both pond and experimental fish highlights the importance of early intervention. Initiating treatment at an earlier stage may avoid feed refusal, which not only impacts the farm's economic sustainability but can also pose an environmental risk due to potential leaching of uneaten medicated feed into the system. Early intervention may also prevent parasite loads from reaching harmful levels, avoiding the establishment of parasite populations in the ponds and consequentially minimizing health and welfare impacts on reared fish. The results of this internship contributed to the adjustment of the farm's protocols for managing ectoparasitic infections. They also provided a starting point for future research, such as optimizing dosage or evaluating environmental safety. Overall, these findings supported improvements in fish health and welfare, with long-term benefits expected.

5. Conclusion

The number of parasitic infections in aquaculture continues to grow. With the climate change leading to rising temperatures, it is expected that the life cycle of the parasites will be accelerated and their distribution modified. The fluctuating water conditions will affect the fish immune system, and consequentially, the number and intensity of outbreaks are expected to rise. The shift towards replacing protein of marine source with plant-based ingredients in the feed may be ethically and environmentally friendly, however, it can increase the stress levels in fish, as this dietary changes negatively affect their immune system, making them more susceptible to infections.

To worsen the problem, the available treatment methods are limited, with many facing licensing restrictions. Furthermore, many antiparasitic compounds are toxic to the environment and are associated with resistance development, especially when applied incorrectly. It then becomes urgent to invest in the development of reliable and accessible solutions that are simultaneously economically viable and environmentally friendly. For example, the use of natural substances with antiparasitic properties as medicine or in functional feeds, or the use of preventive strategies, may become viable alternatives. For that to be possible, collaboration between researchers, producers, and regulatory institutions is essential to study, develop, and implement such strategies in a commercial setting.

In this context, this work contributed valuable insight into *Caligus sp.* and *L. kroyeri* biology and treatment strategies, contributing to a better understanding of how to optimise intervention practices. These findings can support the transition to more sustainable treatment methods by aiding the hosting farm in future research and integration of alternative control measures.

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Webgraphy

Fig. 6- *Dicentrarchus labrax* illustration

https://fish-commercial-names.ec.europa.eu/fish-names/species/dicentrarchus-labrax_pt - consulted in May, 2025

Fig. - 7 *Dicentrarchus labrax* distribution

<https://www.fishbase.se/summary/dicentrarchus-labrax.html> - consulted in April, 2025

Fig. 21 – Adult female *Ceratothoa oestroides* settled in the buccal cavity of European sea bass

https://en.wikipedia.org/wiki/Ceratothoa_oestroides#/media/File:Fig_1_Ceratothoa_oestroides_parasite_in_European_sea_bass.jpg – consulted in June, 2025

Fig. 22 – Cleaner fish *Cyclopterus lumpus* and *Labrus bergylta*

<https://www.biodiversity4all.org/photos/14791?size=original> and
<https://www.biodiversity4all.org/photos/59055985?size=original> – consulted in June, 2025

Fig. 23 - Scheme of a skirt used in cage aquaculture to avoid encounters between fish and copepod parasites

<https://industrial.cunninghamcovers.co.uk/sea-lice-skirts-for-fish-farms/> -
consulted in June, 2025

Fig. 28 – *Caligus* sp.

<https://www.google.com/url?sa=t&source=web&rct=j&opi=89978449&url=https://nathistoc.bio.uci.edu/crustacea/Copepoda/Caligus/index.html&ved=2ahUKEwiesz5nolp-OAxXj87sIHe9EJxkQFnoECB0QAQ&usg=AOvVaw2ekpePBlohWHlwRAZo-9Ng> –
consulted in April, 2025

Fig. 30 – Earthen ponds from Piscicultura do Vale da Lama

<https://www.aqualvor.pt/sobre-nos/> - consulted in June, 2025