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Evaluation of Ballan wrasse (*LABRUS BERGYLTA*) juvenile development with two commercial feeds at Mowi Rensefisk



UNIVERSIDADE DO ALGARVE
Faculdade de Ciências e Tecnologia
2022-2023

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Mestrado em Biologia Marinha

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Acknowledgements

Firstly, I would like to thank MOWI Rensefisk Stord for the opportunity to use their facilities and production as a subject for my master thesis. I would also like to thank my supervisor, PhD Ingrid Overrein for the guidance provided, and PhD and Professor Catarina Cortes Valente de Oliveira for her guidance, help, patience, and motivation to finish my thesis. Finally, I would thank the head of Juvenile department and friend Vaidas Zarnauskas for helping me balancing my workload in order to collect the necessary data for my thesis. Last but not least, a big thank you to my boyfriend, Tiago Lopes for all the help and support through my master.

Abstract

Farmed Ballan wrasse (*Labrus bergylta*) is an efficient cleaner fish used for non-medicinal de-licing of Atlantic salmon in sea cages. The idea is replacing the use of wild caught individuals due to considerations for biodiversity and risk of overfishing local wrasse populations. Low growth rate during production has been a common struggle, resulting in fish not reaching the desired minimum size when they are to be shipped to different facilities for further development before being deployed to the salmon sea cages. Another difficulty is fins development, with the increased demand for Ballan wrasse in commercial applications, Norwegian food authority is improving welfare regulation concerning this species. Due to pectoral fin erosion the mortality rate is exceedingly high during the first weeks after deployment to the salmon pens, considering that fin erosion begins at the rearing facilities it becomes a point of important focus. The aim of this study was to find a more suitable feed for Ballan wrasse as well as ideal rearing conditions to improve survival and performance. This trial consisted in three tanks: control tank, Biomar II and Biomar III with 116 000, 88 000 and 90 000 fish respectively, with regular samples of 30 individuals over a three-month period. Length, average weight, pectoral and caudal fin erosion were analyzed. Two commercial feeds were employed: Otohime, a well-established finfish feed, and Biomar Symbio Plus, a relatively new and custom developed feed for Ballan wrasse. Also, hidings were introduced in the Biomar II tank for a portion of the trial. Results showed an even growth rate in the three groups during the first two months of the trial, and a pronounced growth was registered in the Biomar groups during the last month. It was distinctly observed that Biomar tanks held better fin health throughout the trial, in contrast to Otohime. This study showed that by keeping a lower density during production the use of a specialized feed could improve the rearing quality and robustness of Ballan wrasse.

Resumo

O Bodião-reticulado (*Labrus bergylta*) é atualmente utilizado na aquacultura de salmão atlântico como uma alternativa mais eficiente de tratamento não medicinal contra o piolho do salmão (*Lepeophtheirus salmonis*). A produção comercial desta espécie tem como objetivo reduzir a utilização de indivíduos selvagens, cuja captura tem vindo a aumentar nos últimos anos devido ao aumento da procura por parte dos produtores de salmão, respondendo assim a preocupações de pesca excessiva do Bodião-reticulado e a problemas de biodiversidade. Deste modo tentando também reduzir a aplicação, em muitos casos excessiva, de medicamentos contra o piolho do salmão que se está a tornar exponencialmente mais resistente aos mesmos, e tem efeitos adversos nos ecossistemas marítimos próximos das jaulas de salmão. No entanto, a produção de Bodião-reticulado é relativamente recente e ainda enfrenta algumas dificuldades por falta de conhecimento específico sobre o desenvolvimento desta espécie. Taxa de mortalidade elevada, taxa de crescimento lenta e a erosão das barbatanas são os principais problemas que afetam a produção comercial desta espécie. Devido à taxa de crescimento lenta (> 3 anos até poderem ser utilizados nas jaulas de salmão) e ao longo período de desenvolvimento há a necessidade de transferir os peixes para instalações onde possam atingir o tamanho e robusteza necessários para serem eficazes dentro das jaulas de salmão, onde irão precisar de nadar contra as correntes marítimas, lidar com alterações de temperatura mais acentuadas, e onde é necessário de certa forma acompanharem o ritmo do salmão, um peixe notavelmente maior e mais forte. O facto de os peixes não atingirem tamanhos necessários para o transporte afeta a sua taxa de sobrevivência mesmo antes de poderem ser empregues nas jaulas, onde se deparam com outro problema bastante comum: a erosão das barbatanas peitorais. Tendo em conta o impacto que isso tem na taxa de mortalidade do Bodião-reticulado ao longo da produção, que também se mantém excessivamente alta nas primeiras semanas após ser implantado junto do salmão em alto mar, e considerando também que a erosão das barbatanas começa nas instalações de aquacultura em terra, torna-se fundamental procurar soluções para este problema. Deste modo, o objetivo deste estudo foi encontrar uma alimentação adequada para o desenvolvimento do Bodião-reticulado, assim como as condições de produção ideais para aumentar a sua sobrevivência e performance. O estudo teve início no departamento das larvas quando estas atingiram os 50 DPH sendo então transferidas aproximadamente aos 65 DPH para

o departamento Juvenil onde se deu continuidade ao teste. Este consistiu em três tanques de 130 000 peixes cada, de onde foram retiradas amostras regulares de 30 indivíduos. Através das amostras foram calculadas as médias de peso e comprimento, e foram observadas as condições das barbatanas caudais e peitorais para determinar a erosão das mesmas. Foram utilizadas duas alimentações comerciais: Otohime, uma ração comum em aquacultura de peixes; e Biomar Symbio Plus, uma ração relativamente recente e desenvolvida especificamente para esta espécie. Otohime foi usada no tanque de controlo por já ser uma alimentação bem estabelecida na indústria, e por ser a alimentação padrão nestas instalações. Biomar Symbio Plus foi utilizada nos restantes dois tanques, Biomar II e Biomar III. Os três tanques estiveram sob regime de alimentação de 24H ao longo do teste. Os três grupos apresentaram níveis de mortalidade altos ao longo do teste. Quando comparados com o tanque de controlo ambos os tanques Biomar registaram uma mortalidade mais elevada imediatamente após a transferência para o departamento juvenil. O grupo Biomar II apresentou uma descida de mortalidade acentuada na terceira semana, mantendo depois um registo de mortalidade baixo até ao final do teste. No entanto o grupo Biomar III registou uma mortalidade elevada comparando com o grupo Biomar II, possivelmente por ser o grupo mais novo dos três tanques e assim o mais afetado pela avaria na bomba da água. O grupo de controlo registou um aumento na mortalidade após dois meses, isto coincide com a alteração do tamanho da ração Otohime, aumentando de B2 para C1. Isto implica que o aumento da mortalidade possa estar relacionado com as larvas menos desenvolvidas morrerem nesta fase por não se conseguirem alimentar com este tamanho de ração. Relativamente ao comprimento, durante os primeiros dois meses registou-se um desenvolvimento relativamente equilibrado entre os três tanques, sendo que entre os 110 DPH e os 125 DPH se assinalou um aumento acentuado nos dois tanques da Biomar. Enquanto o grupo de controlo registou um aumento 7,5% no comprimento, o grupo Biomar II registou 43,2%, e o Biomar III 31,2%. Na fase inicial os três grupos apresentaram um crescimento similar, no entanto a partir dos 110 DPH observou-se um crescimento acentuado nos grupos Biomar. O crescimento assinalado aos 110 DPH pelos grupos Biomar traduziu-se num aumento de peso médio, registando ambos aproximadamente 0,55g em contraste com 0,35g do tanque de controlo. Ao longo do teste foram analisadas as barbatanas das amostras retiradas em intervalos de aproximadamente um mês, sendo que a primeira amostra foi realizada no departamento das larvas aos 50 DPH. Registou-se maior erosão das barbatanas no tanque de controlo em relação aos dois tanques Biomar, sugerindo que a

alimentação Biomar Symbio Plus poderá estar a promover condições mais indicadas às necessidades do Bodião-reticulado em aquacultura. A interação entre o número de dias após a eclosão e os dois tipos de ração tem um efeito significativo na erosão da barbatana peitoral (Two-way ANOVA, $p < 0.001$). No entanto é importante referir que durante a transferência inicial dos peixes houve uma falha técnica da contadora, sendo que esta registou um número superior de peixes em relação ao real. Este erro apenas foi revelado no final do teste quando se realizou a triagem com uma contadora mais exata, e utilizando os dados da mortalidade cumulativa, concluiu-se que na realidade os tanques Controlo, Biomar II e Biomar III continham aproximada e respetivamente 116 000, 88 000 e 90 000. Isto poderá ter influenciado a condição das barbatanas, considerando que a densidade por tanque deveria ter sido aproximadamente 8000 peixes/m³, e na realidade foram bombeados cerca de 7000 peixes/m³ no grupo de controlo, e 5500 peixes/m³ nos grupos Biomar. Os resultados sugerem que a utilização de uma alimentação especializada, e a redução de densidade nos tanques durante a produção podem melhorar a qualidade e robusteza do Bodião-reticulado.

Keywords: Cleaner fins; Ballan wrasse; Fin erosion; Biomar; Welfare.

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1 INTRODUCTION

1.1. Overview of Salmon production in Norway

Global aquaculture production has increased rapidly over the past decades and has major prospects of further expansion, with the industry having an average annual growth rate of 4.5 percent between 2011 to 2018, achieving a considerable total fish production of 82 million tons in 2018 (Figure 1.1), which was valued at USD 250 billion (Fao, 2018; APROMAR, 2019). As such, it is often viewed as one of the most important sources of food for the growing human population. However, depending on the nature, location, volume of production, and farmed species in question, aquaculture may elicit a variety of negative effects on wild populations and the surrounding environment (Seljestad *et al.*, 2020).

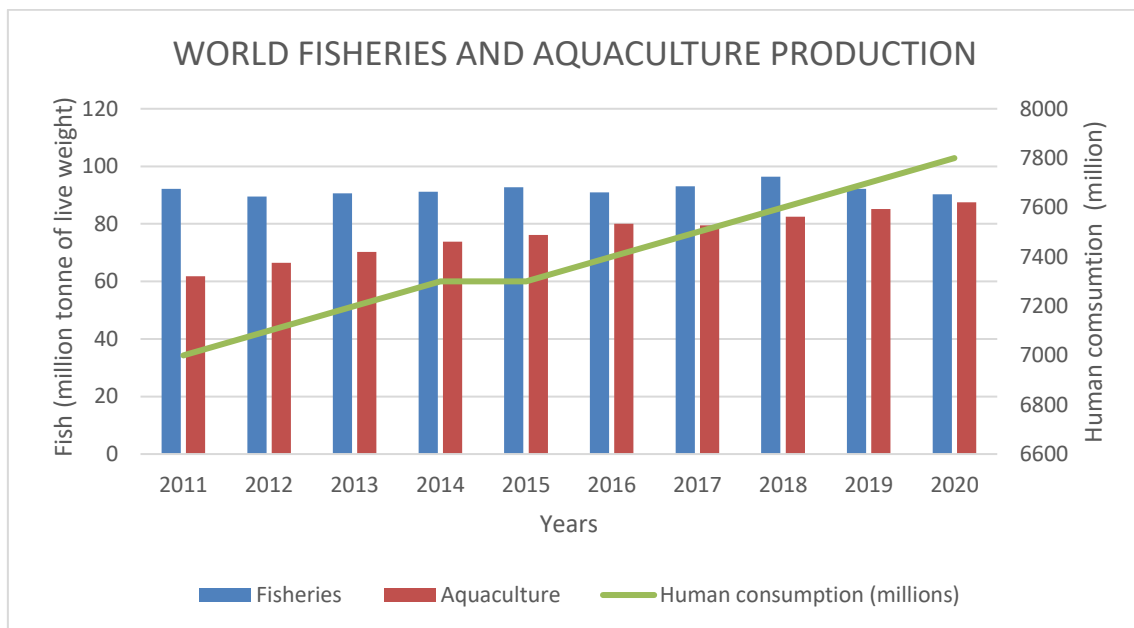


Figure 1.1 Comparison between fisheries and aquaculture production along with the global population (Fao, 2018; FAO, 2021).

Salmon is the common name for several species of the family Salmonidae; however, it is more associated to *Salmo salar* (L.), the Atlantic salmon. Atlantic salmon farming started in the 1960s in Norway, and since that time, it has become a strong industry in Chile, the United Kingdom and Canada (Torrissen *et al.*, 2013). In 2019, FAO estimated that total consumption of farmed Atlantic salmon was around 2.3 million tons (GWT) (Harvest, 2015; Gonzalez and de Boer, 2021). The majority of salmon farms in Norway are in open

sea cages, and the whole cycle of production takes around three years (24-40 months) to complete as it is represented in figure 1.2 (Initiative, 2021). About 70% of the world's salmon production comes from farming. It can be reared in offshore nets or placed in sheltered waters such as fjords or bays. First, the eggs are fertilized with milt and then incubated in shelves for approximately 30 days (1). In the first year, juveniles grow until they weight approximately 100g in freshwater tanks (2). The fish is then transported to sea cages (3) and remains there for 14-24 months, growing to 4-5kg (4). Upon reaching harvest size, the salmon is transported to processing units, where it is slaughtered and gutted (5). Following customer preferences the salmon is usually sold as a whole fish or as a fillet in ice packages (6) (Harvest, 2015).

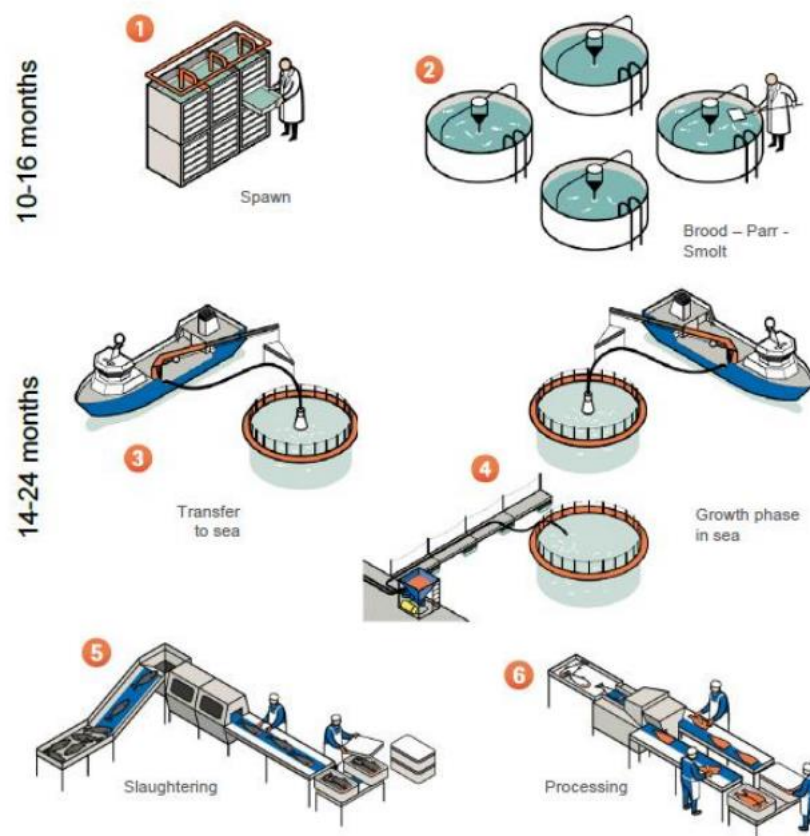


Figure 1.2 Farmed salmon cycle, (adapted from Harvest, 2015).

Despite being still an expanding industry, Atlantic salmon aquaculture in Norway has been controversial regarding the impact on marine ecosystems. Environmental pollution; eutrophication; ecological and genetic interactions between escaped farmed fish and wild stocks; increase of parasites and diseases; or lack of development in infrastructure to

reduce physical damage of sea cages, are the major technological and environmental challenges in salmon aquaculture (Naylor *et al.*, 2005; Taranger *et al.*, 2015). Salmon farming industry faces the problem of the ectoparasite sea lice, which if left untreated can cause significant losses of fish, both in aquaculture, and among wild populations. Outbreaks of sea-lice remain among the most damaging health issues in marine salmonid aquaculture (Wootten, Smith and Needham, 1982). According to the IntraFish Sea Lice Report 2022, annual costs associated with sea lice management were estimated at \$1 billion (Wootten, Smith and Needham, 1982; Jensen *et al.*, 2020).

1.2. Sea lice dilemma in salmon farming

Aquaculture industry has been struggling with several serious biological threats, such as bacterial and viral outbreaks, which threatens its production, and as mentioned above outbreaks of sea lice, which represents one of the major challenges in marine salmonid production. Ambitious sustainability criteria in the Norwegian industry hinder the goals to increase production (Fiskeridepartementet, 2014). The severe biological conditions faced by Norwegian farmers in recent years is reflected by the stagnation in production volumes (Taranger *et al.*, 2015). Frequent outbreaks of pancreas disease, as well as infectious salmon anaemia, a virus which is endemic and widespread in Norway, is the result of intensive salmonid farming in net-pens (Jansen *et al.*, 2017; Hjeltnes *et al.*, 2018). However the primary cause to the stagnation of production is the ectoparasitic sea lice *Lepeophtheirus salmonis* along with the required treatments strictly enforced by Norway to control sea lice levels (Abolofia, Wilen and Asche, 2017). To protect wild salmonids, farmers must perform delousing treatments whenever concentrations exceed 0.5 female lice per salmon in net-pens (or 0.2 when juvenile wild salmon is migrating to the ocean in the spring). In 2014, in Norway it was estimated for salmon lice to have cost about \$380 million to the industry. Because of the huge amount of money spent in lice treatments the price of slaughtered salmon produced in 2014 increased to $\approx 0,54\$/\text{kg}$ (Iversen *et al.*, 2015). In 2019 salmon lice treatments were estimated to cost between \$600 million and \$1 billion (*Business intelligence: The secret lives of sea lice | IntraFish.com*, no date).

1.3. Sea lice

The copepod *Lepeophtheirus salmonis* (Figure 1.3) is the most abundant species of sea lice on salmonid farms in the Northern Hemisphere, and infests both farmed and wild salmonids in the marine environment (Costello, 2006). The life span of this louse is still in debate among researchers, but it is predicted to be between 25-45 days, depending on the temperature. During spring/summer their reproduction is substantially high and it has been reported that adults can survive over-winter on wild salmon (Treasurer, 2002; Hamre *et al.*, 2013).

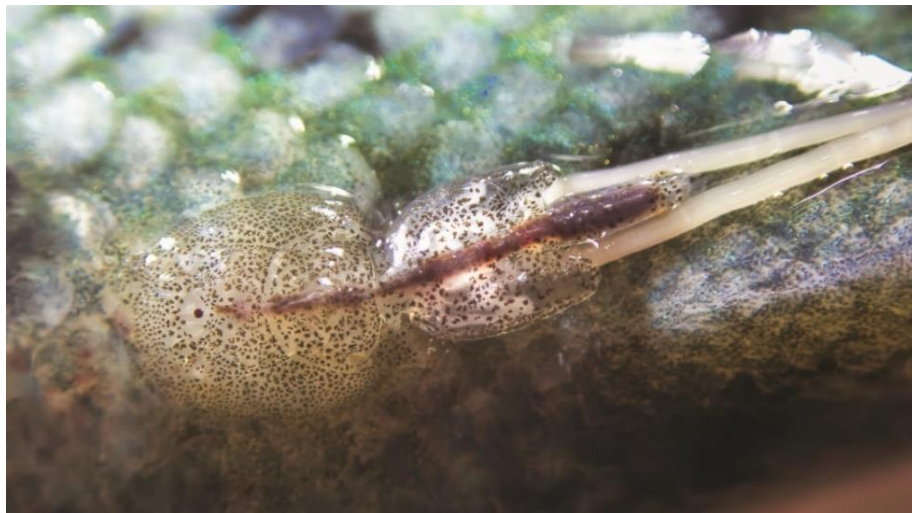


Figure 1.3 Adult Sea lice (*Lepeophtheirus salmonis*) adapted from (Sea Lice Biology and Control, 2022).

The life cycle of this louse (Figure 1.4) includes several phases, starting by two initial larval stages called Nauplius (nonfeeding and planktonic). The third stage is as copepods, where the louse attaches itself into the host by a thread ('frontal filament'). At the attachment site, copepods start to turn into four sessile chalimus stages. The louse undergoes two immature pre-adult stages, where they move freely along the host to feed, until reaching adulthood. The female carries a pair of external egg sacs or "egg strings"

with 100-500 eggs, producing six to eleven broods over her lifetime. Afterwards, the eggs float on the surface hatching into the first stage (Whelan, 2010).

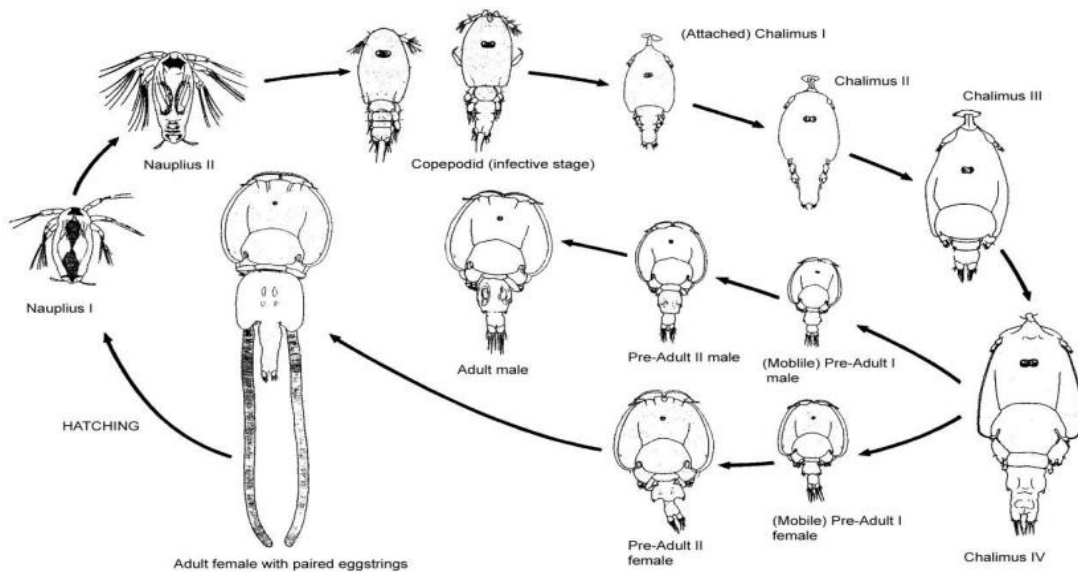


Figure 1.4 Life cycle of the sea lice *Lepeophtheirus salmonis* (Whelan, 2010)

Salmon is harmed by sea lice in various ways. After attaching itself to its host surface it develops to mobile stages and begins actively feeding becoming potentially harmful by causing skin lesions when grazing the host's skin, disrupting mucus biochemistry, causing underlying tissue necrosis. The potential to secondary microbial infection increases as this damage spreads, and results in osmoregulatory imbalance, lower immune capability and higher stress levels (Treasurer, 2002; Stien *et al.*, 2005; Dean *et al.*, 2021).

1.4. Sea lice treatments

The fight against sea lice has become an international concern and the use of chemicals is at its maximum in the industry history (Leclercq, Davie and Migaud, 2014; Geitung *et al.*, 2020). A variety of methods has been used to remove sea lice: chemical, mechanical, and biological. Chemical treatments through the feed or in bath, have been used in the salmon industry for decades (Burrige *et al.*, 2010). In 2017 the most common mechanical treatment used was thermal delousing, by using lukewarm seawater and turbulences (Overton *et al.*, 2019). The most commonly used chemical treatments in 2018

were emamectin benzoate in feeds, and hydrogen peroxide through baths (Jansen *et al.*, 2018). These chemical methods have led to animal welfare problems, environmental spill, higher production costs and sea lice becoming resistant to these treatments (Liu, Olaf Olaussen and Skonhoft, 2011). The need for pest control in salmon farming has led to new strategies being proposed such as lice removal technologies by using brushes or water jets (e.g., hydrolicer), bath treatments with warm or fresh water (e.g., thermolicer), passive lice control via laser technology and manipulation of salmon swimming behavior using light regimes. The biological treatment consists in using cleaner fish feeding on sea lice, and despite also being a method used by farmers for decades, only in recent years has it gained momentum as organized supply chains grow (Brooker *et al.*, 2018).

1.5. Cleaner fish

Since 2010 cleaner-fish has been considered one of the most widely adopted methods, and the only sustainable and environmentally friendly, in alternative to topic treatments on de-lousing salmonids (Imsland *et al.*, 2014a). The increasing demand for cleaner fish due to salmon industry expansion, together with increasing sea lice pressure and the possibility of the depletion of cleaner fish stocks along the Norwegian coastline, has led to industrial farming of these species. Cleaner fish production allows to control both the quality and health of deployed species and ensures the environmental sustainability of this pest management strategy (Brooker *et al.*, 2018). Currently there are two species being farmed in North Atlantic countries (UK, Ireland, Norway, Iceland, and Faroe Islands), Ballan wrasse and lumpfish (*Cyclopterus lumpus*). Even though being used for a common purpose, Ballan wrasse and lumpfish are quite distinct. Although typically cohabiting in salmon sea cages, these two species differ widely in their biology and life history (Larkum, Drew and Ralph, 2006; Bolton-Warberg, 2018). Both species are diurnal and neither species are fast swimmers such as Atlantic salmon (Geitung *et al.*, 2020). Lumpfish are relatively easy to culture and can be transferred into salmon cages at 6 to 8 months of age, however, due to health challenges during the warm summer and autumn temperatures, commercial production of lumpfish has decreased significantly over the later years. Temperatures above 10°C are detrimental for lumpfish, which will shift their distribution northwards as sea-temperatures rise due to climate change (Rodríguez-Rey and Whittaker, 2023). This holds significant consequences for aquaculture, fisheries, and

conservation of the species (Hjeltnes *et al.*, 2018). Ballan wrasse has the greatest potential as a biological preventive measure, but there is still a dependence on wild-caught fish due to difficulties associated with their culture, including production time (>1 year). Opposed to lumpfish, wrasse becomes inactive at low temperatures (6°C); thus, lice eating efficacy is minimal during winter months. However wrasse are more active and efficient in warmer temperatures when the lice is also more prevalent (Imsland *et al.*, 2014b; Bolton-Warberg, 2018; Geitung *et al.*, 2020).

1.6. Ballan wrasse

Wrasses are an extensive family of marine fish (Labridae) counting more than 500 species many among inshore areas (Skiftesvik *et al.*, 2015). In the late 1980's wrasse species from wild stock such as *Labrus bergylta*, corkwing (*Smphodus melops*) and goldsinny wrasse (*Ctenolabrus rupestris*) were introduced in salmonid aquaculture (Seljestad *et al.*, 2020). From 2008 with 1.7 million wild wrasse, to 24 million in 2017 (Winfield, 2018), Norway saw a dramatic increase in the harvest of wild stock, raising sustainability concerns among the scientific community, as there is little knowledge of the ecological consequences of such harvesting in Norway (Espeland *et al.*, 2010; Skiftesvik *et al.*, 2015; Halvorsen *et al.*, 2017). Ballan wrasse, known as bergylt in Norwegian, is the largest wrasse species found in continental waters of the north-eastern Atlantic presently ranging from southern Norway to the west coast of Morocco and the Macaronesia archipelagos (Almada *et al.*, 2017). It has a lifespan of 17 years, has the fastest growing rate among cleaner wrasse species, and can grow to a maximum length of 30 – 50 cm (Jobling, 1996; Gonzalez and de Boer, 2021). Currently Ballan wrasse is the only wrasse species farmed, and despite production being still relatively low in numbers, the production is increasing (Statistic Norwegian Directorate of Fisheries: <http://www.fiskeridir.no/Akvakultur/Statistikk-akvakultur/Akvakulturstatistikk-tidsserier/Rensefisk> , “Accessed 10 November 2021”). Ballan wrasses display a high variety of body coloration with two major color patterns, plain and spotted (Figure 1.5) (Villegas-Ríos *et al.*, 2013). Like many wrasse species, it is a protogynous hermaphrodite, sex changing species, which means that all individuals are born as females and at the age of 6 years old, some change into males. They show territorial behavior and react with aggression against competitors, especially during the spawning season, which occurs in early summer from April until August, depending on

latitude (Olsen *et al.*, 2019). Female fish exist in harems within the reproductive territory of a dominant male, with which they will mate, spawning their benthic eggs over temporary nests providing parental care and building nests (Muncaster *et al.*, 2010). Eggs are demersal and coated with a sticky external layer, with a light-white color (Ottesen, Dunaevskaya and Arcy, 2012). The preferred habitat for wrasse is shallow rocky areas (<5m) with macroalgal cover (Costello, 1991).



Figure 1.5 Spotted Ballan wrasse. (Adapted from IMR.com info site about Ballan wrasse).

1.7. Fin Welfare

Since the 1970s fin erosion has been recognized in rainbow trout (Boydston and Hopelain, 1977; Ellis *et al.*, 2009) and Atlantic salmon (Schneider and Nicholson, 1980) and is considered an important measurement of welfare of the animals (Noble *et al.*, 2007). In intensively reared fish it is a common and serious situation (Ellis *et al.*, 2008). Treasurer and Feledi (2014) suggested it to be one of the most common physical injuries in Ballan wrasse. There is a higher occurrence of fin erosion in farmed fish than in natural fish stocks. Density, feeding regime and environmental conditions are likely to be contributing factors (Mork, Järvi and Hansen, 1989). The dorsal fin is the most affected by fin erosion, it is clinically described as a degraded, damaged fin, usually shortened in length, due to friction in the tank, pathogenic infection, or aggressive attack (nipping) from other fish. In the initial phase when juveniles are offered commercial fish feeds, the levels of several dietary components, such as fatty acids, amino acids, vitamins, and minerals were recognized to affect fin erosion (Castell *et al.*, 1972; Woodward, 1984; Sprague, Dick and

Tocher, 2016). Kindschi, Shaw and Bruhn (1991) questioned if aggressive nipping could be related to nutritional deficiencies. It has been observed in trout that dorsal fin erosion is nearly eliminated with a krill meal-based feed, instead of a fishmeal-based diet. Dietary improvements could reduce aggressive behavior (Kindschi, Shaw and Bruhn, 1991; Lellis and Barrows, 1997). A behavior often seen in aquaculture with juvenile Ballan wrasse is the formation of large dense “carpets” (Figure 1.9), this can lead to fin damage and impede healing. The most common tactics to break these formations and prevent nipping have been increased water flow, altered light environments and hidings (Figure 1.7) (Dahle, Hagemann and Stenstad, 2013). Bacteria have been found to play a role in fin erosion. By isolating bacterial cultures from samples of eroded fins, bacteria were found in much greater numbers on eroding fins than on healthy fins of Atlantic salmon, however they were found to be a secondary cause of fin erosion by two separate studies examining the prevalence and distribution of bacteria on Atlantic salmon and rainbow trout (Ellis *et al.*, 2008). As described by Turnbull, Richards and Robertson (1996), seriously injured fish do not feed well, becoming weaker and liable to attack from other fish, and secondary infections (as furunculosis, caused by *Aeromonas salmonicida*). Being a recent industry, Ballan wrasse lacks the welfare privileges applied to, for example, salmon. Therefore in the last years the authorities have become more concerned with its welfare indicators (Norwegian Directorate of Fisheries, 2019, no date). This industry seeks to become self-sufficient with farmed cleaner fish. Despite being seen as the most efficient salmon lice eater, Ballan wrasse production fails to meet demand. This is due to lack of biological knowledge of the species, and its nutritional needs along the production cycle. Currently production is still troubled by slow growth, elevated mortality levels from lack of robustness, poor feeding performance and costly feeds (Barrett *et al.*, 2020).



Figure 1.6 Pectoral fin damage in juvenile Ballan wrasse (category 2).



Figure 1.7 Tank Biomar II with hidings for Ballan wrasse.



Figure 1.8 Tail fin erosion on the top fish.

1.8. Feed technology and feed source

Feed source plays an important role for nutritional condition, health, and survival for cleaner fish (Imsland *et al.*, 2020). Contrasting with most of the cultured marine fish species, Ballan wrasse lacks a stomach (agastric), pyloric caeca and also has a relatively short intestine (Le *et al.*, 2019). The expansion of aquaculture has greatly benefited from the development of formulated aquafeeds. Nowadays aquafeeds have allowed the formulation of more complex diets, using a variety of raw ingredients and additives that contribute to a better tailoring of the diets to each species (marine, freshwater), life stages (broodstock nutrition, weaning, on-growing, etc.) or to exceptional circumstances such as disease outbreak (Roques *et al.*, 2020; Hardy and Kaushik, 2022). The nutritional requirements of most farmed species (European seabass, Atlantic salmon, rainbow trout, gilthead seabream) have been extensively investigated which comprehensively gathers the nutritional requirements of each farmed species. Nevertheless, commercial production of farmed Ballan wrasse only began in the last decade and as such there is limited research on the nutrition and feed requirements for this species. The intensification of Ballan wrasse farming over recent years has now triggered the launch of research programs aiming at identifying the nutritional requirements of the species in order to adapt the current diet formulation and ultimately improve growth performance and robustness. Ballan wrasse hatcheries have historically used commercial diets for species like Atlantic cod, rich in fish meal and specifically formulated for piscivorous marine species, thus possibly lacking essential nutrients for Ballan wrasse (Hamre *et al.*, 2013; Kousoulaki *et al.*, 2015). In present time, Ballan wrasse stocked in commercial salmon pens are being fed extruded pellets placed in fine mesh bags. Challenges with this method are that the feed has been found to dissolve within hours leading to substantial wastage, compromising the validation and quantification of feed intake (Leclercq, Graham and Migaud, 2015). A recent study also demonstrated that conventional extruded feeds negatively affect survival and condition during the on growing phase for Ballan wrasse (Kousoulaki *et al.*, 2021). The extrusion process involves high temperature and pressure, which affects the physicochemical properties of the nutrients (Kousoulaki *et al.*, 2015). The agglomerated feed production technology uses lower processing temperatures and results in feed particles that apparently allow higher uptake rate of minerals in Ballan wrasse juvenile (Kousoulaki *et al.*, 2021). These authors suggest that Ballan wrasse feed

for all stages should be processed using low temperatures, as is done for cold extrusion or agglomeration technologies.



Figure 1.9 Formation of dense "carpet" in the juvenile department.

2 MATERIALS AND METHODS

2.1. Ballan wrasse Larve culture at Mowi

Mowi is one of the world's largest seafood companies and a global leader in its business. Since their establishment in 1964 they have become the largest producer of Atlantic salmon with a turnover of EUR 4.1 billion in 2019 (Mowi, 2020). Mowi Rensefisk AS is located in Stord (Figure 2.1), an island situated in the west coast of Norway. The facilities were initially built as a full cycle cod farm in 2006 and in 2010 was turned to growing only halibut (*Hippoglossus hippoglossus*). Ballan wrasse production started in 2015 proving to be challenging, with several bottlenecks hampering the deployment of high numbers of fish. Currently, Ballan wrasse aquaculture relies on acquiring wild caught adult fish for breeding stock during Spring to spawn indoors and then rear their larvae until adult phase. At hatching, the larvae are capable of swimming and feed on their yolk sac until approximately 10 days post hatch (DPH). The first feeding tanks are stocked at stocking densities varying from 111 larvae L⁻¹ (Hansen *et al.*, 2013) to 114 larvae L⁻¹. Each tank will not have larvae with more than 4 days difference in hatching. This ensures an easier weaning from dry feed later. From 3 DPH to 49 DPH larvae are fed a regime of live feed such as rotifers (*Brachionus sp.*), Cryoplankton Large (*Semibalanus balanoides*) and *Artemia*. (D'Arcy *et al.*, 2012). From 49 DPH to 53 DPH, the larvae are co-fed *Artemia* and dry formulated feed. From 54 DPH larvae are exclusively fed with formulated dry feed (Trofi/Otohime and Trofi/Biomar Symbio). Once the Ballan wrasse have been weaned onto dry feeds, the fish are commonly referred to as juveniles, meaning that they have reached a similar physiological stage to that of the adults but still lack mature sexual organs. The whole cycle takes approximately 18 to 22 months when Ballan wrasse reach 40 to 80 g, including 2 to 3 months with live prey feeding and weaning, with the prolonged length of production being seen as one of the main bottlenecks.

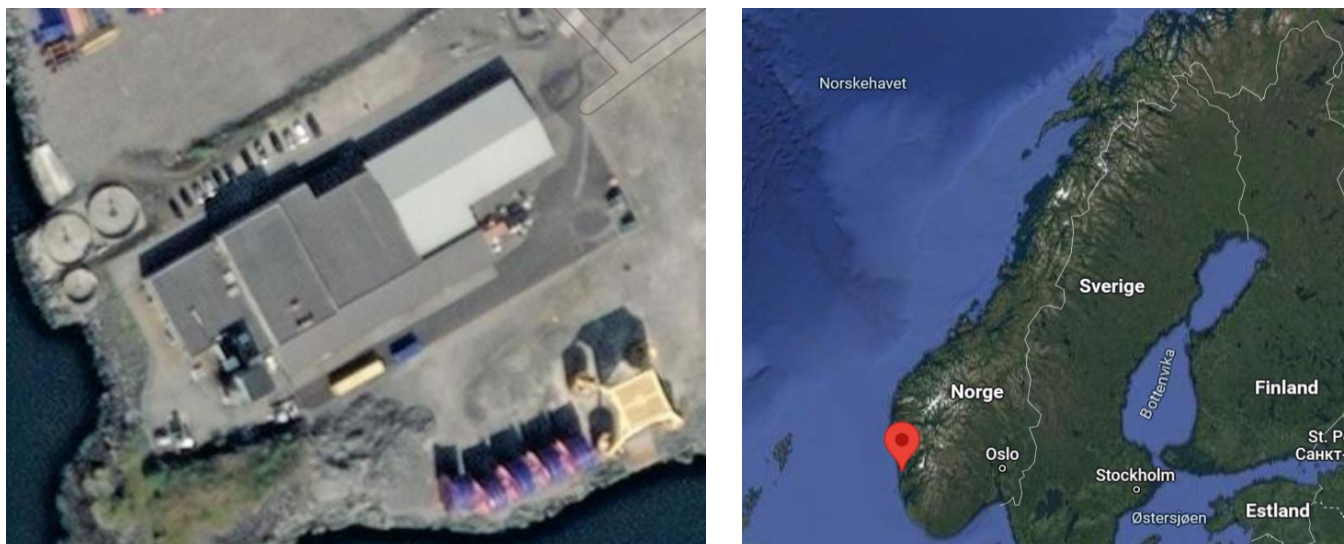


Figure 2.1 Mowi Rensefisk AS at the right picture and Stord at the left picture (Simple view, 2022).

2.2. Experimental design

The experiment began in mid-April 2023 and lasted until the end of July 2023. The first part of the experiment started when larvae were 50 DPH in the larvae department, using three tanks with 7 m³ at the beginning of the weaning phase. The second part continued in the juvenile department when the fish were with a nano counter pumped into 16 m³ juvenile tanks and simultaneously counted with a Vaki nano counter until reaching circa 130 000 fish per tank. Fish were divided in two different groups: two tanks being fed with Biomar SYMBIO (Biomar II and Biomar III) feed and one tank with Otohime feed (control tank). In Biomar II hidings were inserted during the juvenile phase to follow the behavior of the fish, considering that hidings are commonly used in salmon sea cages. During this phase, the juveniles were reared until approximately 0,7g. Thereafter juveniles were graded with an automatic grading machine and separated into 2 groups (>0,6 g and <0,5 g). The department where these tanks are held is equipped with LED light comprehended between 500 to 600 lux. Fish were fed during 24h of the day, with an automatic dosing TORP feeder, where the dosage was given according to the volume and appetite, using a commercial food pellet (Otohime, Pacific Trading Aqua, Ireland and Biomar SYMBIO). Throughout the four-month period in the juvenile department the daily routines were as follows: tanks were cleaned, and dead fish were recorded, levels of dissolved oxygen, temperature and salinity were determined with an Oxyguard Handy probe and a VWR EC300 salinity meter (ca.35 ppt). The juvenile department used a flow-

through system supplied with sea water at temperature 14 ± 1 °C. Daily and periodic measures were implemented to ensure the health and welfare of the fish throughout the whole cycle of production, so that fish can express their natural behavior as much as possible. During the whole production cycle this species was not vaccinated. At an early stage of life, it is vital to fulfil larval nutritional requirements during larval development. Following larval growth, feed was adapted to different particle sizes and nutritional values. The composition of the feeds used is described by table 2.1.

Table 2.1 Composition of Trofi, Otohime and Biomar feed.

Feed	Type	Size (µm)	Proteins (%)	Lipids (%)	Ash (%)	Fiber (%)	Phosphorus (%)
Trofi	AgloNorse Extra	300 to 500	59	18	10	?	6
Otohime	B2	360-650	55,8	14,9	15	2,8	2,2
	C1	580-840	55,1	14,3	16,4	2,9	2,4
Biomar	Symbio plus	500	52	10,2	13,1	2,3	1,98
	Symbio plus	800	52	10,2	13,1	2,3	1,98
	Symbio plus	1100	52	10,2	13,1	2,3	1,98

Ingestion of microparticles is triggered by visual and chemical stimulation, therefore extrinsic characteristics might influence differently larvae appeal (Cahu and Zambonino Infante, 2001). Trofi and Otohime feeds were part of the protocol already used at these facilities, while Biomar was the test feed. These feeds are very distinct from each other: Trofi is an agglomerated feed while Otohime and Biomar are cold extruded feeds, a type commonly used in production. Biomar Symbio is a recently developed feed, specifically designed with a complete nutritional profile for hatchery reared Ballan wrasse. This feed also contains Bactocell that is a specific probiotic based on viable cells of a strain of *Pediococcus acidilactici* that will stabilize microbiota balance and enhance digestive function. Whereas Otohime is specially formulated to meet the needs of marine fish, such as flatfish and red sea bream. The feeding protocol was adapted accordingly to each feed and larval/juvenile stage. In larval department, each tank is equipped with two different feeders. The Micro feeder feeds under water and the TORP feeder feeds on the surface of the water. One supplies feed to the fish under water for 24 hours, while the second is placed on the surface of the water, and releases determined amount of feed within hourly intervals. All feeding transitions were made roughly at the same fish size for each feed. When larvae were sampled for the determination of gut content, more than 80% of the samples should present consumption of the current feed, before changing to the next feed. The tanks L2 (Biomar II) and L3 (Biomar III) start with a mix of Trofi/Biomar, and tank L1 (control tank) with a mix of Trofi and Otohime. Mixed feeds are used due to the agglomerated characteristics of Trofi, which will clog the TORP feeder if not mixed with a drier feed.

2.3. Sampling procedure

Since the beginning of the weaning period (50 DPH), samples of 30 fish were taken regularly to follow the length (mm), and the progression of the fin erosion as a welfare indicator, since this has been one of the major problems in the juvenile department (Table 2.2 and Figure 2.2). The fish were caught randomly using a small fish net and placed in a bath with an overdose of powdered Fiquel anesthetic (0,14 g/l) for euthanizing. In each sample collection, additional information from each tank was recorded for average weight (g) by weighing each sample, followed by counting the individuals in each sample, then

dividing the sample weight by the number of individuals (average weight of the sample/number of individuals in the sample = average weight of the fish in the tank).

2.4. Weight determination

At 50 DPH in larvae department dry weight was determined, and at 65 DPH wet weight was determined after moving into juvenile department. At 50 DPH, 30 larvae were euthanized, rinsed with freshwater, and then put in pre-weighed tin capsules, one larva in each capsule. The samples were dried at 60°C for 24 hours, and afterwards carefully set on a plate (previously tared) and weighted on a microscale. During juvenile phase, weekly samples were taken to find the average weight. A bucket was filled with sea water and tared; afterwards fish collected with a net were placed inside the bucket for weight determination and counted back into the original tank.

Table 2.2 Scores and definitions of fins welfare indicators used in Ballan Wrasse.

	Score	Definition
Fins (tale and breast)	0	No erosion, splitting or rays exposed.
	1	Minor erosion or split damage.
	2	Erosion and spilt damaged are more widespread. Be consider at high risk.
	3	Clear evidence of erosion or split damaged on fins, recovery unlikely, health status compromised.

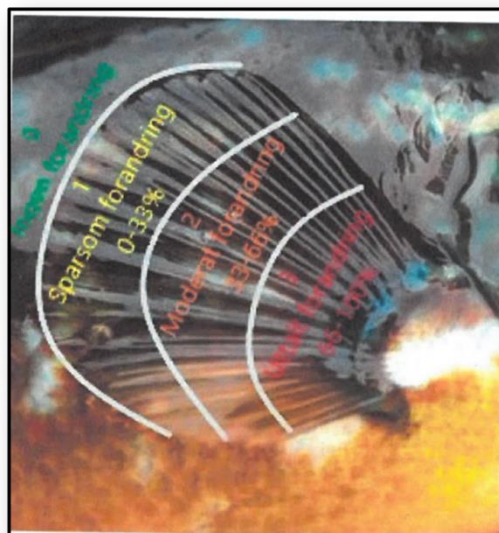


Figure 2.2 Fin erosion categories for Ballan wrasse as a welfare indicator.

2.5. Standard length (SL)

Ballan wrasse larvae were sampled for standard length (SL; mm and cm) every week since the beginning of the weaning period until sorting. More specifically, SL samplings took place at 50, 65, 70, 85, 95, 110, 125 and 135 DPH. First measurement was carried out using a Zoom Stereomicroscope SMZ1000 (Nikon Instruments Inc., NY, USA). Afterwards, when sampled fish reached an average of 0,7g, they were measured with a ruler. SL was measured along the midline of the body from the tip of the snout to the posterior end of the last vertebra (Figure 2.3).

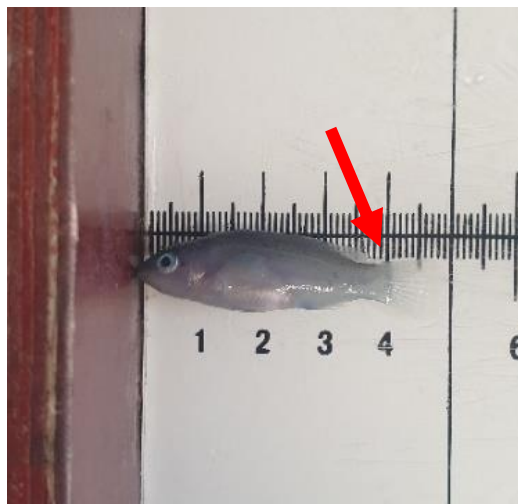


Figure 2.3 Ballan wrasse juvenile being measured with a ruler until the last vertebra.

2.6. Mortality rate

The mortality in all tanks was registered daily throughout the whole experiment. While the tanks were cleaned the dead were filtered into a bucket, afterwards they were counted and subtracted from the initial total amount of the respective tank. The dead fish were also checked for deformities two times per week.

2.7. Statistics Analysis

Statistical analysis and data analyzed were performed using Microsoft 365 Excel 2022, and R-studio 2021 (version 4.1.2). Normality of variances in the data were confirmed using Shapiro-Wilk test (Zar, 1999). Data were analyzed by two-way Analysis of Variance (ANOVA), with days post hatch and type of feed as factors. Tukey's post-hoc test was applied when relevant ($P < 0,001$), for fins status. The student's t -test was performed for average weight and length at 135 DPH, to determine the presence of significant difference between treatments. All treatment effects were considered significant at a significance level of $P < 0.05$. Besides, specific growth rate (SGR) (Kottmann *et al.*, 2023) and condition factor (K) (Cavrois-Rogacki *et al.*, 2019) were calculated according to the formula:

$$\text{SGR (\% day}^{-1}\text{)} = 100 * (\ln (\text{final biomass, g}) - \ln (\text{initial biomass, g})) / (\text{time, days})$$

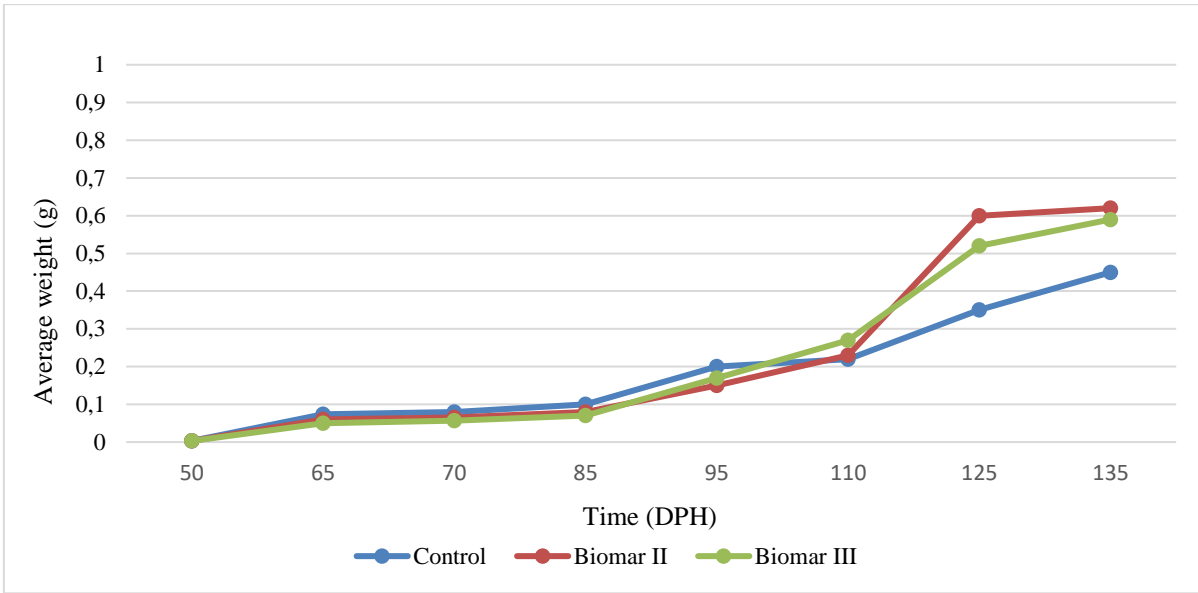
$$\text{Fulton's condition factor (K)} = (\text{weight}) / (\text{length}^3) * 100$$

3 RESULTS

3.1. Growth rate performance

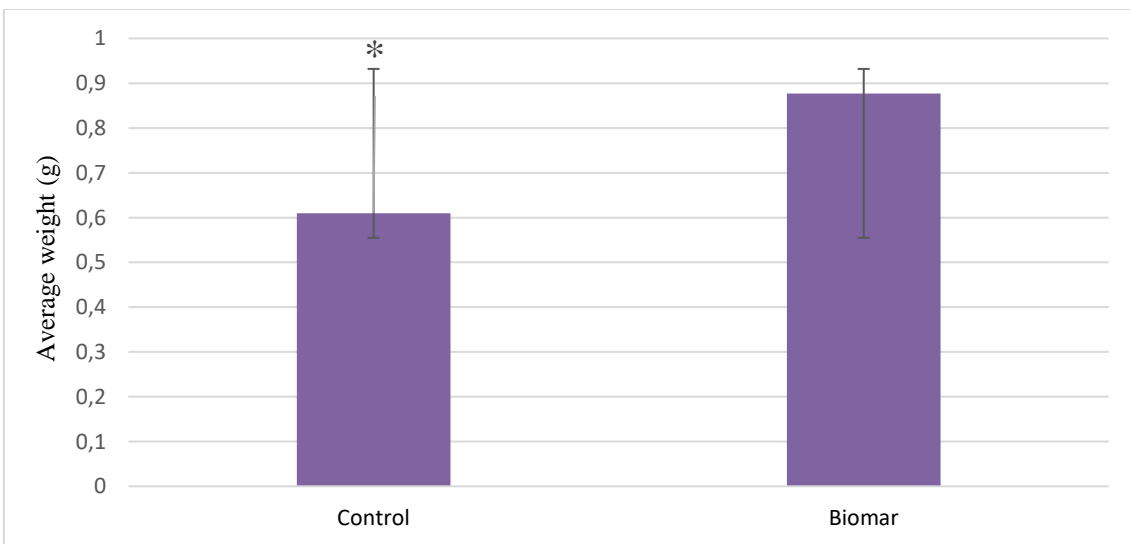
3.1.1 Average weight (AW)

While all fish groups began the study having an equivalent average weigh, at 65 DPH the control group had an increase of 0,07g when compared with the group Biomar II and III which both had an increase of 0,05g. However, despite Biomar groups showing a slower growth rhythm in the beginning these recovered after two months (110 DPH) reaching an average weight of 0,55g contrasting with 0,35g in control group (Graph 3.1). Growth rate (%/day) was calculated for all tanks, considering the whole experimental period. The groups Biomar II and III had a grow rate of, 6,62% day⁻¹ and 6,66% day⁻¹ respectively, while the control tank presented a growth of 6,25% day⁻¹ (Graph 3.3).

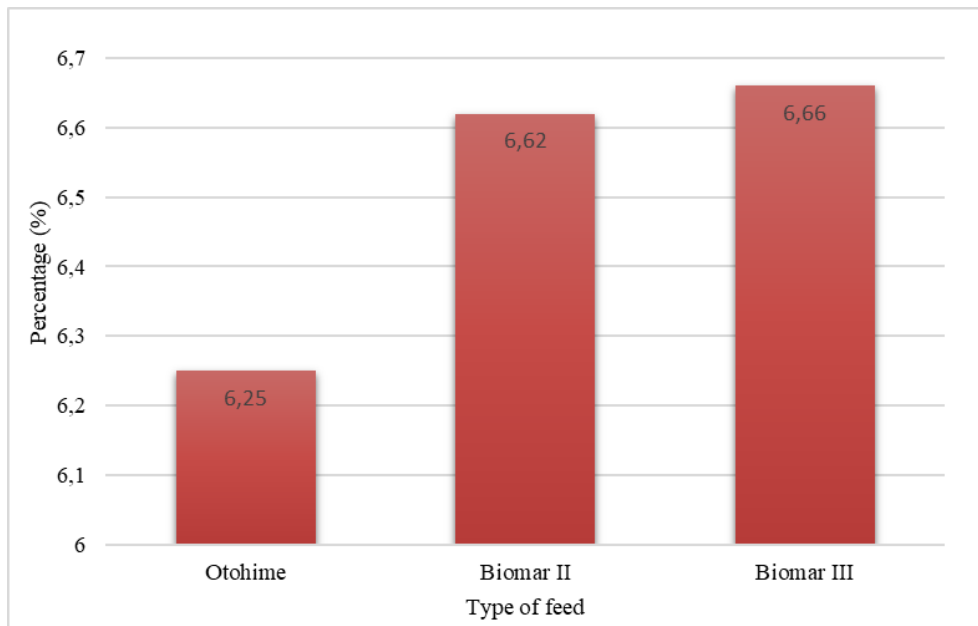


Graphic 3.1 Average weight of Ballan wrasse during juvenile development for control and Biomar tanks during the trial.

At 135 DPH a Student *t*-test was performed and it was proven that average weight of Biomar fed juvenile fish were significantly higher than control group (Student *t*-test, $p < 0.001$) (Graph 3.2).



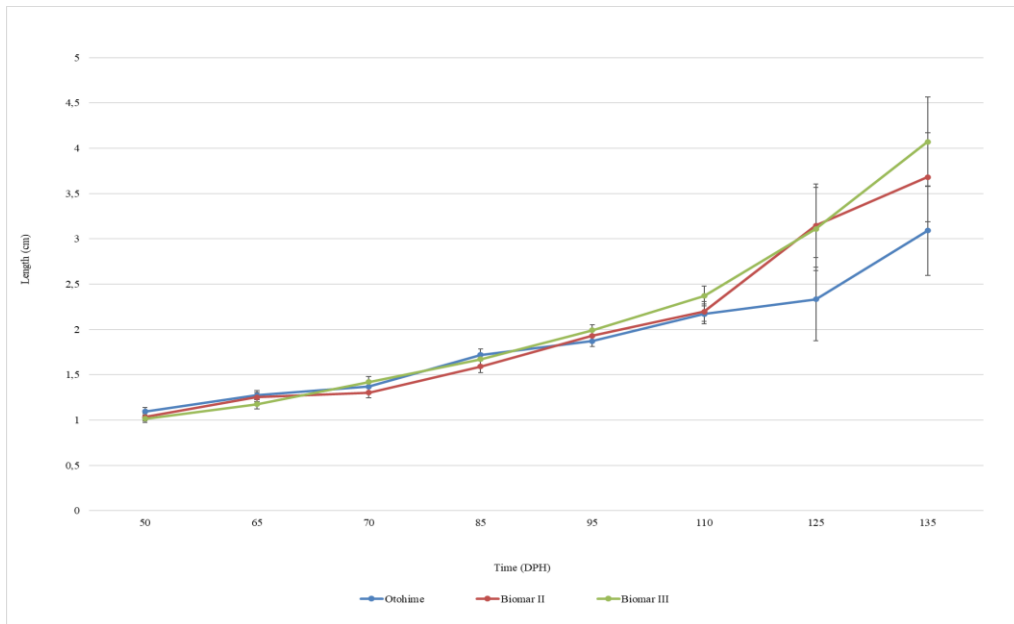
Graphic 3.2 Ballan wrasses mean weight at 135 DPH, after being fed with two different diets. Data represented as mean \pm standard deviation. * Stands for statistically significant differences between treatments (Student *t*-test, $p < 0.001$).



Graphic 3.3 Ballan wrasse Specific Growth Rate (%) for control and Biomar tanks during the trial.

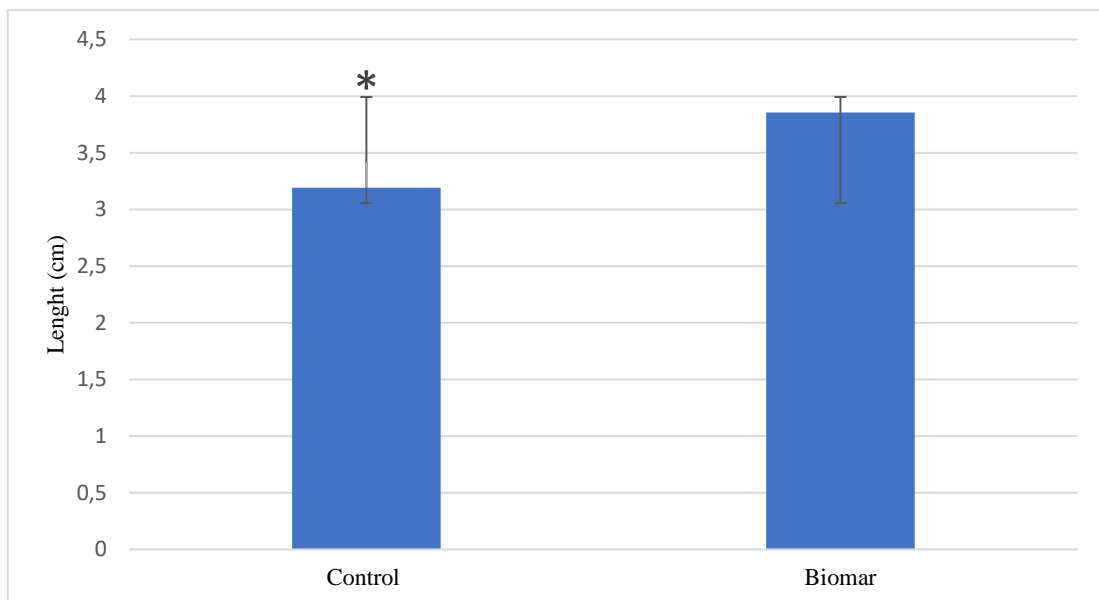
3.1.2 Standard Length (SL)

From the beginning of the experiment, until 95 DPH, all tanks showed a similar gradual growth rhythm in terms of SL (Graph 3.4). From that point onwards the fish in the Biomar treatment registered a substantial increase in growth compared with the Otohime group, reaching a difference of 0,06 cm between treatments. At 125 DPH the control tank showed a slight decrease in SL due to an incorrect sample. At the end of the trial, the fish in the Biomar treatment showed a considerable size difference, when compared with the control tank.



Graphic 3.4 Ballan wrasse larvae standard length for control and Biomar tanks during the trial.

It was possible to prove that the average standard length of Biomar fish was significantly higher than the control group, fed with Otokime at 135 DPH. (Student *t*-test $p < 0,001$) (Graph 3.5).



Graphic 3.5 Ballan wrasses mean length at 135 DPH after being fed with two different diets. Data represented as mean \pm standard deviation. * Stands for statistically significant differences between treatments (Student *t*-test, $p < 0.001$).

3.1.3 Condition factor

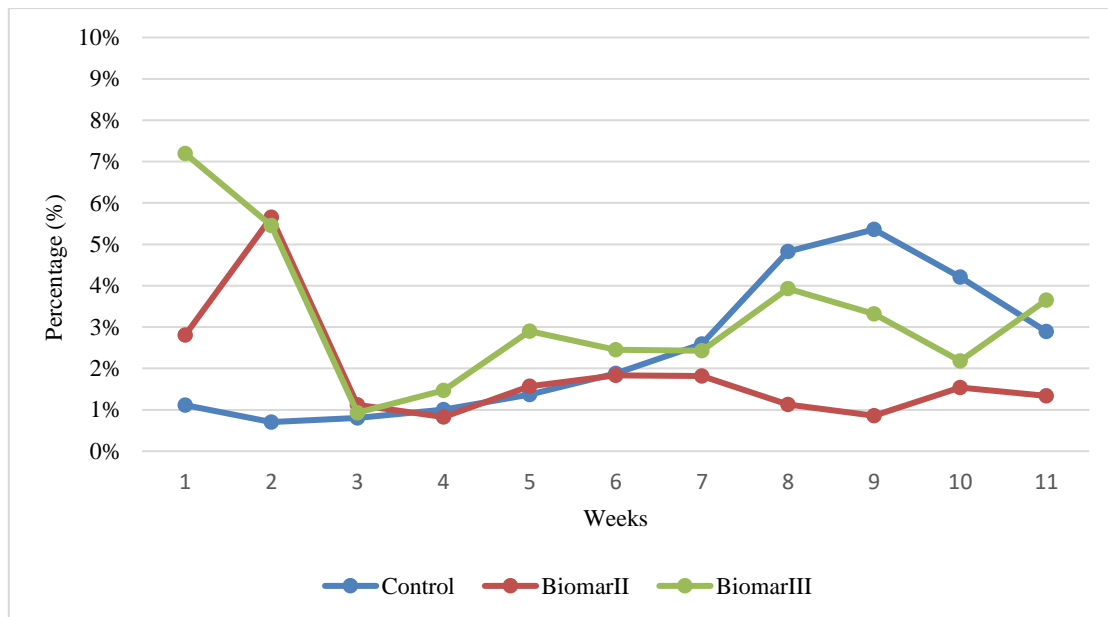
When calculating the condition factor, it is possible to observe that the fish in the control tank had a significantly higher K-factor than fish fed with Biomar until 95 DPH (table 3.1). Afterwards this pattern reversed and the fish in Biomar tanks showed an increase in condition factor from 0,046 to 0,115 g cm⁻³ (SD: 0,48) in Biomar II, and from 0,062 to 0,147 g cm⁻³ (SD: 0,35) in Biomar III, whereas the condition factor for the control group decreased from 0,122 to 0,104 g cm⁻³(SD: 0,30).

Table 3.1 Condition factor between control tank (Otohime) and Biomar tanks.

	Otohime	Biomar II	Biomar III
50	0,00000286	0,00000287	0,00000291
65	0,01959346	0,01099624	0,00770126
70	0,01989556	0,01242400	0,00647363
85	0,01965236	0,01273734	0,00736452
95	0,12233907	0,04694635	0,06234298
110	0,10420507	0,11478656	0,14785849
125	0,33750000	0,69304833	0,46744322
135	0,88851443	1,32133915	1,01079897

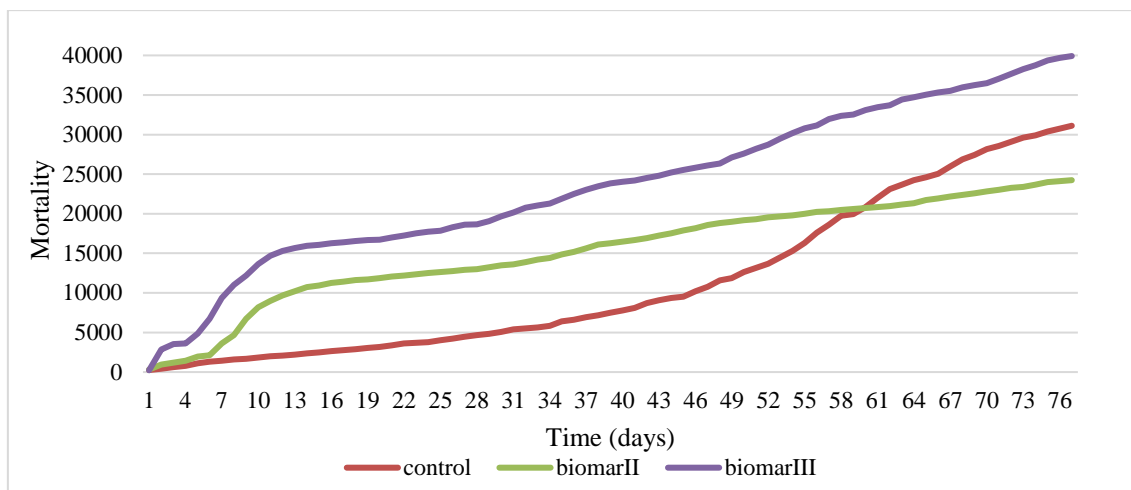
3.2. Mortality rate

In Ballan wrasse, higher mortality rates during production are expected, due to the lack of knowledge on this species biology and culture protocols. Dead fish were counted every day from each tank, registered and subtracted from the total number in the tank. Mortality rates were monitored, and the dead fish were categorized into four groups, according to the main injury: losers, tail bites, inflamed anus and unspecified.



Graphic 3.6 Weekly mortality rates (%) of Ballan wrasse for control and Biomar group feedings after moving into juvenile department until reaching 1g.

Both groups exhibited high peaks of mortality (Graph3.6). Biomar tanks showed higher mortality rates after moving into the juvenile department (circa 65 DPH) compared with the control group. Control group had an increase in mortality after ca. 8 weeks. This coincides with the size change of the Otohime feed (control group), from B2 size to C1, meaning that the stronger larvae will out compete the slower growing larvae in the tank, possibly translating into this increase in mortality. The Biomar II showed a pronounced drop of mortality on the third week, right after the first peak, continuing with a low mortality rate until the end. Biomar III began with a very high mortality rate during the first week, dropping drastically during the second week, continuing with a moderately high mortality rate, and presenting some minor peaks until circa 140 DPH (Graph 3.6).



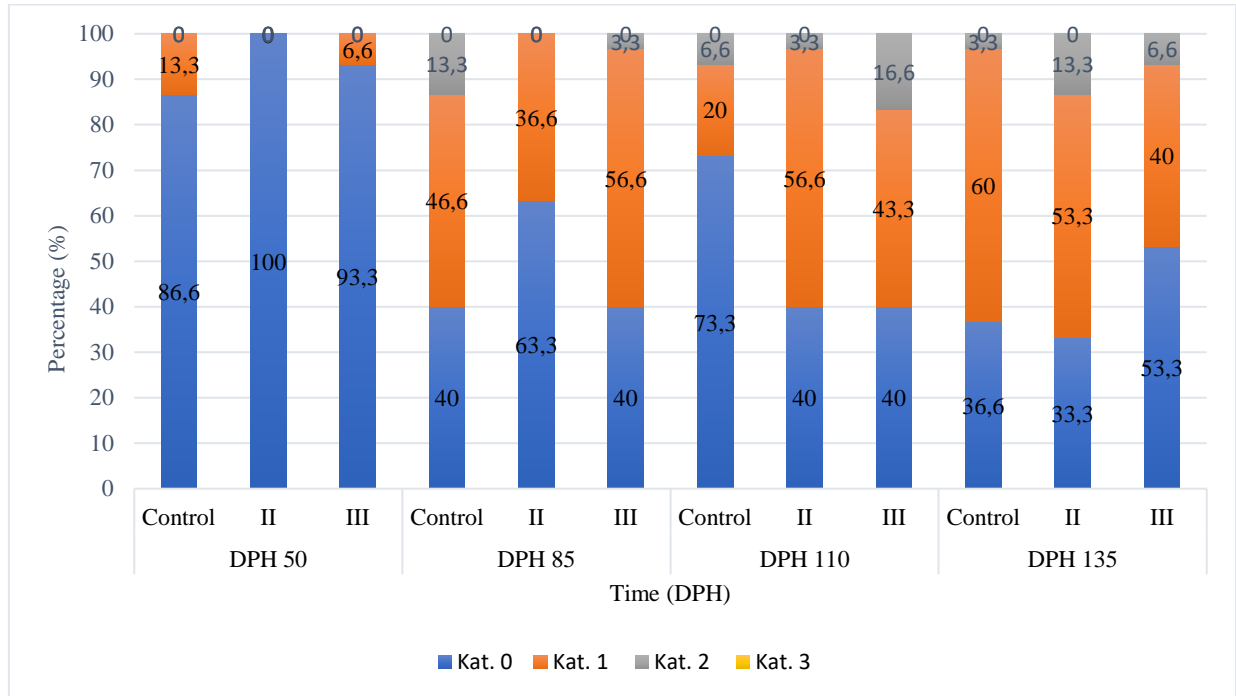
Graphic 3.7 Cumulative Ballan wrasse larvae mortality, after transfer to juvenile department onwards.

During the trial mortality was present throughout the time, however the control tank had a lower mortality rate than the Biomar groups immediately after the transfer to juvenile department. However, when plotting the cumulative mortality (Graph 3.7) the control group showed slightly higher mortality than Biomar II after 58 days (8 weeks on the daily mortality graph 3.6). Biomar III showed the highest cumulative mortality compared to the other tanks.

3.3. Fin quality categories

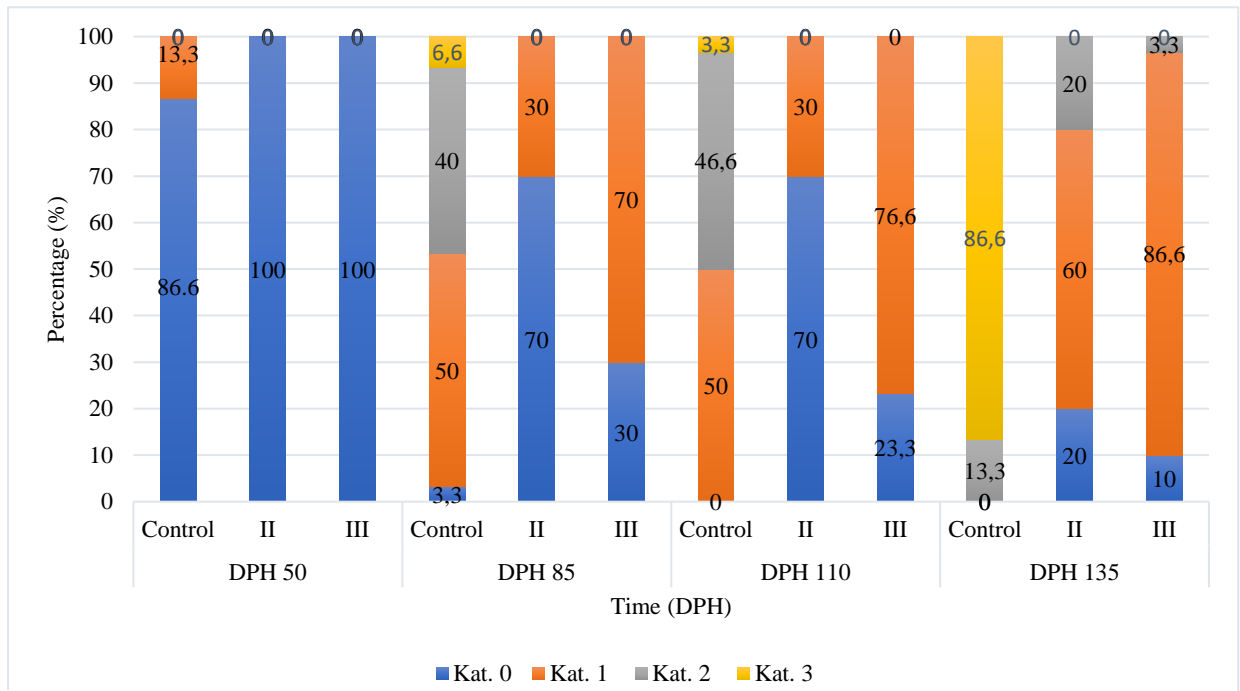
Juvenile period is the phase when most problems related to fin erosion occurred. During 2,5 months fin erosion was followed up and recorded, according to table 2.2. The graphs representing caudal fin damage (Graph 3.8) show a trending decrease of category 0 (Figure 3.1) fins from the first sampling to 135 DPH. Despite different treatments presenting similar patterns, the Biomar II treatment showed a more stable decrease of category 0 fins, compared with control tank and Biomar III, where more pronounced peaks were registered. Fish in all three tanks showed little signs of caudal fin damage (Figure 3.2) in the beginning of the trial, with only category 0 and category 1 fins being registered in control tank and Biomar III. However, control group showed a higher rate of category 2 fins at 85 DPH, and Biomar III treatment at 110 DPH. Biomar II group

showed higher percentage of category 2 fins at 135 DPH, this could be related with stress caused by the removal of the hidings at circa 110 DPH.



Graphic 3.8 Percentage for caudal fin damage categories, of Ballan wrasse fed with different feeds and along the whole the experiment (Monitored at ages 50, 85, 110 and 135DPH).

Regarding pectoral fins, there was no marked differences registered between left and right pectoral fins within the same tanks (Graph 3.9 and 3.10). Fish in the control treatment showed a major decrease in fin quality, with practically no occurrences of category 0 fins within the first month and a stable number of category 1 and category 2 (Figure 3.4) fins until 110 DPH, before a dramatic deterioration in fin quality occurred at 135 DPH, after which the majority of the fish showed category 3 fins (Figure 3.3).



Graphic 3.9 Percentage for pectoral fin (left) damage categories, of Ballan wrasse fed with different feeds and along the whole the experiment (Monitored at ages 50, 85, 110 and 135DPH).



Figure 3.1 Ballan wrasse juvenile (pectoral fin category 0).



Figure 3.2 Ballan wrasse juvenile with caudal fin erosion.



Figure 3.3 Ballan wrasse juvenile with pectoral fin erosion category 3.

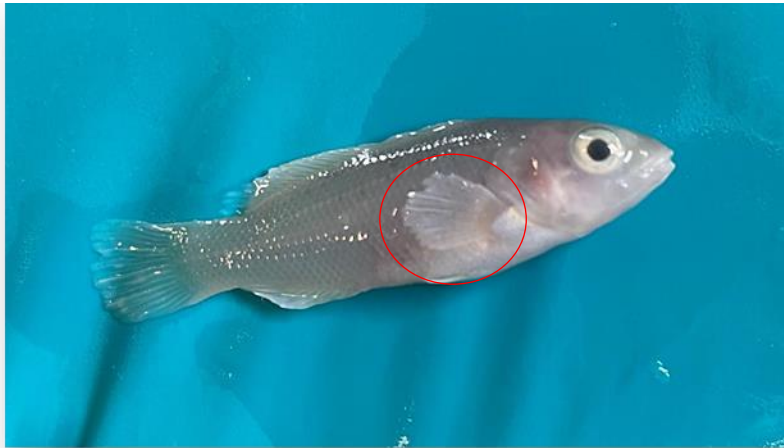
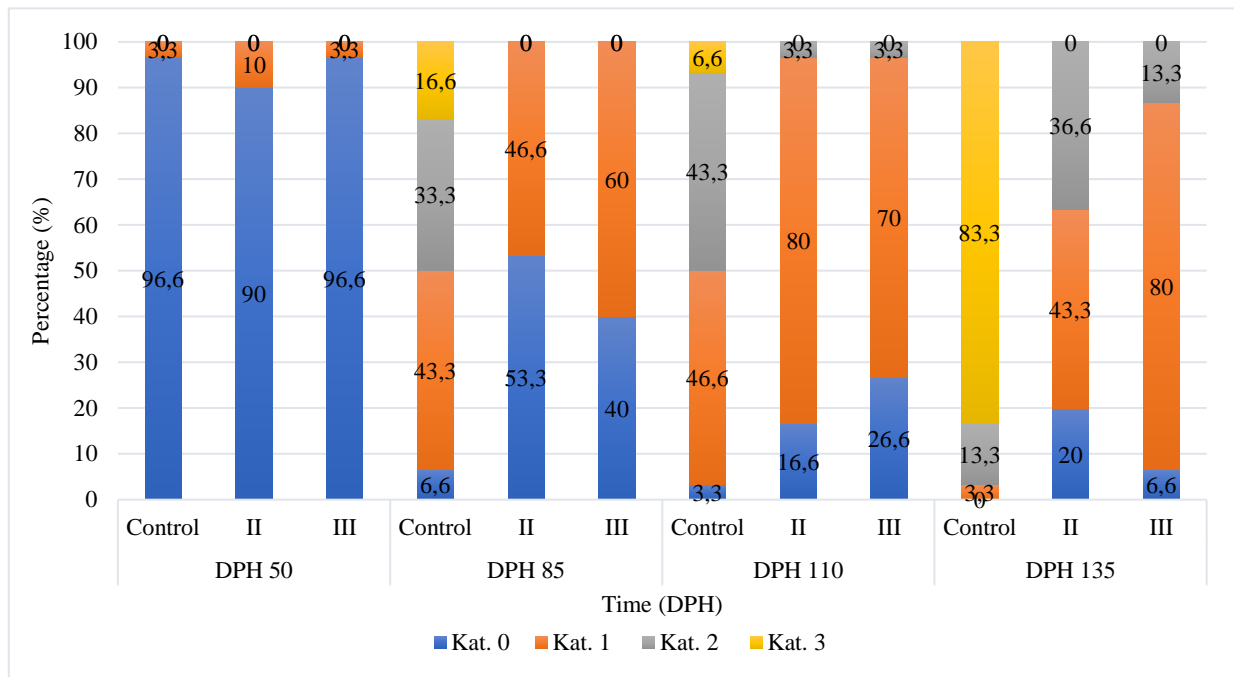


Figure 3.4 Ballan wrasse juvenile with pectoral fin erosion category 1.



Graphic 3.10 Percentage for pectoral fin (right) damage categories, of Ballan wrasse fed with different feeds and along the whole the experiment (Monitored at ages 50, 85, 110 and 135DPH).

Fish in Biomar tanks showed a slower decrease in fin quality, with Biomar II showing an overall better-quality registry. However, both tanks presented a slow decrease of category 0 alongside an increase of category 1 and 2 fins with time, followed by a stable percentage of category 1 fins until the end of this phase. The interaction between the days post hatch and the two types of food has a significant effect on the pectoral fin damage (Two-way ANOVA, $p < 0.001$) (Table 3.2 and 3.3).

Table 3.2 Two-way ANOVA results for the right pectoral fin category data.

	Df	Sum Sq	Mean Sq	F Value	Pr(>F)
DPH	1	116.66	116.66	386.36	< 2e-16 ***
Tank	2	59.01	29.50	97.71	< 2e-16 ***
DPH: Tank	2	24.10	12.05	39.91	2.34e-16 ***
Residuals	354	106.89	0.30		
Signif. codes:					
0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					

Pectoral fin data, for right and left fin, was further analyzed through by Tukey's post-hoc test, revealing significant differences between Control group and Biomar groups ($p < 0,05$). Besides, between Biomar tanks II and III, statistical similarity was proven in both cases (table 3.4 and 3.5).

Table 3.3 Two-way ANOVA results for the left pectoral fin category data.

	Df	Sum Sq	Mean Sq	F Value	Pr(>F)
DPH	1	110.06	110.06	448.18	<2e-16 ***
Tank	2	70.52	35.26	143.58	<2e-16 ***
DPH: Tank	2	25.60	12.80	52.12	<2e-16 ***
Residuals	354	86.93	0.25		
Signif. codes:					
0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					

Table 3.4 Tukey multiple comparisons of means for left pectoral fin damage and DPH between control and Biomar tanks.

	diff	lwr	upr	p adj
Tank II-Control	-0.96666667	-1.11723743	-0.8160959	0.0000000
Tank III-Control	-0.90833333	-1.05890409	-0.7577626	0.0000000
Tank III-Tank II	0.05833333	-0.09223743	0.2089041	0.6331813

Table 3.5 Tukey multiple comparisons of means for right pectoral fin damage and DPH between control and Biomar tanks.

	diff	lwr	upr	p adj
Tank II-Control	-0.84166667	-1.0086334	-0.6746999	0.0000000
Tank III-Control	-0.87500000	-1.0419667	-0.7080333	0.0000000
Tank III-Tank II	-0.03333333	-0.2003001	0.1336334	0.8854794

4 DISCUSSION

To fight sea lice outbreaks the salmon industry is looking to increase the use of Ballan wrasse, and in order to reduce pressure on wild stocks of this species it is now turning to farmed fish. The increasing demand for farmed Ballan wrasse raises the awareness concerning the quality of the fish being farmed. However, production is facing several challenges with slow growth, poor welfare and high mortality preventing farmed Ballan wrasse from being more widely used (Hamre *et al.*, 2013; Kousoulaki *et al.*, 2015). As with other marine finfish species, intensive production often brings increased number of malformations and abnormalities which can lower the fish condition and its growth performance (Boglione *et al.*, 2013). After deployment to salmon cages, fin deformities can compromise their welfare and performance and lead to death. The increase in production of this species has raised the attention by the Norwegian Food Authority who are following up the companies producing Ballan wrasse to ensure improved welfare of this species. Currently deformities in jaws, operculum, spinal malformations, and fin erosion are being reported by the hatcheries and salmon farmers. However, there is to the authors knowledge no published data describing fin erosion and its prevalence among farmed Ballan wrasses. This study has focused on Ballan wrasse fin welfare, considering the lack of specialized feed for this species, by comparing the effects of a feed not specific for Ballan wrasse against a newly developed feed for the specie. Since this trial took place in a commercial production, it was not possible to have three replicants of each treatment due to the risk it represented to the company.

Growth rate and average weight were similar between the three tanks until circa 110 DPH when the feed was mixed with a bigger grain to accommodate for the fish growth. Here, fish in the Biomar tanks showed more robustness by swimming against the current and had a better development from calculating the growth rate. When analyzing the samples for each tank at 135 DPH, the control group showed a higher range of fish below 0,5 g compared with the Biomar groups. More precisely, in the 30 fish sampled from each Biomar group, there was only one fish below 0,5 g in each sample, whereas the control group showed ten fish below 0,5g in the same number of 30 sampled individuals. As the minimum weight considered worth keeping for production is 0,5 g, this means the control group is producing a higher number of fish which will later be discarded during sorting

due to size. At this point Biomar groups were transferred to a different facility without being sorted due to lack of space for the current production volume, therefore not allowing to follow their development. According to Leclercq *et al.*(2010) heavier individuals of Atlantic salmon tend to display a positive allometric relationship often resulting from a higher condition factor. Data from this study showed that Ballan wrasse somatic growth and following weight gain was improved in the Biomar groups compared to the control tank, producing heavier individuals and proportionally growth. Farmed fish which are fed freely can still present a reduced swimming activity compared to their natural habitat, therefore resulting in a higher condition factor (Skiftesvik *et al.*, 2013).

Mortality rate during juvenile phase of Ballan wrasse, as for most other marine farmed fish species, is a critical issue which restricts hatchery productivity. When compared to other intensive farmed species, Ballan wrasse has higher mortality rates. During the experiment both Biomar groups registered high mortality during the first weeks following the transfer to the juvenile department, contrasting with the control group which presented a lower mortality rate during the same period. A likely reason for this difference in mortality may be the age of each tank at the time of the transfer. Control, Biomar II and Biomar III groups were transferred at 67 DPH, 63 DPH and 61 DPH, respectively. Larvae are usually transferred at 67 DPH up to 70 DPH when they are considered more robust, and they are big enough that they will not pass through the sieve (1,5 mm) covering the water drainage at the bottom of the tank, suggesting that Biomar groups could benefit from staying longer in the larvae department, and a longer weaning phase before transfer to the juvenile department. The reason why Biomar groups were transferred earlier was due to the cleaning schedule of the larval department that needed to be performed in time to receive the new generation of larvae, which at this point was close to spawning. Also, the high mortality rate in group Biomar III was possibly due to an incident of water stoppage that happened in consequence of a malfunctioning pump during the experiment, considering that the larvae in Biomar III were younger and not as resilient as the ones in Biomar II and control groups. Hidings were introduced in tank Biomar II to study the fish behavior in this phase, seeing as hidings are used for other species such as Grouper (Teng and Chua, 1979). The tail fin evaluation showed that the hidings were helpful in reducing tail bites, a common behavior among Ballan wrasse which can be aggressive towards weaker individuals (Jones, 1984; D'Anna *et al.*, 2012; Brooker *et al.*, 2018). By comparing Biomar II with Biomar III and control groups the data showed a considerable

improvement in tail fin quality until circa 110 DPH. At this time the hidings were removed due to water quality deterioration in Biomar II tank, as well as to combat the formation of fish clumps above the hidings. After this, it is possible to observe on graph 3.4 that the tail fin quality slightly declined in Biomar II fish. It was observed that caudal fins in the control group were slightly more eroded compared to Biomar groups during the beginning of the trial. However, there were no significant differences between the three groups throughout the trial that could be attributed to the feed. Separate evaluations were performed on left and right pectoral fins with the intention of determining if there were any major differences in damage between them. The idea was to see if there was more damage on one of the sides, which could be connected to the fish rubbing against each other while clumping. However, no major differences were observed between left and right pectoral fins. Since the beginning of the weaning (50 DPH) there could be observed some damage, however small, to the pectoral fins. In the span of the first month of the trial (85 DPH) there was a somewhat dramatic increase in pectoral fin erosion on fish from all tanks, however fish in the control tank had an exceedingly high increase, including all four damage categories. In contrast, neither of the Biomar groups registered more than two damage categories (categories 0 and 1) during the same period. At 110 DPH the fish in the control tank continued its deteriorating tendency with category 0 being practically nonexistent. At this time fish in both Biomar tanks began showing category 2, however this coincided with a dramatic increase in biomass compared to the control tank. At 135 DPH fish in the control tank was worsening with the majority of the fins being category 3, while both Biomar groups registered a visible increase of category 2 and no category 3. The pectoral fin damage between fish from control and Biomar tanks had a drastically visible difference, however it is unknown if this is due to feed quality which may be promoting better conditions for mucosa maintaining good fin health, or due to the discrepancy in the number of fish in each tank due to counting error during transfer (Ellis *et al.*, 2008; Mori *et al.*, 2021; Nimalan *et al.*, 2022). Overall, when comparing to the control group, Biomar groups showed positive results with a faster development from 110 DPH. Both Biomar groups had a pronounced increase in biomass, better fin health and higher length and average weight, seeming to be more robust. During this test there were some technical problems such as a failing water pump and transferring the test subjects at an earlier age than the standard, which may be related to the high cumulative mortality rate in Biomar III group. The failing water pump which needed replacement caused the water circulation to stop in all tanks for 4 hours, this caused stress

and in addition, feeding was interrupted in advance to prevent water quality from deteriorating during the repairs. Furthermore, this was the first production round using the fish nano counter during the transfer from larval department to the juvenile department and the newness of the system caused a problem with overcounting the larvae. Later during sorting with a micro counter that has been in regular use and the department technicians were accustomed to, the following discrepancies were found; the three tanks were supposed to have circa 130 000 fish each (8000 fish/m³), however the control tank had circa 116 000 fish, Biomar II had circa 88 000 fish, and Biomar III had circa 90 000 fish. Regarding the fins, when the fish were transferred to juvenile department the water flow was greatly reduced in order to help them adjust to the tank and to prevent them from being sucked down by the water drainage at the bottom of the tanks. Water flow is then gradually increased by hand via a valve, and only after approximately one month is the water flow accurately measured and corrected to reach circa 50 minutes water exchange. This is a possible factor concerning the fin health because this may be worsening the existing damage from biting (Ellis *et al.*, 2008). Also, the cleaning arms circulating around the bottom of the tanks needed replacement, slightly reducing their effectiveness, and affecting the water quality therefore increasing the possibility of bacterial damage to the fins. Despite there not being a noticeable difference in mortality between control and Biomar groups, it is worth noting that Biomar groups produced fish that seemed overall visibly healthier, and it would be relevant to continue testing Biomar feed to determine its efficacy in Ballan wrasse farming.

5 CONCLUSION

Currently, the commercial production of Ballan wrasse juveniles is hampered by low survival, slow growth, poor feed efficiency and lack of robustness. It appears, given that this is a new species to culture, that these bottlenecks are the consequence of sub-optimal rearing conditions and non-optimized diets. A lot needs to be done to improve the farming of this fish in order to make it an economically viable option to sea lice treatments, especially considering that Norwegian authorities are tightening regulations concerning the amount of chemicals used in salmon cages to fight sea lice. Unfortunately, it was not possible to further develop this study due to budget limitations, and production targets. Also due to the high survival rate in the larval department, keeping the total amount of

the produced fish in these facilities was unsustainable, which in the end determined the need to transport a portion of the production to other rearing facilities. It is important to mention that these tanks were especially attributed to this study and that it was allowed to use a lower number of individuals (130 000 per tank) than standard (180 000 per tank). At the end of this trial, it was possible to conclude there were notable growth differences between the fish in Biomar tanks and the control group, which could allow to reduce the total production time of Ballan wrasse, therefore allowing a faster deployment of this cleaner fish into salmon sea cages and an economically viable production. Concerning the fin damage, Biomar groups were visibly better compared to the control group, improving Ballan wrasses performance and survivability after deployment. Hides were showing good results, however further research is needed to find the ideal hiding for Ballan wrasse in an intensive production. Overall, this research contributes to the development and optimization of Ballan wrasse aquaculture as a more sustainable alternative source of cleaner fish, reducing the pressure on wild stocks, as well as the amount of chemicals used in salmon sea cages which affect marine ecosystems.

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