Production of
ballan wrasse

Science and practice

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Production of ballan wrasse *(Labrus bergylta)*

Science and Practice

Table of Contents

Foreword

Science

Science (cont'd)

Practice

Participants

Chapter 1

The 'LeppeProd' project

Introduction

The salmon louse *(Lepeophteheirus salmonis)* is an ectoparasite copepod that uses salmonid fish as a host and has always been seen to be present on wild salmonids in Norwegian waters. Salmon lice feed off the skin, mucus and blood of the salmon and may, if present in high numbers, cause illness or death through secondary infection and osmoregulatory difficulties.

In the early 1990s, it was discovered that wrasse fish demonstrated a cleaning behaviour of these lice and a few organic farms started to use wrasse in the role of cleaner fish. However, this practice was not adopted by the industry at large since commercial delousing treatments were available. In recent years, salmon lice infections became a more important issue for European salmon farmers, increasing in magnitude and persistence. Such infestations are also perceived to be a potential threat to small migrating salmonids, which could affect wild salmon stocks.

The present lice problem poses serious risks to the viability of salmon aquaculture due to a build-up in resistance and the limited availability of control methods, reducing economic profitability. Controlling potential environmental impacts in Norwegian coastal areas is also important for the public image of both the profession and the final product. Demonstrating responsible management and best practice control procedures are integral to all modern livestock farming activities and the Norwegian salmon industry is well aware of this.

The salmon farming industry currently applies several different but interlinking strategies for combatting lice infestation in the farms:

- Production zone management
  (synchronised production and fallowing)

- Use of cleaner fish (wrasse and lumpfish)

- Biological management (breeding, functional feed
  including louse-attachment inhibitors)

- Strategic and optimised use of medicines and chemicals

- Technical innovations

Challenges exist with all known methods that are used for removing lice. Chemical treatments are of particular concern as the lice now show resistance to the commercially-available delousing chemical treatments, also recently including hydrogen peroxide (Fallang et al., 2004 ; Paul Negård and Frank Nilsen at www.kyst.no). Furthermore, we have far too little basic knowledge about the response of the salmon's own immune system to delousing procedures, especially with regard to responses by mucus cell populations in the gills and skin (pers. comm. Professor Karin Pittman, University of Bergen).
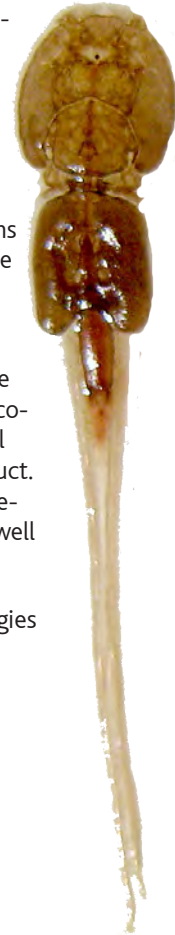
Farmers have to be aware of the risks related to cleaner fish and diseases, pinpointing the need to screen the cleaner fish before use in the salmon cages. This is a routine procedure for farmed fish but not so easy for wild-caught wrasse and lumpfish.

Research is being done on developing vaccines against sea lice but a pharmacological solution is believed to be many years from becoming a commercial reality (Frank Nilsen, www.intrafish.no).

Nonetheless, salmon farmers need tools to use now so as to keep the lice levels as low as possible. This realisation has led to an increased interest in the use of wrasse fish species as a primary solution to combat lice infections.

Several species of wild-caught cleaner fish are now in use; corkwing wrasse (*Symphodus melops* or grønngylt), goldsinny wrasse (*Ctenolabrus rupestris* or bergnebb), and ballan wrasse (*Labrus bergylta* or berggylt).

The fisheries catch of wild cleaner fish is seasonal, and was recently regulated further by the Directorate of Fisheries due to concerns on overfishing the wild stocks. Catching is forbidden during winter/spring, and fisheries do not open before June/July - conditions that are too late to meet the sector's needs for cleaner fish supplies.

Since year-round use is needed, a supply of cleaner fish must be based on a combination of wild-caught and farm-raised fish. It is therefore timely that a commercial industry to cultivate ballan wrasse has started and, more recently, also the rearing of another species of cleaner fish, the lumpsucker (*Cyclopterus lumpus* or rognkjeks).

Although in its infancy, the aim of this new aquaculture industry is to meet the urgent requirement of the salmon farming industry for disease-free delousing fish as well as to protect wild wrasse stocks.

The production of wrasse juveniles was developed with support from the Institute of Marine Research for 20 years, and this Institute has developed experience that has been very useful in LeppeProd, being the background for undertaking comprehensive developmental work, aiming to establish the commercial farming of ballan wrasse.

In 2009 Marine Harvest started a commercial activity to produce wrasse and other producers started up the following year.

This new aquaculture industry has needed strong research support to be successful, representing the principle reason for the LeppeProd project that started in 2010-2011.

The LeppeProd steering group selected the most competent scientific collaborators, with a solid knowledge base in marine fish research, using the best professionals to work on the bottlenecks identified. The researchers selected have been crucial to the success of LeppeProd.

Acknowledgements

The role of the industry

The foundation Norwegian Seafood Centre (SNS) has served as the Project Manager for LeppeProd, and governed the contract with FHF on behalf of the consortium. The Project Manager (SNS) exercised the daily responsibility for the project and reported to the steering committee, and was assisted by Grete Adoff.

The project has also published 12 newsletters during the project, which are available at the www.fiskerifond.no in the Norwegian language. All the main reports are available on www.rensefisk.no .

LeppeProd had a FHF-appointed steering group with members selected from the producers in the consortium and consisted of these members: Harald Sveier (Lerøy Seafood Group ASA, heading the group), Olav Breck (Marine Harvest), Espen Grøtan (Marine Harvest Labrus), Helge Ressem (Profunda, until 2012) , Tone Vassdal, Elin Eidsvik, Ingrid Overrein (Nordland Leppefisk, each of them in parts of 2011-2012), Lars Jørgen Ulvan (Nordland Leppefisk, from 2013), and Erling Otterlei (Cleanfish AS, until 2012).

The steering group for the consortium had 4-6 yearly meetings, discussing more than 90 issues on the agenda, and convened thematic and dialogue meetings in the consortium.

This manual "Production of ballan wrasse (Labrus bergylta) – Science and practice" has been prepared on the basis of the research activities in LeppeProd, using the expertise of the personnel at the hatcheries, and the researchers that have used their knowledge gained from working with other marine fish species (e.g. halibut and cod). The manual has been prepared by the authors cited for each section, while editing was done by Synnøve Helland, Stine Wiborg Dahle, Courtney Hough and Jørgen Borthen.

This manual is a compilation of the knowledge gained during the LeppeProd project, and its goal is to be a help for future ballan wrasse farmers, students and the professional stakeholders of European aquaculture.

Jørgen Borthen

LeppeProd project leader
Bergen, February 2014

Nordland Leppefisk AS

Nordland Leppefisk, located on Lovund, an island close to the polar circle, has been working closely with SINTEF on live feed issues. They also have a close cooperation with R & D groups at the University of Nordland, which communicated new knowledge outside this consortium. The company reports great benefit from its collaboration with Nofima regarding broodstock knowledge. The analysis of the live feed that was done at NIFES has been very important to improve the quality of rotifer production. Nordland Leppefisk also tried the production of goldsinny in 2011 and 2012. As an alternative to a more tolerant species for cold water, they also made a trial production of lumpfish in 2012 and succeeded well. This will be the main species in the coming years for Nordland Leppefisk, reports the Manager - Lars Jørgen Ulvan.

Profunda AS

Profunda is a hatchery that has produced several marine fish species, located at Barstadvik on the north-west coast of South Norway. The company decided to join the LeppeProd project to take advantage not only of the R&D but also the horizontal exchanges of knowledge with the other industrial partners in the consortium. Helge Ressem, who is the manager of Profunda, says that their participation in the LeppeProd has been fruitful, not only within the formal meetings but also through the visits to the different hatcheries.

During the project period, Profunda developed a close cooperation with Nofima and its personnel, and Ressem thinks that this contributed significantly to the success of the cooperation, focusing on brood stock management and gender determination. Testing of live feed done at SINTEF was also productive.

Cleanfish AS

Cleanfish (LeppeProd participant until 2012) is located south of Bergen. The Research Manager, Erling Otterlei, pinpoints the scientific information from meeting activities (R&D and industry) as being very useful. Larvae production was supported by Nofima with sampling of juveniles (at 1 g wet weight) for X-ray evaluation of deformities. The live feed was sent to NIFES for evaluating the quality and nutritional status.

Marine Harvest Labrus AS

Marine Harvest Labrus, outside Bergen, started in 2009 with the goal of producing 3 million wrasse juveniles each year. They are close to reaching this target in 2013-2014.

The most relevant R&D cooperation for Marine Harvest Labrus was:

• Developing better and more affordable dry feed (Nofima / NIFES / IMR)

• Malformations (Nofima)

• Developing better weaning feed (NIFES/ Nofima)

• Focus on a better understanding of the lumping behaviour, possibly harming fish health (IMR)

• Recirculation workshop and testing new anti-fouling materials for wrasse shelters (SINTEF / NTNU)

The manager, Espen Groetan, reports that the work on dry feed has highlighted the needs for phospholipids and hydrolysed protein. Moreover, IMR has measured light spectra in all production departments, which again has given advice on which light sources and light colour one should choose. In experiments, the red light can be used to observe the wrasse undisturbed, as the wrasse are insensitive to red light.

Skretting AS

The feed company Skretting became a partner in the consortium in 2012, participating in the development of dry food with their own funding. Skretting had a close cooperation with Nofima on formulated feed development.

A comprehensive summary of the progress and status of the production of ballan wrasse is presented on the following page, demonstrating achievements and challenges.

It is necessary to recognise that the achievement of this complex project could not have been made without the valuable inputs, professionalism and enthusiasm of these different project partners.

LeppeProd
February 2014

Harald Sveier — *Leader of Steering commitee*

Jørgen Borthen — *Project coordinator*

Synnøve Helland — *Editor of this Final Report*

Ballan wrasse production in commercial hatcheries Status early 2013		
	Marine Harvest Labrus	**Profunda**
Contact	Espen Grøtan espen@marineharvest.com	Helge Ressem profunda@online.no
Broodstock	3 spawning groups	3 spawning groups
Spawning and larvae production	Spawning tanks with egg collectors	Sufficient for small scale production
Production capacity	8 million juveniles 2 million produced (2013)	2 million juveniles
Survival rate	0 - 0.5 g: good 0.5 g – seacage (25 – 75 g) "less good"	0 – 0.5 g: good 0.5 – 2 g: low
Live feed	Rotifers, day: 5 – 30 *Artemia*, day: 25 – 45 Weaning from day 45	Rotifers, day: 5 – 63 *Artemia*, day: 30 – 100 Weaning: from day 50
Disease	Some disease problems have resulted in early mortalities	
Main challenges	• Stagnant growth in nursery stage after 0,5 g • Deformities in larvae seen initially are currently scarce • Clumping in larval tanks after first 50 days can cause fin rot and related problems • Insufficient nursery capacity • Fin rot in tanks • Identify major desease causes and vaccine development	• Limited warm sea water capacity • Shelters distort water circulation in tanks • Need vaccines for atypical furunculosis
Major improvements	• Better formulated feed gives improved growth • Less deformities due to improved feed quality • Improved survival – better survival in tanks due to better production protocols • *Artemia* gives better results	

CHAPTER 2

Establishment of ballan wrasse broodstock

Ingrid Lein and Synnøve Helland

INTRODUCTION

To date, only wild caught broodstock has been used in the farming of ballan wrasse. Brood fish has been caught either by fish traps or nets. Catch by fish traps is gentler to the fish, but the number of fish per catch is relatively low. Therefore, nets have been used for catching large numbers of brood fish for farming purposes. After catch, injured fish might need treatment with iodine and/or antibiotics to heal. Brood fish of ballan wrasse are most often stocked at low densities, with an approximate female:male relationship of 4:1 – 10:1. In nature, ballan wrasse males maintain harems of reproductive females, and courtesy the females before a pairwise spawning takes place (Sjolander et al., 1972). Higher numbers of males than one to four females seem to cause aggressive behaviour in males (E. Grøtan, pers. com.)

Ballan wrasse, in general, seems rather picky about its food. In Norway, a high quality protein rich dry feed mixed with water, binder and whole shrimps is currently used as broodstock diet. Shrimps seem to be critical for the palatability of the feeds for ballan wrasse. Additional feeding with fresh or frozen shrimps has been used by several hatcheries. In Scotland, Norway lobster has been successfully used as additional feed for wrasse brood fish (J. Treasurer, pers. comm.). Feed intake in terms of energy can influence the fecundity of brood fish considerably. Lein and Berge (2008) showed that an insufficient energy intake in Atlantic halibut females caused reduced numbers of spawning per season, and also smaller egg batches per spawning. For wild stocks, total length is a common measure for fish size, and is also used for gender estimation. For other species, such as cod, there is a good relationship between length and weight in small fish but, in larger fish and farmed fish with higher condition factors, total length is not a good measure for body weight and condition. In the present work performance, wild-caught broodstock was followed from catch in June 2011 until April 2012. Spawning in June 2012 was also monitored.

EXPERIMENTAL SETUP

In mid-June 2011, 617 net-caught ballan wrasse brood fish, taken off the coast of mid-Norway, arrived by truck at the Nofima research station in Sunndalsøra. The fish were stocked in two tanks (2x2 m, 5 m³, green walls, and overhead light, plastic shelters) and treated with formaldehyde the day after arrival. 4-5 days after arrival, all fish were individually tagged with electronic tags (Pit-tag), weighed, and length measurements were taken. Fish with catch injuries (Figure 1) were treated with injections of antibiotics and the wounds were sprayed with an iodine solution. Gender was determined by ultrasound (Figure 2).

Three weeks later the fish were distributed to three 5 m³ tanks and two 3.5 m³ and fish with injuries that had not healed were treated with antibiotics and/or iodine once more. Stocking density was ~ 12 and 6 kg/m³ in the 5 m³ and 3.5 m³ tanks, respectively.

Figure 1. Newly-caught ballan wrasse broodstock resting inside plastic shelters in the spawning tank (left). Typical gill net injury is shown to the right. The majority of such wounds healed quickly. Photo: Synnøve Helland, Nofima

Figure 2. Brood fish were individually tagged (Pit-tag), weight and length were recorded, gender was determined by ultrasound. Fish with wounds were given an injection with antibiotics. All injuries were sprayed with an iodine solution.
Photo: Nofima

After the fish were distributed to five tanks, two photoperiods were applied; natural spawning occurred in three tanks (June) and one delayed spawning in two tanks (August/September). The temperature was controlled at 10.5°C until July 15; thereafter ambient temperature was applied.

The brood fish were fed a moist diet (75% Skretting Vitalis, 25% shrimp, binder, fresh water) every 3rd day for 4 months, thereafter they were fed daily except for Sundays. Mid-April 2012 all brood fish were identified by the electronic tags before weighing, measurement of total length and determination of gender. The results presented below constitute data from the individuals that were examined both mid-June 2011 and mid-April 2012. In the meantime, some fish died while others lost their electronic tags and could not be identified.

Results and discussion

Survival and healing of catch injuries

78 fish died during the period from arrival June 15 until October 1 , i.e. 12.5% mortality. This is lower than anticipated since commercial wrasse producers reported mortalities of up to 50% during the first months after catch by nets.

Almost all mortality (70 fish) occurred in the first tank sampled, probably because the concentration of anaesthetics used during handling was too low. No established procedures for anaesthesia of wrasse existed at the time. It turned out that the dosis of Finquil recommended for salmon needed to be almost doubled to give satisfying sedation and wake-up time in adult wrasse (150 mg/L vs. 80 mg/L recommended for salmon). After this initial occurrence, mortality during the following nine months was very low except for one tank which experienced a break in water supply.

The catch wounds (see Figure 1, right) healed surprisingly well after treatment with antibiotics and iodine. After about 2-2.5 months it was difficult to trace which fish had been injured at catch.

Gender determination and spawning

All 78 fish that died during the first 6 weeks after catch were autopsied, and 82% had been correctly evaluated. 10 fish classified as immature or unknown turned out to be small females while the remaining 7 larger fish with unknown gender probably were inter-sex, i.e. in the process of changing sex (Muncaster, 2008).

Table 1. Mean, maximum and minimum weights and total lengths of females, males and immature fish in June 2012. Larger fish classified as immature were probably in the intersex stage.

Gender Ultrasound	No. of Fish	Mean Weight (g)	Max (g)	Min (g)	Mean Length (cm)	Max (cm)	Min (cm)
Female	460	401	1122	177	296	440	230
Male	75	664	1355	252	348	430	260
Immature	82	268	792	106	263	370	200

Fish were spawning daily in all tanks for a little more than two weeks after arrival to Sunndalsøra. The fertilisation rates were high, near 100%, for all groups, and eggs that were incubated hatched well. The short spawning period is probably due to stress caused by catch and transport. This corresponds with experiences reported in commercial wrasse hatcheries.

Growth

The mean body weight of females increased by 17% during the 10-month growth period while males increased their mean weight by 14%. The increase in total length was minor in both males and females during this period. Hence the condition factor of males and females increased during the 10 months (Table 2). The real increase in body weight for females during this period was higher than the number shows because most females had larger ripe gonads during the first weighing in June 2011. Fish classified as immature had a decrease in mean body weight and did not increase their mean total length (Table 2 and Figure 3). The stocking density was high in the present study compared to what is being used by some commercial producers (6-10 kg/m³ versus ~5 kg/m³). It was anticipated that the high stocking density could brake down hierarchies within tanks. However, some aggressive behaviour was observed during feeding times. This indicates that smaller fish might benefit from being stocked in separate tanks. No differences in performance between the two stocking densities were observed.
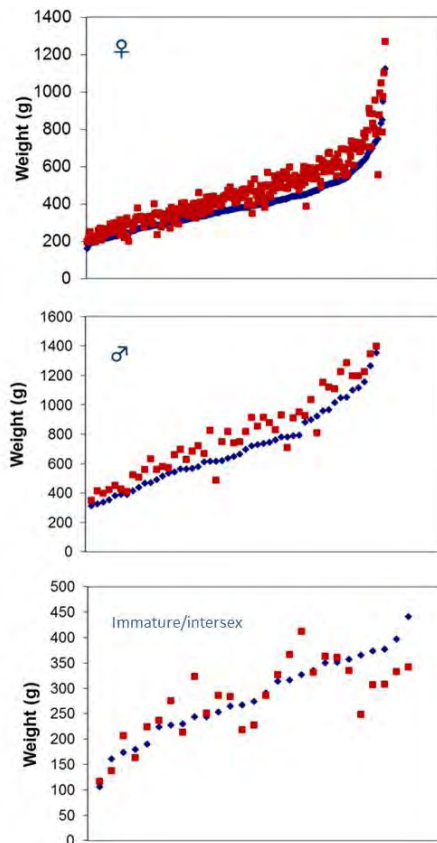
Figure 3. Size distribution of individually tagged brood fish in June 2011 (blue) and April 2012 (red). Each dot represents one individual.

Table 2. Mean weight, length and condition factor of female, male and immature or intersex broodstock at the Nofima Research station at Sunndalsøra in June 2011 and April 2012.

	Females (n=295)	Males (n=49)	Immature (n=27)
Mean body weight June 2011 (g)	395	699	282
Mean body weight April 2012 (g)	461	793	277
Body weight increase (%)	17	14	–2
Mean total length June 2011 (cm)	29.5	35.4	26.6
Mean total length April 2012 (cm)	30.1	35.8	26.6
C-factor June 2011	1.47	1.50	1.46
C-factor April 2012	1.61	1.66	1.44

Weight-Length relationship

When plotting the weight-length relationship there is a good correlation up to approximately 25 cm total length (Figure 4). Thereafter measurement of total length correlates less well with body weight and, from 35 cm and upwards, total length is a poor indicator for body weight and condition. For example, at 40 cm length we recorded weights from 800-1300 grams. Table 2 shows that the condition factor of females and males increased from June to weighing ten months later. These findings show that regular weighing of broodstock should be done to make sure the growth is satisfying. Good growth between spawning seasons is important for the fecundity in multiple spawners like wrasse.
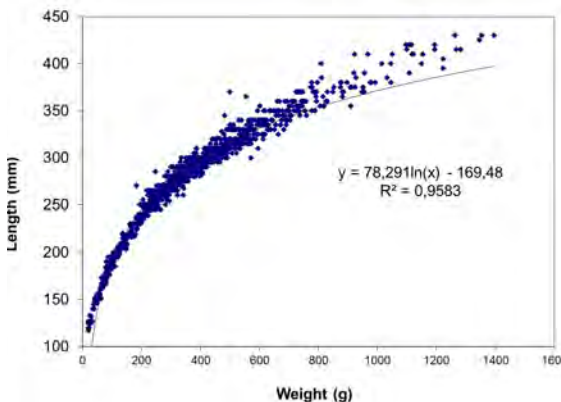
Figure 4. The weight-length relationship of all broodfish of ballan wrasse at the Nofima research station at Sunndalsøra in April 2012, approximately two months prior to the spawning season.

References

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Sjolander, S., Larsson, H.O., Engstrom, J., 1972. On the reproductive behavior of two labrid fishes, the ballan wrasse (*Labrus bergylta*) and Jago´s Goldsinny (*Ctenolabrus rupestris*). Rev. Comp. Animal.6, 43-51.

Recommendations

Weight and condition factor of broodstock should be checked at regular intervals, a minimum of once a year, to make sure that the broodstock is in good condition before spawning.

Total body length is not a good measure for growth and condition in adult fish over 25 cm.

Total body length is not a good method for gender determination because there is an overlap between females and males, and also with fish in the intersex stage (i.e. in the process of changing sex). Ultrasound for sex determination has an accuracy of approximately 80%.

Adult wrasse seem to tolerate anaesthetics and gentle handling well, and wrasse broodstock caught by net heal quickly and well when treated with antibiotics and iodine shortly after catch.

The first spawning period after catch of a new broodstock is short, probably due to stress. However, eggs fertilize and hatch well.

Wrasse broodstock can be stocked at fairly high densities without negative interference with spawning activity and fertilisation.

Gender determination of ballan wrasse broodstock

Ingrid Lein, Yoav Barr and Synnøve Helland

INTRODUCTION

In ballan wrasse, all fish first reproduce as females before some fish undergo gender change to develop a functional testis (protogynous hermaphrodism). This change is strongly correlated with fish size and occurs most often in fish between 34-41 cm total length during the months following spawning (Muncaster, 2008). The male wrasse is known to maintain harems of females with which they can breed. Competition between males for access to females or breeding sites favours large body size. So far, the total length of individual wild-caught brood fish has been used as the main criterion for gender determination in commercial farming of wrasse. In a farming situation, a high fraction of females is desired for egg production, and to mimic the wild with a male:female relation of 1:10. Thus many fish >36 cm total length have been judged as males and hence removed from the broodstock. It appears that there is an overlap in size between males and females, and there are indications that not all fish undergo sex change (Muncaster, 2008). While some farmers have too many males, other farmers experience a shortage. Clearly, there is a need for simple and reliable methods for determining the gender of ballan wrasse brood fish. Several methods for gender determination in ballan wrasse are currently being investigated such as morphometrics, external coloration, hormonal profiling and genetic markers. In this project we looked to find a simple and rapid method that can be used by commercial farmers and the two selected methods were ultrasound and biopsy.

ULTRASOUND

617 wild ballan wrasse caught by net along the coast of mid-Norway were brought to the Nofima research station at Sunndalsøra the first week of June, i.e. at the beginning of the spawning season. Approximately a week after arrival, all fish were anaesthetised and electronically tagged (AEG ID). The fish were weighed and total length was measured. At the same time the gender was determined using a portable UV-machine (LOGIQ Book XP Vet, Figure 1). All fish that died during the first two months after catch (78 in total) were autopsied for verification of gender determination.

In females, the gonads can be observed as a tubular homogenous mass (Figure 1) while for males the picture is much less structured. 82% of the fish were correctly evaluated using ultrasound. One fish was wrongly evaluated while 17 fish (22%) were classified as "unknown gender". These fish were mainly small females (23-28 cm) with undeveloped or very small gonads.

Ultrasound was also used when brood fish were weighed and measured during the autumn and spring. In the beginning of April, i.e. approximately two months before the start of spawning, ultrasound gave good indications of male or female gonads while the differences were less distinct during autumn, at least in smaller fish (23-28 cm total length).

Figure 1. Determination of gender in ballan wrasse using ultrasound. Female gonads are shown. Photo: Synnøve Helland, Nofima

This method gives a good, but not 100% determination of gender in ballan wrasse. However, the equipment is quite expensive (~20.000 €).

Determination of sex in ballan wrasse by biopsy of gonads

Biopsy of gonads was done using a disposable cat catheter (diameter 1.3 mm) that was inserted into the gonads through the urogenital opening (Figure 2). It turned out to be quite easy to retract oocytes from the gonads of females during the spawning season. Good hygiene is important to prevent inflammation of the gonad. Disposable gloves were used, and the disposable catheter was washed with 70% alcohol before it was inserted into urogenital tube.

In females, it was found that the catheter could easily be inserted all the way into the female gonad (Figure 3) while, in males, the catheter stops approximately halfway. This was very consistent and gives a simple and cheap method for sex determination in ballan wrasse that can be done by hatchery staff without special training. The danger of damaging the gonadal walls in females is probably reduced when no eggs are retracted. An advantage of this method over ultrasound is that it can probably be used all year round. However, since the sex change into males happens during the 4-5 months following spawning, gender determination of fish expected to spawn the coming season should be done later than that.

In another test, biopsy was done on 68 females confirming that the catheter could be inserted the full length of the gonads. For males, the method was tested for a number of fish that had released milt before, confirming that only approximately half the length of the catheter can be inserted into the testinal tube through the urogenital opening. The method should preferably be tested on a higher number of males but so far the results have been consistent for all tested males.

Figure 2. Determination of sex in ballan wrasse using a cat catheter (1.3 mm diameter). Photo: Synnøve Helland, Nofima

Figure 3. Biopsy of female gonads using cat catheter (1.3 mm). Photo: Synnøve Helland, Nofima

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Recommendations

Ultrasound can be used with relatively high precision (>80%) to determine the sex of ballan wrasse brood fish a least two months before the spawning season. However, this method requires expensive equipment and trained personnel. Fish under 28 cm total length are most probably females. Cat catheters inserted into the gonads through the urogenital opening can be used for determination of the sex of wrasse brood fish. In females, the catheter can easily be inserted all the way into the gonad while in males the catheter stops half way in. The biopsy method can probably be used all year round, but since the sex change into males occurs during the 4-5 months following spawning. The sex determination for fish expected to spawn the following season should be tested later than this period. Biopsy of gonads is a simple method that can probably be done by hatchery staff without special training.

Initial tests of procedures for stripping of eggs and sperm and induction of final maturation in ballan wrasse

Ingrid Lein, Synnøve Helland and Helge Tveiten

INTRODUCTION

Reliable methods for stripping eggs and sperm from wrasse broodstock have been requested by commercial wrasse producers. Artificial fertilisation allows more efficient egg disinfection, and also selection of parent fish. Ballan wrasse is a multiple spawner that spawns numerous batches of eggs over several years. In nature, males maintain harems with several females. In captivity, brood fish are stocked with a female:male relation of 4:1-10:1 for pairwise spawnings. Spontaneous spawning in broodstock tanks is to date the common method for egg collection. Eggs are collected on mats of different materials placed at the tank bottom. Using this procedure, a lot of eggs are lost down the drain while another challenge is dirt clinging to the sticky layer surrounding fertilised wrasse eggs. A few attempts have been made to strip wrasse eggs and sperm in commercial hatcheries. It has been reported that the males release little or no milt when stripped (Grøtan, pers. comm.).

The captive brood fish of many species do not produce sufficient amounts of gonadoreleasing hormones. (GnRH). These hormones stimulate the final maturation of oocytes and sperm. In a range of cultured fish, such hormones are routinely used to induce and/or synchronise spawning times in mature fish. In males, GnRH treatment increases the sperm production and it helps to complete the maturation of oocytes in females. For both sexes stimulation with GnRH synchronises the spawning time.

The timing of injection of GnRH according to oocyte development can be important for the response to hormone treatment. In a multiple spawner, like ballan wrasse, the ovaries consist of oocytes at different maturation stages. Diameter of oocytes and location of the germinal vesicle can be used as indicator for maturation status in females. According to Muncaster (2008), who studied reproductive status in wild wrasse, the leading cohort oocytes in mature wrasse females have an oocyte diameter of 651-720 µm and the germinal vesicle has started to migrate toward the animal pole. In the present work, the first attempts to strip eggs and sperm from wrasse is described as well as initial work on the induction of final maturation by injection of gonadotropin releasing hormone (GnRH).

Test 1: Stripping of eggs and sperm

Setup

The first attempts to strip eggs were done in 2012 with wild-caught brood stock at the Nofima research station at Sunndalsøra. The broodstock was captured in June 2011, but was allowed to spawn spontaneously during the first short spawning season to avoid additional stress to fish that was already stressed from catch, catch-related injuries and transport.

A procedure previously successfully applied to Atlantic cod was tested. Brood fish were anaesthetised and checked for identity and gender (Pit-tag). To avoid damage to the skin, disposable gloves were used and the fish were placed on a silicone pad during the stripping procedure.

Results and discussion

Sperm

All males were checked for running milt by gently stripping the anaesthetised fish and collecting the sperm from the genital papilla into a 1 mm sterile syringe. Only a few males released running milt; several released only a few droplets of milt while a few males released larger volumes. The volumes varied from 0.05 ml to 0.8 ml. The same individuals were tested several times, but a male releasing sperm one day would not necessarily release sperm the following day.

Eggs

Attempts were made to strip eggs from all females with swollen bellies, which usually mean that an egg batch has been hydrated and is ready to be released.
Some fish were quite easy to strip eggs from (Figure 1) but others retained the eggs even though females appeared mature.

Figure 1. Stripping of eggs from ballan wrasse. Photo: Synnøve Helland, Nofima

The same individuals were inspected over several days, but no regular spawning rhythms were observed.

Eggs and sperm stripped during these tests were fertilised and used for other experiments; the quality of the eggs was good as high fertilisation rates were obtained (>90%). The predictability, however, was too low, and sometimes it was not possible to strip eggs and sperm on the same day. To develop artificial propagation as an option in commercial wrasse production, a procedure that secures a more predictable access to eggs and sperm must be developed.

Test 2: GnRH injections

Setup

Initial testing of effects of GnRH-injections on the spawning in ballan wrasse was started in 2013. Five females and six males were injected with the commercial GnRH-product Ovaprim. Before noon, the fish were anaesthetised and weighed before injection of a dose of Ovaprim according to their body weight (0.5 ml Ovaprim/kg body weight) and stocked in a common tank. At the same time as the fish were given Ovaprim injections, attempts were made to strip eggs and milt.

No females released eggs at day 0, but all the males released milt.
The fish were then stocked in one common tank (1500 L), and attempts to strip eggs and milt were made again on the three consecutive days. The volume of milt collected from the six males was measured. The milt was centrifuged before spermatocrit values were measured.

Results and discussion

The plan was to strip females and males for eggs and sperm the following day. However, there was a massive spontaneous release of eggs and sperm during the night following injection with Ovaprim. The tank was full of eggs, indicating that several females had released large amount of eggs. The fish were inspected as planned, but only a few eggs were released from three out of five females one day after injection with Ovaprim. Two days after injection, one female released 95 ml of eggs while the other four females released only small amounts of eggs. On the last day no females released eggs. This indicates that the Ovaprim injection induced final ovulation within few hours, and that four out of five females spontaneously released almost all their eggs in one portion. The eggs spawned during the first night after injection stayed in the spawning tank and hatched successfully, indicating that the egg quality was good.

Four out of six males released sperm on day 0, and the sperm volume increased in 4 out of 6 males both one and two days after injection with Ovaprim (Table 1). Three days after injection however, the volume dropped in 4 males. There was no clear increase in spermatocrit values after injection with Ovaprim, except for 1 male. An explanation could be that also males spawned spontaneously within a few hours after injection and therefore had less sperm left when checked two and three days later.

Table 1. Body weight, sperm volume and spermatocrit values for male wrasse injected with 0.5 ml Ovaprim per kg body weight at day 0. Due to technical problems no spermatocrit values were obtained for day 1.

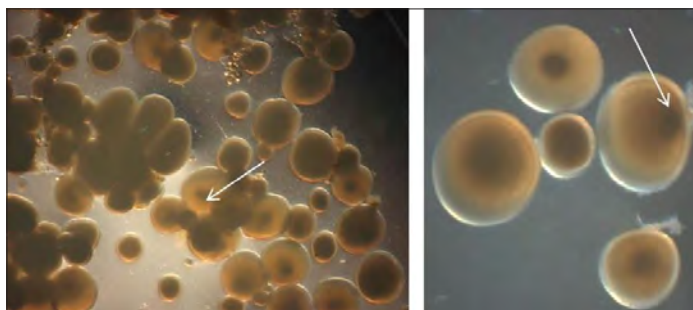| Male no.	Weight gram	Sperm volume (ml)				Spermatocrit			
		Start	Day 1	Day 2	Day 3	Start	Day 1	Day 2	Day 3
1	421	0.11	0.39	0	0.15	51	-	0	<10
2	491	0.08	0.11	0.26	0.13	35	-	57	45
3	873	0.29	0.45	0.25	0.23	80	-	24	25
4	536	0	0	0.12	0	0	-	7	0
5	591	0.35	0.22	0.31	0.30	74	-	17	21
6	736	0	0.12	0.27	0	0	-	23	0

In a repeated test, no females released eggs after injection with Ovaprim. It turned out that several females had poor and over-ripe eggs in their gonads. In 2013, temperature of the broodstock was accidentally increased abruptly from <7°C to 11°C which probably affected the egg quality negatively. In 2012, the temperature was gradually increased over one month from 7°C to 10.5°C before spawning, resulting in regular spawning of good quality eggs. Thus the main cause for poor egg quality in 2013 was probably the abrupt increase in temperature, resulting in stressed broodstock.

Figure 2. Example of variation in oocyte development within one wrasse female (left). The eggs had been been stained with Serr's solution, and the germinal vesicles can be seen as dark spots in the center of the oocyte (arrow). Migration of the germinal vesicle is seen to the right (arrow). Photo: Ingrid Lein, Nofima

Test 3: Oocyte development

Setup

To evaluate the maturity status of females, eggs were sampled from 69 females using a cat catheter (see "Gender determination of ballan wrasse brood stock"). Samples of eggs were treated with Serr's solution for staining of the germinal vesicle. Serr's solution is made up of ethanol, formalin and acetic acid in the relation 6:4:1. A small volume of Serr's solution diluted x 20 in a physiological solution (Ringer) was added to oocyte samples in a petri dish under the microscope.

Results and discussion

After 10 minutes exposure to Serr's solution the germinal vesicle was visible (stained dark, Figure 2). Diameter of oocytes samples from all 69 females were measured (Table 2), and location of the germinal vesicle was recorded. Migration of the germinal vesicle towards the animal pole was observed in 9 of the 69 females (Figure 2).

The majority of the oocytes had a diameter in the range 300-650 μm, i.e. the eggs were in the early to late yolk stage. This either means that the females had not yet reached the mature stage, or that the final maturation process was arrested. The latter is probably the case since very few females released eggs in the spawning tanks after this date.

Arrested final maturation is often caused by stress, which in our case may be due to a sub-optimal temperature regime that year. However, a large variation in oocyte diameter was observed, which is typical for a multiple spawner like the wrasse where batches of eggs mature at different times.

Table 2. Diameter of oocytes from five females. Mature oocytes have an oocyte diameter >654 μm. Standard deviation per female is shown (Std).

	Female 1	Female 2	Female 3	Female 4	Female 5
Oocyte diameter (μm):					
<300	0	0	10	7	2
300-450	19	17	29	33	48
400-650	16	17	17	24	28
>650	7	13	6	16	9
Number of oocytes	42	47	62	80	87
Mean	16.8	18.8	24.8	32.0	34.8
Std.	14.3	15.4	20.2	25.5	30.6

## Test 4: Selection of mature females for GnRH injections

Setup

For the last testing of injections with Ovaprim, 22 females with the high proportions of larger oocytes were selected for the test. 11 females and 4 males were injected with Ovaprim as described above while the same number of females and males were injected with Ringers' solution (saline) as control. One female selected for injection with Ovaprim died before start of the test, thus only 10 females were injected with Ovaprim. After injections, fish injected with Ovaprim were stocked in one common tank, and the control group injected with Ringer's solution in another tank. The Ovaprim injection was done late after-noon to avoid spontaneous spawning during the night. Attempts were made to strip eggs and sperm from all fish in both treatments on the day of injection, the next morning and the day after.

Results and discussion

No females released eggs on the day of injection. The next day nine out of 10 of the females injected with Ovaprim released eggs when stripped while only 3 of 11 females injected with Ringer's solution (control) released eggs. The next day 6 out of 10 injected females released eggs while 4 out of 10 fish injected with Ringers' solution released eggs (Figure 3). The eggs were not of good quality (not all were transparent), but this was similar in fish treated with Ovaprim or with Ringers' solution (control).

The results show that injections with Ovaprim can be used to induce spawning in ballan wrasse, but more knowledge is needed about timing of injections and the effects on egg and larval development before this method can be implemented in a commercial wrasse production.
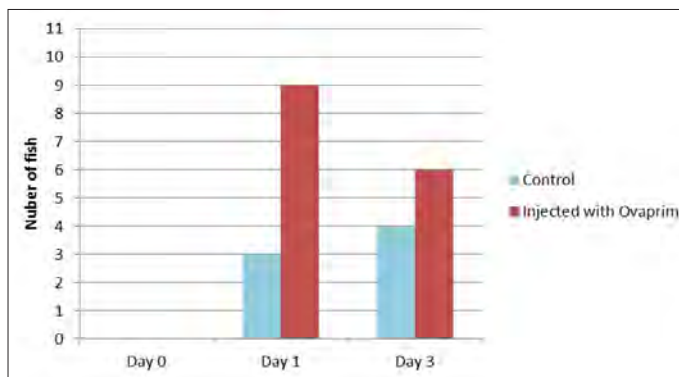
Figure 3. Number of fish releasing eggs when injected with Ringers' solution (control), or with Ovaprim (GnRH). The number of females was 11 in the control group and 10 in the group injected with Ovaprim.

References

Muncaster, S., 2008. Reproductive Physiology of ballan wrasse (*Labrus bergylta*). PhD dissertation, University of Bergen, Norway. ISBN 978-82-308-0532-9.

Recommendations

Size distribution of oocytes and staining of the germinal vesicle with Serr's solution for evaluation of maturation sta-tus in individual female wrasse is a relatively simple and practical procedure that can be used by commercial wrasse producers when selecting fish for spawning.

Eggs and sperm can be stripped from ballan wrasse, but stimulation of final maturation with gonadotropin releasing hormone (GnRH) might be necessary to obtain a predictable production of high quality eggs.

However, more knowledge is needed about timing of treatment and effects on egg and larval development before this method can be implemented in commercial wrasse production.

Procedure for short-term storage of ballan wrasse milt

Ingrid Lein, Synnøve Helland, Inger Grevle and Katarina Nordtun Ruud

INTRODUCTION

To date, only naturally spawned eggs have been used in the commercial production of wrasse. However, the producers have asked for a method for stripping eggs and milt from wrasse in order to gain better control of both their egg and larval production. It appears that wrasse males release varying or mostly small amounts of milt compared to other marine fish species, such as halibut and cod, when stripped. This might be due to stress in wild-caught fish since it has been reported that larger amounts of milt can be obtained at the time of catch. The problem of limited access to milt when the females are spawning calls for procedures for short or long term storage of milt. In this experiment, two different extenders for milt on fertilisation of wrasse eggs were tested. The effect of storage on milt quality was also assessed.

EXPERIMENTAL SETUP

EVALUATION OF TWO DIFFERENT EXTENDERS

Eggs from one female and milt from one male were stripped and stored at 4°C for about 60 minutes during which the extenders (provided by Cryogenetics) were prepared in the laboratory. Milt was then diluted (1 part milt: 2 parts extender) in two different extenders (A and B). Approximately 1 ml of eggs was transferred to microscope slides and carefully spread in a thin layer. The slides with eggs were placed in petri dishes, 3 petri dishes per extender, and 3 for the control group. 2.5 µl of the diluted milt was spread evenly over the eggs before 25 ml of filtered and UV-treated sea water was added. For the control, 1 part of milt was mixed with 2 parts sea water and spread over the eggs before addition of 25 ml extra sea water. After 10 minutes, excess milt was removed by rinsing the eggs three times with filtered and UV-treated sea water. The eggs were placed in a temperature-controlled room at 10.5°C until the next morning when the fertilisation rates were checked under the microscope.

EVALUATION OF STORED DILUTED MILT

Milt was diluted according to the procedure described above, but with a mix of extender A and B. The milt was stored in a fridge at 4°C until they were used to fertilise newly stripped eggs 5 days later. The eggs were fertilised as described above, and the fertilisation rates were checked the day after.

RESULTS AND DISCUSSION

The fertilisation rates were high in all groups, but the fertilisation rates were significantly higher in the two groups of eggs fertilised with diluted milt (A or B) than in the control group ($p<0.05$).
The fertilisation rate of eggs fertilised with milt stored for 5 days was 90% (Table 1), and was not statistically different from the control fertilised with fresh milt 5 days before.

Chemical components of the extenders may have improved the sperm motility and thereby improved the fertilisation rates in eggs fertilised with diluted sperm. The motility of the sperm was, however, not checked in this experiment.
In this experiment diluted milt was tested after 5 days of storage.

Diluted ballan wrasse milt could probably be stored for more than 5 days, but this could not be tested in this experiment due to lack of females releasing eggs at the time.

Cryogenetics has proved that sperm from salmon can be diluted and stored for 16 days without any dramatic reduction in the fertilisation rate. Based on this knowledge Cryogenetics has developed a product called AquaBoost™ Extender for Atlantic salmon, and is presently running trials to adjust this product to other species.

Table 1. Fertilisation rates of eggs fertilised with two different extenders (A or B), and eggs fertilised with milt which was first diluted with a mix of A and B, and then stored cold for 5 days prior to use. Means with different number of stars (*) are significantly different.

Treatment	Fertilization (%) +/-STD	Significant differences
Day 0:		
Control	85.8 ± 3.5	*
Extender A	94.5 ± 0.5	**
Extender B	91.3 ± 1.1	**
Day 5:		
Mix A and B	89.9 ± 3.5	*

Acknowledgements

Recommendations

A sperm extender can be used to increase the gain of small amounts of ballan wrasse milt both by increasing the volume of the milt and by improving the fertilisation rates.

A sperm extender can also be used for storing milt for at least 5 days, which will improve the utilisation of available sperm from each male.

Broodstock nutrition

Kristin Hamre and Espen Grøtan

Introduction

The starting point for successful farming of fish is the production of high quality eggs. The nutrient composition of the yolk, which will support growth and development of the embryo and yolk sac larvae, is strongly affected by the broodstock diet. This diet must therefore be nutritious and balanced to promote both egg and larvae quality.

In classical nutritional studies it is common to measure the requirements of one or a few nutrients at a time. Here we have used an alternative approach. Assuming that wild fish have a good nutrient status, we analysed the whole nutrient profile in the female gonad of wild fish and compared it to the nutrient profile of gonad from fish that had been held in captivity and fed a broodstock diet for one year. The composition of the broodstock diet was also analysed. Lower concentrations of individual nutrients in the farmed fish would then indicate a dietary deficiency, while higher concentrations may indicate over-supplementation. This last point is most important for nutrients that may be toxic at high concentrations. The data have been published in Hamre et al. (2013).

Experimental Set-up

The broodstock diet was made by crushing 100 kg Vitalis cal (Skretting) and mixing in 40 kg minced Greenland shrimp (local supplier), 4.8 kg wheat gluten for binding and 22 L fresh water. The dough was then pelleted. Captive broodstock (718±91g, mean±SD, n=14) were sampled from the production line of MHL. The fish had been held in 40 m3 tanks at a density of 5 kg m3 for more than one year.

Water temperature was 8 - 10 °C, oxygen 7.5 – 8.0 mg L^{-1}, the light regime was set for delayed spawning and the tanks contained shelters made of plastic sheets where the fish could hide. The fish were scheduled to spawn in December and were sampled on 14/12/2011. The wild fish (521±155 g, mean±SD, n=17) were obtained from a local fisherman and moved to MHL, where they were held for less than 1 week without feeding and sampled on 5/05/2010. The spawning season for ballan wrasse in the wild lasts from May until July.

The nutrient analyses were performed at NIFES, using routine and certified methods.

Results and Discussion

The water-soluble vitamins, vitamin E, Ca, Mg, P, Cu, Se, Mn and Zn were similar or higher in the captive, compared to the wild group. Astaxanthin was not detected in gonads from wild fish, but was present in gonads from captive fish. The electrolytes, Na and K, were also higher in captive that in wild fish (Table 1).
The concentration of vitamin D was approximately half in captive compared to wild fish.

Vitamin K was not analysed in the captive group due to a shortage of sample material, vitamin A because of methodological difficulties with egg samples. Iodine was present in captive fish at about 1/3 and taurine at 1/2 of the concentration in wild fish.
The most important differences in fatty acid composition were those of arachidonic acid (20:4n-6, ARA), eicosapentaenic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA). ARA was lower, EPA higher and DHA lower in gonads from captive compared to wild fish. This resulted in large differences in essential fatty acid ratios, DHA:EPA being 1.53 and 2.53 and ARA:EPA ratios being 0.10 and 0.49 in gonads from captive and wild fish, respectively.
Of the nutrients analysed in female gonads, the least need for further investigation is in water-soluble vitamins, vitamin E, and minerals except iodine and Zn. However, the vitamin levels were high in farmed fish and could be lowered to save costs. The high levels of electrolytes in cultured fish were probably due to wounds and fin erosion and are of minor interest when it comes to nutrition. An optimum level of ARA has been shown to promote good spawning performance and egg quality in a number of fish species. ARA should therefore be adjusted for farmed ballan wrasse to become more similar to the wild fish. Adjustments should also be done in iodine. Vitamin D was higher in wild fish than in cultured fish. This vitamin does not usually show a clear relationship between body level and requirement, so it is unknown if the requirements were covered in the cultured fish and requirement studies are recommended. Taurine and zinc are other possible candidates for more in-depth investigations, zinc because it was considerably higher in farmed than in wild fish and is usually quite well regulated.

Table 1. Nutrients present at different levels in female gonads of captive and wild caught ballan wrasse (several other nutrients were measured as similar).The captive fish had been held at Marine Harvest Labrus for more than one year and fed a diet consisting of 75% Vitalis (Skretting) and 25% cooked and minced shrimp meal±SD. (Taurine, g/kg; Micro-nutrients mg/kg; fatty acids % of total fatty acids).

N	Captive 6	Wild 10	P t-test	P Mw-u test
GSI*	7.0±1.9	8.7±2.7	ns*	ns
Taurine	5.6±1.8	10.4±1.8	0.002	0.014
Vitamin D	0.31±0.23	0.66±0.33	0.04	0.04
Vitamin K	na	0.058±0.027	-	-
Astaxanthin	0.8±0.2	0.0±0.0	ns	0.0014
I	0.61±0.17	1.48±0.58	0.003	0.004
Zn	262±47	169±24	0.0001	0.001
20:4n-6 ARA	2±0	6±2	0.00002	0.001
20:5n-3 EPA	17±1	12±1	$<10^{-7}$	0.001
22:6n-3 DHA	25±1	30±4	0.01	0.015
DHA:EPA	1.5±0.1	2.5±0.5	0.0004	0.0014
ARA:EPA	0.10±0.03	0.49±0.13	$<10^{-5}$	0.0014

* GSI: Gonadosomatic index (gonad weight/total weight*100%), ns: not significant

ACKNOWLEDGEMENTS

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Hamre, K., Nordgeen, A.H., Grøtan, E., Breck, O. (2013). A holistic approach to development of diets for ballan wrasse (*Labrus bergylta*) – a new species in aquaculture. PeerJ 1:e99; DOI 107717/peerj.99

RECOMMENDATIONS

The levels of nutrients in the broodstock diets should be adjusted so that the nutrient composition of farmed fish female gonad matches that of wild fish.

This may be difficult to achieve for vitamin A, D and K (A and K are probably also low based on the results from juveniles) and requirements studies should be performed for these vitamins.

CHAPTER 3

Wet and dry fertilisation of wrasse eggs

Ingrid Lein and Yoav Barr

INTRODUCTION

Artificial fertilisation of stripped eggs will give commercial ballan wrasse producers better control of the egg production by a higher gain of eggs per female because a high amount of eggs are lost during natural spawning, eggs are dirty and efficient disinfection is difficult. However, the wrasse males release mostly small amounts of milt which must be utilised in the best possible way during artificial fertilisation. Due to this, two experiments were designed so as to check whether a wet or a dry fertilisation procedure result in different fertilisation rates and to check how much sperm is needed to obtain the highest fertilisation rates.

EXPERIMENTAL SETUP

WET AND DRY FERTILISATION

Eggs were stripped from one female and two males. The motility of the sperm from both males was checked under the microscope before start of the experiment. Approximately 15 ml of eggs were distributed to two plastic beakers (Figure 1).

Figure 1. Experimental units used for fertilisation of ballan wrasse eggs. The picture also demonstrates coagulation of eggs immediately after addition of sea water (right beaker). Photo: Yoav Barr, Nofima

WET FERTILISATION:

A couple of droplets of milt from each male were diluted in 150 ml sea water, mixed quickly and then poured over the eggs. The eggs were gently stirred for 5-6 seconds before they were left to harden for 10 minutes.

DRY FERTILISATION:

A couple of droplets of milt from each male were added to the eggs without addition of water. Two minutes later 150 ml of sea water was added. The eggs were then gently stirred for 5-6 seconds before they were left to harden for 10 minutes.

After 10 minutes the eggs were rinsed three times with filtered and UV-treated sea water before incubation in experimental units (16x12x3.8 cm) resembling hatching trays used for salmon (Figure 2).

The fertilisation rates were checked under the microscope after 19 hours.
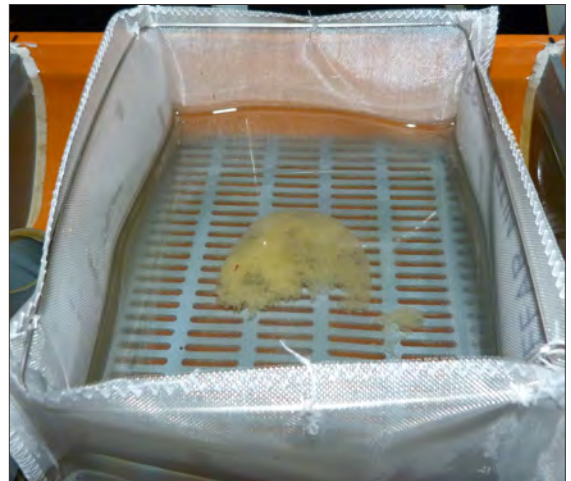
Figure 2. Ballan wrasse eggs incubated in experimental units resembling traditional hatching trays used for salmonids. Photo: Yoav Barr, Nofima

SPERM CONCENTRATION

Milt was stripped from 3 males and sent to Cryogenetics at Hamar for dilution 1:2 in an extender before being returned to Nofima 3 days later. Eggs were then stripped from one female and approximately 1 ml of eggs was transferred to each of 5 scintillation vials (volume 50 ml). 10 ml of seawater was added to each of 5 other scintillation vials and 5, 10, 20, 50 or 100 µl of diluted milt was added to these glasses in sequence. After a quick stir, the milt/water mixture was poured over the eggs, and the vials were closed and stirred. The fertilisation of eggs with the different milt concentrations was done in sequence to ensure that the milt stayed motile after contact with sea water. The vials with fertilised eggs were held in a temperature controlled room at 11.5°C, and fertilisation was checked after 19 hours.

Results and discussion

Wet and dry fertilisation

The stripped eggs appeared clean and of good quality, and the milt from both males had good motility when examined under the microscope. The fertilisation rates were high in both treatments; 99% for wet fertilisation and 96% for dry fertilisation. The results suggest that both methods can be applied with good results for ballan wrasse eggs.

Sperm concentration

For the second experiment using different concentrations of sperm, a few fertilised eggs were observed in the groups fertilised with the three highest concentrations of milt (20, 50 and 100 µl diluted milt). There could be several reasons for the low fertilisation rates; the milt motility was not as apparent in this experiment as for the milt used in the wet/dry experiment, the transportation of the milt could have affected the viability of the sperm cells, although we obtained high fertilisation rates in a later experiment using diluted milt that had been stored for 5 days.

We suspect milt motility as one important factor. However, the eggs became sticky immediately after contact with sea water, and eggs were sticking to the wall of the vial. Therefore the vials were turned upside down several times, and also stirred to loosen the eggs. We suspect this handling of the eggs before hardening may have been the main reason for the poor fertilisation in this experiment.

Recommendations

Both wet and dry fertilisation can be applied to ballan wrasse eggs.

The motility of sperm used for artificial fertilisation of ballan wrasse eggs should be checked under the microscope before use to make sure that the sperm cells are of good quality.

Rough handling of eggs should be avoided, especially before hardening.

Eliminating egg stickiness
Spawned and fertilised ballan wrasse eggs

Ingrid Lein and Yoav Barr

INTRODUCTION

Ballan wrasse spawns eggs that stick together after exposure to seawater. So far, only naturally-spawned eggs are used in the commercial production of ballan wrasse. The eggs are collected on rubber mats placed on the tank bottom. Thus eggs collected from spawning tanks are dirty (Figure 1a), and the stickiness of the eggs makes it difficult to disinfect wrasse eggs as efficiently as for other pelagic marine eggs, such as from halibut or cod. Elimination of egg stickiness can also make easier both the egg incubation and the removal of dead eggs during the incubation period. Many methods for removing adhesiveness have been applied for other fish species with sticky eggs. Some involve placing the eggs in solutions such as: milk, talc or sludge (clay), whereby the eggs placed in such solutions are coated with a fine film and, therefore, do not stick together. Milk protein (either as milk powder or fresh milk), and/or treatment with clay suspensions prevented stickiness in eggs of tench (Linhart, 2000) and common carp (Billard, 1995). Protease enzyme treatment has also been successfully used to eliminate stickiness in fertilised tench eggs (Linhart et al., 2000, 2003, 2006). Other chemical methods for elimination of egg stickiness involve urea/sodium chloride (NaCl) or sodium sulphite (Na_2SO_3) treatment followed by a tannic acid wash (Kowtal et al., 1986). In the present work clay suspension, sodium sulphite and enzyme treatments were tested on eggs that were spawned spontaneously and fertilised in the broodstock tanks.

EXPERIMENTAL SETUP

A series of small scale experiments were done to investigate the effectiveness of the different treatments on elimination of egg stickiness in naturally-spawned wrasse eggs.

CLAY SUSPENSION

20 g of clay was mixed in 1 L of sea water with a hand blender. Eggs spawned during the night were scraped into a petri dish, and clay suspension was added to approximately 5 mm depth. In a control dish, only seawater was added. The petri dishes were rotated by hand every minute. After 10 minutes, the eggs were sieved, returned to the petri dish and a new clay solution was added. After another 10 minutes with almost continuous rotation, the eggs were sieved again, returned to the petri dish and rinsed with seawater. The stickiness of the eggs was checked using a microscope after 10, 20 minutes and 20 hours.

SODIUM SULPHITE (Na_2SO_3)

1.5 g of Na_2SO_3 were dissolved in 100 ml of distilled fresh water. A sample of eggs spawned the night before was transferred to a 200 ML glass beaker, and 90 ML of sea water were added, and oxygen was measured. Then 10 ML of the Na_2SO_3 solution were added. The eggs were held in Na_2SO_3 solution for 10 minutes before rinsing with sea water, and then checked in the microscope for stickiness.

ENZYME TREATMENT

Sigma-Aldrich alkalase with >2.4 units/ml was used to prepare a stock solution of 1:10 in seawater. Eggs spawned the night before were transferred to a graded cylinder, and seawater was added until 40 ml. Thereafter the eggs were transferred to a plastic beaker and 6 different volumes of the enzyme stock solution (0.31, 0.62, 1.24, 1.86 2.48 and 3.49 ml) plus water were added until a total volume of 50 ml was obtained. Eggs with seawater only were used as control. The beakers were rotated immediately and after 1 minute to ensure good mixing. After 2 minutes, the eggs were filtered, rinsed with sea water and transferred to a clean beaker with sea water. The eggs were photographed after 10 minutes and 20 hours. The process was repeated for all concentrations to ensure two replicates.

Results and discussion

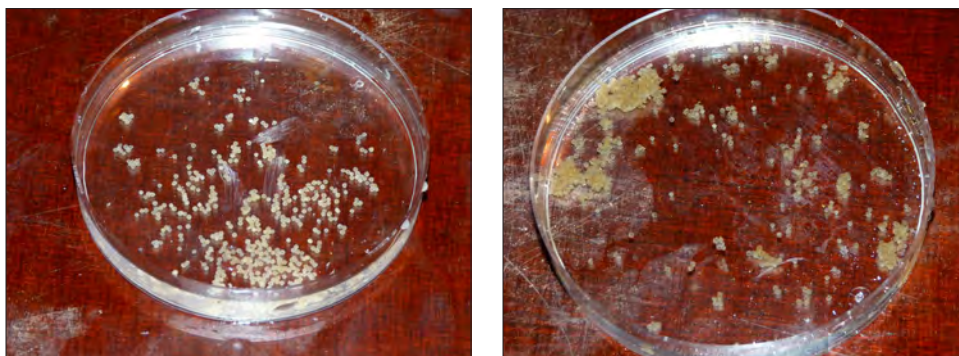## Clay treatment

The clay treatment did not decompose the gum layer nor loosen the dirt sticking to the gum layer, i.e. the eggs did not appear to be any cleaner (Figure 1).
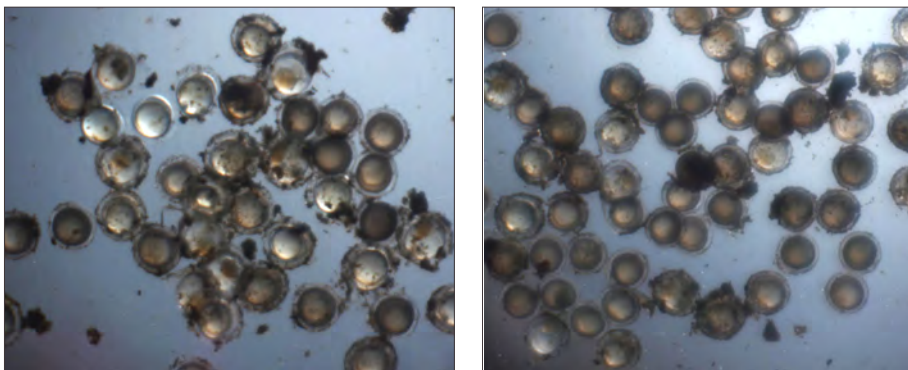
Figure 1. Naturally spawned eggs collected from a spawning mat (a) and eggs treated with a clay solution for 20 minutes. The pictures are taken 20 minutes after end of the clay treatment. Photo: Yoav Barr, Nofima

However, after 10 minutes, eggs treated in a clay solution were mostly single eggs and the eggs no longer adhered to the surface of the petri dish. In the control group, the stickiness did not change, and eggs were sticking to the dish walls. After 20 hours the results were the same as after 10 minutes (Figure 2). This means that this method can be used to decrease the egg stickiness and thereby improve the effect of disinfection and the availability of oxygen during egg incubation. However, the risk of contamination is still high as the dirt from the spawning mats is still attached to the eggs and, therefore a proper disinfection protocol is required.

Figure 2. Ballan wrasse eggs treated with a clay solution for 20 minutes before incubation in sea water (a), and wrasse eggs incubated in sea water only (b). The pictures are taken 20 minutes after end of the clay treatment. Photo: Yoav Barr, Nofima

Sodium sulphite (Na_2SO_3)

The sodium sulphite treatment did not disintegrate the gum layer, and the eggs stickiness seemed not much affected by the treatment. From cod we know that use of sodium sulphite in sea water increases the oxygen demand drastically. The oxygen measurements in the present experiment showed that the oxygen content was reduced drastically during the 10 minute treatment; from 98% to 7%. This means that this treatment probably is dangerous for the eggs, and cannot be recommended.
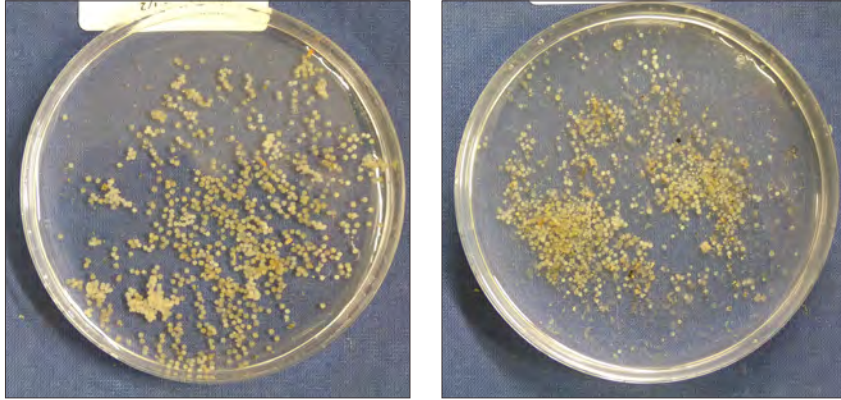
Figure 3. Naturally-spawned ballan wrasse eggs either incubated directly in seawater (a), or treated for 2 minutes with the enzyme alkalase before incubation in sea water. The pictures were taken 20 minutes after end of the enzyme treatment. Photo: Yoav Barr, Nofima

Eliminating egg stickiness
Stripped ballan wrasse eggs

Ingrid Lein, Yoav Barr and Helge Tveiten

INTRODUCTION

Ballan wrasse eggs stick to each other and to substrate after exposure to seawater. This makes egg disinfection and incubation more challenging than for non-sticky fish eggs, such as halibut or cod eggs. Many other teleost fish species such as tench, common carp and wolffish also have adhesive eggs which in nature attach to plants or to hard substrates (rock or gravel). Various methods have been used to eliminate such egg stickiness (see previous article). Most of these procedures involve the treatment of eggs after fertilisation. However, based on the poor results from our previous attempts to remove the stickiness of fertilised eggs, a series of small-scale experiments was set up to treat the eggs before fertilisation. In the present work, tests were made on the effects of treatments before fertilisation, using milk or enzymes (alkalase), on the formation of a gum layer on the egg surface.

EXPERIMENTAL SETUP

PRELIMINARY TEST OF MILK FOR ELIMINATION OF EGG STICKINESS

The experiment was done with unfertilised eggs. Eggs were stripped from one female and approximately 1 ml of eggs was distributed to each of three 20 ml scintillation vials. For the first vial (control) sea water only was added to the eggs. To the second vial, a mixture of 50% sea water and 50% fresh milk was added to the eggs, and to the third vial fresh milk only was added. All vials were stirred for 1, 5 and 15 minutes after addition of sea water and/or fresh milk, and the stickiness of the eggs were evaluated visually at these times.

EFFECT OF MILK ON EGG STICKINESS

The experiment was done in 50 ml centrifuge tubes. Eggs and milt were stripped from one female and one male. 1 ml of eggs was distributed to each of 6 tubes. 1 ml of milt was diluted in 1.5 ml of Ringer's solution. Ringer's solution is a physiological solution that does not initiate the formation of the gum layer or sperm activity. The eggs were fertilised using a semi-dry method; the mix of milt and Ringer solution was split in six and distributed to 6 tubes with unfertilised eggs. Immediately thereafter sea water was added to 3 tubes, and a mixture of 50% sea water and 50% fresh milk was added to the other 3 tubes. The eggs were left to harden for 10 minutes, and then rinsed 3 times with sea water to remove effluent milt. The stickiness of the eggs was evaluated visually after rinsing. The tubes with eggs were then stored in a temperature controlled room (10.5°C) until the next day when the fertilisation rates were checked under the microscope.

ENZYME TREATMENT
– DURATION OF TREATMENT

This experiment was done to clarify whether the stickiness comes into being on the egg surface or in the egg fluid, and whether the duration of the exposure to enzymes is of importance for the formation of the gum layer. To investigate the effect of the egg fluid, the egg fluid was replaced by Ringer's solution which is a physiological solution. Alkalase was used to study the effect of enzyme treatment before fertilisation on the development of gum layer. Four treatments were tested:

1.   Control with egg fluid, no Ringer's solution or alkalase added

2.   Ringer: The egg fluid was removed and replaced with Ringer's solution before fertilisation

3.   Alkalase: Alkalase was added before fertilisation of the eggs

4.   Alkalase + Ringer: The egg fluid was replaced with Ringer's solution, and alkalase was added before fertilisation

For all treatments, 3 different durations were tested; 5, 10 or 20 minutes.
Before the start of the experiment an enzyme stock solution was prepared: 25 ml alkalase : 250 ml of Ringer's solution. Sigma-Aldrich Alkalase with >2.4 units/ml was used for enzyme treatment.

Eggs and milt were stripped from one female and 4 males. Approximately 1 ml of eggs was distributed to each of 36 centrifuge tubes (volume 50 ml). Groups 2 and 4 (18 tubes) were rinsed three times with Ringer's solution to remove the egg fluid. Then 15 ml of the alkalase stock solution was added to groups 3 and 4 (18 tubes), and a timer was set. After 5 minutes, approximately 1 ml of sperm was mixed quickly with 25 ml of seawater and added to each of 3 tubes per treatment. After 10 or 15 minutes, the fertilisation procedure was repeated for the remaining 6 tubes per treatment, respectively. Thereafter the eggs were rinsed 3 times with sea water, and then stored horizontally in a temperature controlled room (10.5°C) until the day after when fertilisation rates were checked under the microscope.

RESULTS AND DISCUSSION

PRELIMINARY TEST OF MILK FOR ELIMINATION OF EGG STICKINESS

After only 1 minute the unfertilised eggs in sea water only were sticking to the wall of the vial. For the 50% mix of sea water and milk, the eggs seemed well separated after 1 minute. However, after 5 minutes a few eggs attached to the vial wall and after 15 minutes some more. For the eggs in 100% milk, no eggs were attached to the wall after 1, 5 or 15 minutes (Figure 1). The best result with regard to reducing the egg stickiness was obtained when 100% milk was added to the eggs. However, for marine eggs like those of ballan wrasse the lack of salinity or changes in pH could be negative for fertilisation and/or further development of the eggs.

CONFIRMATION OF ENZYME EFFECT ON GUM DEVELOPMENT

Two more egg groups were treated according to procedure 4 as described above to confirm that the enzyme treatment prevent gum development and to evaluate hatching percentage. These two experiments were done with eggs from a skewed light regime, i.e. eggs were stripped by the end of August/beginning of September instead of June/July, which is the natural spawning season for ballan wrasse. The brood stock was treated with gonadotropin releasing hormone before stripping of eggs and milt.

Figure 1. Visual evaluation of stickiness of unfertilised eggs of ballan wrasse fertilised in seawater only (left) 50% sea water and 50% fresh milk (middle) or fresh milk only right. Photo: Nofima

EFFECT OF MILK ON EGG STICKINESS

The eggs fertilised in a mixture of 50% milk and 50% sea water seemed somewhat less sticky than eggs fertilised in sea water only. However, the stickiness was stronger than observed in 100% milk in the preliminary trial (Figure 2).

Figure 2. Stripped eggs fertilised with either seawater only (a), or in a mixture of 50% sea water and 50% fresh milk. The picture is taken after rinsing with sea water. Photo: Ingrid Lein, Nofima

The fertilisation rates for eggs fertilised with 50% sea water and 50% milk were significantly higher than in the control where the eggs were fertilised with sea water only (Figure 3, Table 1). This was somewhat surprising, but might be due to differences in ion concentration/composition or pH. The difference between the two treatment was substantial (71.4% versus 91.3%), and should be investigated further.
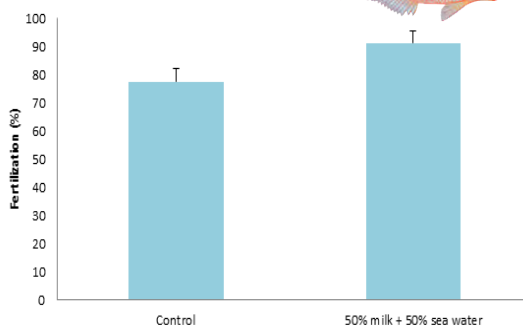
Figure 3. Fertilisation rates of stripped ballan wrasse eggs fertilised either in seawater only (a) or in a mixture of 50% sea water and 50% fresh milk. (Average +SD, n=3).

Table 1. Stickiness of ballan wrasse eggs with replacement of the egg fluid with Ringer's solution and/or addition of the enzyme alkalase.

| Treatment	5 min. treatment	10 min. treatment	20 min. treatment
1. Control	Sticky	Sticky	Sticky
2. Ringer	Sticky	One replicate less sticky	Partly sticky
3. Alkalase	Sticky	Sticky	Sticky
4. Alkalase + Ringer	Free eggs	Sticky	Partly sticky

ENZYME TREATMENT
— DURATION OF TREATMENT

Only treatment 4 where alkalase was added and the egg fluid was replaced with Ringer's solution resulted in totally free eggs (Table 1). In treatment 2, with the replacement of the egg fluid with Ringer's solution, the eggs were less sticky after 20 minutes, but not totally free (Table 1). For eggs treated with alkalase, no gum layer was developed (Figure 4). The control group and group 2, where the egg fluid was replaced by Ringer's solution, both showed similar development of a gum layers. The fact that eggs stored with Ringer's solution only until fertilisation also showed a reduction in stickiness indicates that there is an interaction between proteins of the ovarian fluid and proteins on the egg surface as has described for Eurasian perch by Mansour, 2009.

The fertilisation rates were high in all treatments, but were significantly higher ($p < 0.0001$) when the eggs were treated with alkalase before fertilisation (Table 2). The fertilisation rates were not significantly affected by the duration of the alkalase treatment.

Table 2. Fertilisation rates of ballan wrasse eggs with replacement of the egg fluid with Ringer's solution and/or addition of the enzyme alkalase (means +Std). Different letters indicate significant differences (means ±std, n=3).

Treatment	Fertilisation (%) +STD	Significant differences
1. Control	88.7 ±1.3	a
2. Ringer	89.7 ±0.7	a
3. Alkalase	95.6 ±0.7	b
4. Alkalase + Ringer	95.9 ±1.1	b

The eggs were stored in the hatchery after evaluation of fertilisation rates, but none of the eggs, including the control, developed until hatching despite the high fertilisation rates. It was suspected that the ballan wrasse eggs might depend on water movement to hatch successfully. However, Bridie Grant at University of Stirling obtained high hatching rates in ballan wrasse eggs treated with alkalase after spawning and incubated in petri dishes (pers. med.). This proves that removal of the gum layer or egg incubation in stagnant water should not inhibit hatching. In our experiment, closed vials were used which may have caused oxygen deficiency.
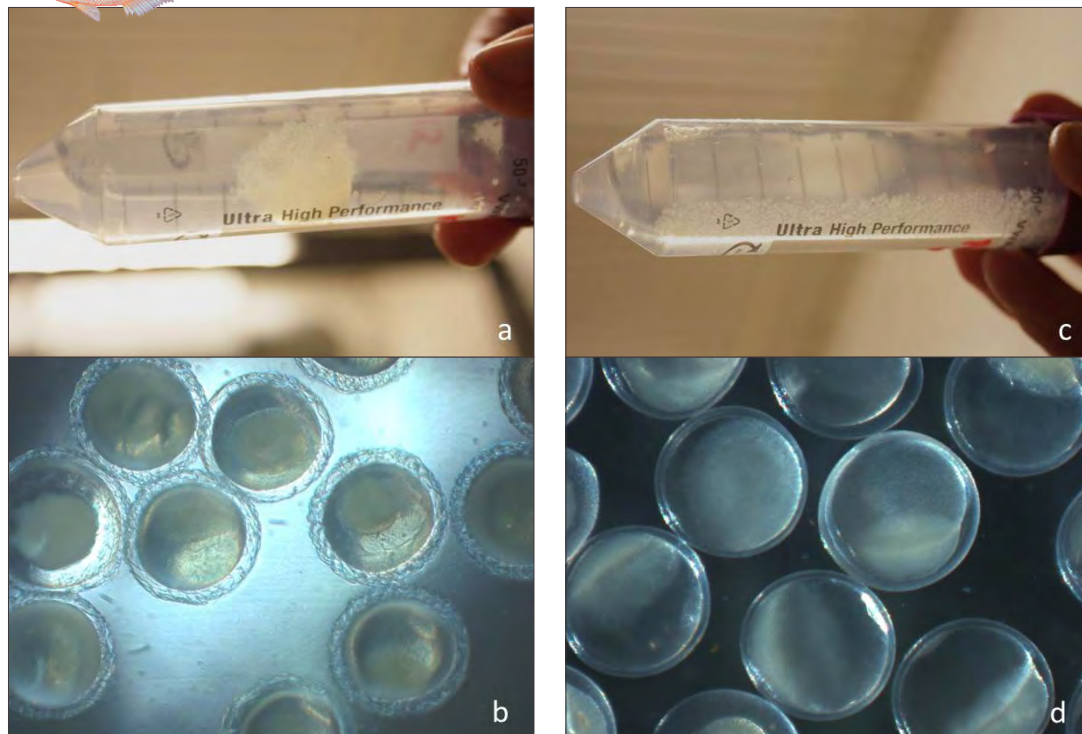
Figure 4. Eggs from treatment 1 (control group) fertilised in the egg fluid with no addition of enzyme or Ringers solution (a, b), and eggs from treatment 4 where the egg fluid was replaced with Ringer's solution, and alkalase was added before fertilisation (c, d). Picture c and d show eggs treated with alkalase for 5 minutes before fertilisation. Photo: Ingrid Lein, Nofima

CONFIRMATION OF ENZYME EFFECT ON GUM DEVELOPMENT

The positive effect of alkalase treatment on preventing gum layer development was confirmed in both follow-up experiments In the first experiment the results were very similar to the main experiment with high fertilisation rates (Table 3), and the fertilisation rates in the control group were significantly lower than in the two other treatments. In the second experiment however, the fertilisation rates were lower (Table 2), and there was no significant difference in fertilisation rates between treatments. However, the fertilisation rates are still high and within a normal variation. As for the main enzyme experiment, the eggs did not survive until hatching in either of the two follow-up experiments.

Table 3. Fertilisation rates in the two follow-up experiments on effect of enzyme and Ringer's solution on development of gum layer (means ±std, n=3).

Treatment	Experiment 1		Experiment 2	
	Fertilisation (%) ±STD	Significant differences	Fertilisation (%) ±STD	Significant differences
1. Control	81.3 ±4.6	a	72.0 ±12.6	a
2. Ringer	90.7 ±0.9	b	71.3 ±3.7	a
3. Alkalase + Ringer	95.0 ±2.8	b	77.3 ±1.2	a

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RECOMMENDATIONS

Fertilisation of ballan wrasse eggs with a 50% mixture of fresh milk and sea water reduced the stickiness of the eggs considerably.

Treatment of ballan wrasse eggs with the enzyme alkalase and replacing the egg fluid with Ringer's solution prevent the development of a gum layer on the egg surface.

However, none of these methods should be used in a commercial production before the effects on the egg and larval development have been clarified.

Surface disinfection of ballan wrasse eggs

Stine Wiborg Dahle, Gunvor Øie and Ingrid Lein

INTRODUCTION

The aim of this study was to establish a procedure that facilitates the surface disinfection of ballan wrasse eggs so as to reduce the probability of disease transfer. Two chemical disinfectants (Pyceze® and glutaraldehyde) were evaluated on the basis of their bactericidal and potential toxic effect on hatchability of ballan wrasse eggs.

EXPERIMENTAL SETUP

Two disinfectants were tested: Pyceze® according to the manufacturer's procedure and glutaraldehyde $(CH_2(CH_2CHO)_2)$ (400 ppm) with contact times of 4 and 8 minutes. In addition, a control without disinfection was included. Fertilised eggs from Nofima broodstock were used, spawned on rubber mats in the tank. The spawning mats were cut in sections (Figure 1A), transferred to the different treatments (Figure 1B) and rinsed with sterile seawater to remove residual chemicals.

The eggs were transferred individually (n=20) to three M65-agar plates for each treatment. In order to cover the egg surface with agar, eggs were gently forced into small depressions in the agar plates (Figure1C) (Salvesen & Vadstein, 1995).

Agar plates were incubated at 12°C in 2 and 14 days for registration of fast and slow growing bacteria respectively.
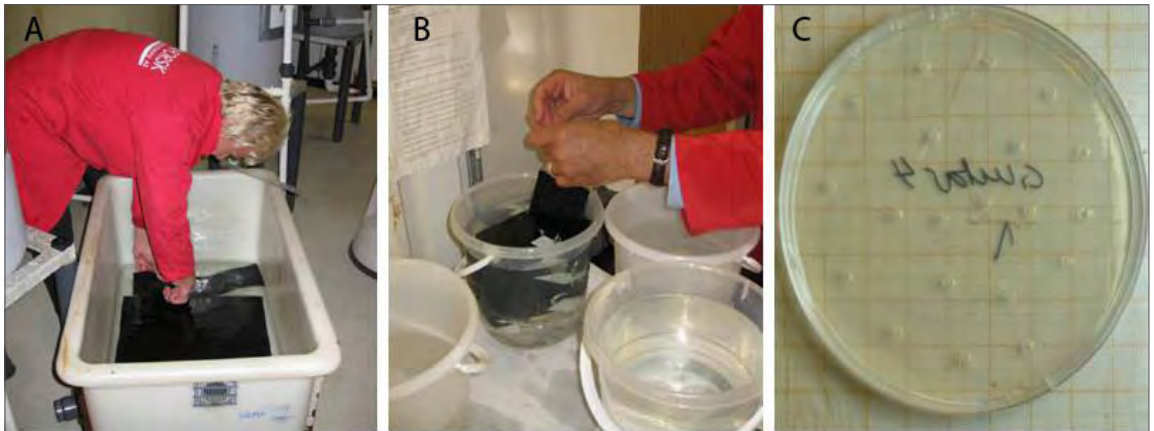
Figure 1. A: Cutting of spawning mats before disinfection. B: Surface disinfection of eggs in different treatments. C: Transfer of eggs to agar plates with depressions, 20 eggs per agar plate. Pictures: Nofima/SINTEF.

Spawning mats from the three different treatments and the control were transferred to incubators of 25 L (Figure 2) to evaluate the effects of different treatments on hatchability.
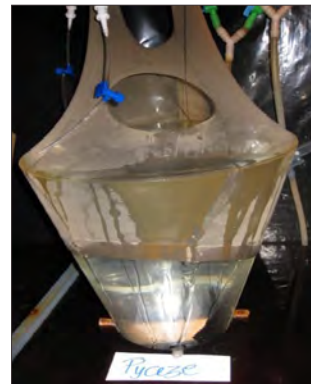
**Figure 2.** Incubator (25 L) of eggs for the Pyceze® treatment.

RESULTS AND DISCUSSION

BACTERICIDAL EFFECT

In the untreated control groups and the treatment with Pyceze®, the percentage of eggs with fast-growing bacteria after 2 days of incubation was 100%. In treatments with glutaraldehyde for 8 minutes the percentage was 27% and glutaraldehyde for 4 minutes 20 %. After 14 days of incubation, all the eggs from the treatment groups had slow-growing bacteria (Figure 3).

HATCHING SUCCESS

Treatments with glutaraldehyde for 4 minutes did not have any significant adverse effect upon hatchability since this group had a higher hatching success (62% ± 20) than untreated eggs (29% ± 17). Glutaraldehyde for 8 minutes contact time seems to be affecting the hatching negatively (Table 1), with a low hatching success (15% ± 16).
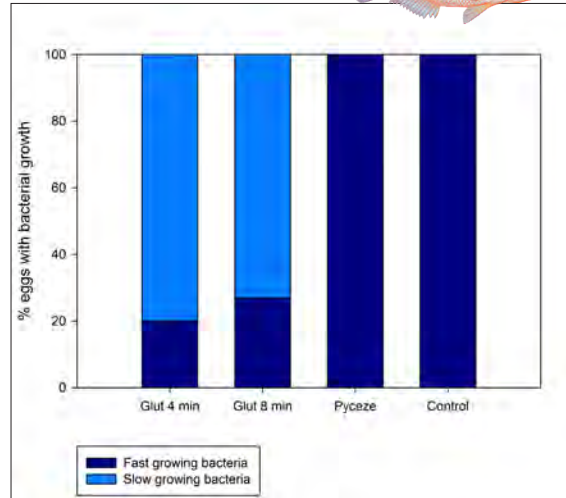
Figure 3. Percentage of eggs with bacterial growth after 2 (fast growing bacteria) and 14 days (slow growing bacteria) of incubation in M65-agar.

Table 1. Hatching success (%) from the different treatments and control, with standard deviation, n=3.

Treatment	Hatching success (%)	Standard deviation
Glutaraldehyde 4 minutes	62,8	20,4
Glutaraldehyde 8 minutes	15,0	16,2
Pyceze®	23,0	19,3
Control without disinfection	29.6	17.0

Of the two tested chemicals in this study, glutaraldehyde is the most suitable disinfectant of eggs from ballan wrasse, since it results in a good bactericidal effect. This is in accordance to other studies with marine fish eggs (e.g. Salvesen & Vadstein, 1995). A 4 min treatment at a concentration of 400 ppm seems to be a safe and effective level for surface disinfection of eggs, without any documented adverse effect on hatchability. For further details, see Dahle et al. (2013).

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RECOMMENDATIONS

Glutaraldehyde with a 4 min treatment at a concentration of 400 ppm seems to be a safe and effective level for surface disinfection of eggs, without any documented adverse effect on hatchability. Glutaraldehyde is an irritant to the skin, eyes, and respiratory system, and precautions should be taken to avoid any health risk to the users. The method is hence suitable for testing the effect of different treatments, but should be developed further, to avoid exposure when used in commercial production of ballan wrasse.

CHAPTER 4

Production Manual for *Acartia tonsa* Dana
The copepod *Acartia tonsa Dana* as a live prey for ballan wrasse larvae

Andreas Hagemann

INTRODUCTION

Copepods are the natural prey organisms for fish larvae in the marine food web and are believed to be ideal as first feed organisms for the intensive production of marine fish larvae. Traditionally, copepods have been harvested directly from the sea or produced in semi-extensive and extensive systems. However, these methods rely on blooms of natural phyto- and zooplankton organisms and allow limited control over species composition and the introduction of diseases and parasites. Intensive production of copepod eggs from the calanoid copepod *Acartia tonsa* Dana has been running successfully at SINTEF SeaLab for over a decade. These eggs are cleaned, disinfected and stored in a refrigerator at 2°C. Thereafter, the eggs can be shipped world-wide to end-users and used as an inoculum for live feed production in commercial hatcheries.

NUTRITION

Copepods have naturally high levels of essential n-3 highly unsaturated fatty acids (HUFA) such as docosahexaenoic acid (22:6n-3; DHA) and eicosapentaenoic acid (20:5n-3; EPA). Moreover, copepods have a macro- and micronutrient composition including high phospholipid fractions of total lipids and balanced amino acid profiles, vitamins and pigments that satisfy the marine fish larvae's requirements for optimal growth and development. As replacement for, or complimentary to traditional live feed organisms such as *Artemia* sp. and rotifers in marine juvenile fish production, copepods will reduce the rate of deformities and malpigmentation and improve growth, survival and stress tolerance of the fish larvae.
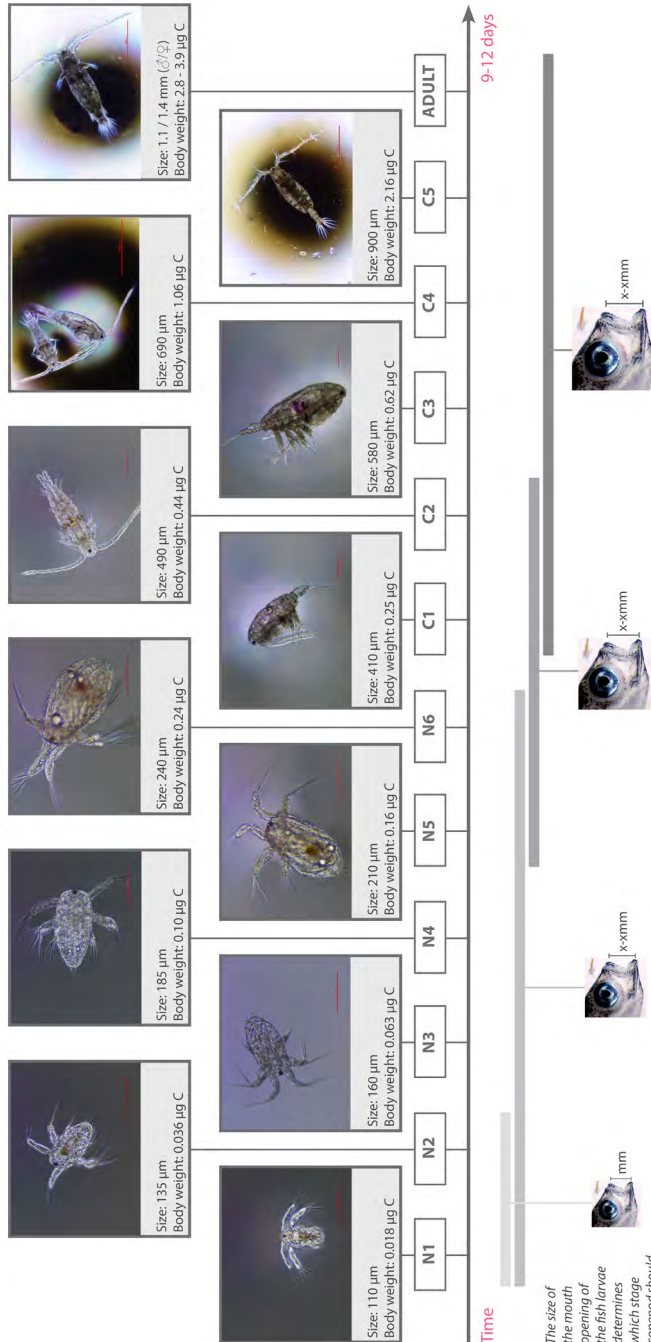
PRODUCTION MANUAL

Acartia tonsa Dana eggs can upon arrival at the hatchery be hatched immediately, or alternatively placed in cold storage for up to a year before use. Newly hatched nauplii can be fed immediately to the fish larvae, or cultivated to larger sizes on a diet of live microalgae.

The production manual describes the basic know-how for hatching and cultivating *Acartia tonsa* Dana , from eggs to adults, with charts and illustrations showing size distribution and dry weight content for the different developmental stages.

DEVELOPMENTAL STAGES OF ACARTIA TONSA DANA

At 22°C it takes approximately one day for the copepod to evolve into the succeeding developmental stage. N = nauplii, C = copepodid.

Size: 110 µm
Body weight: 0.018 µg C

Size: 135 µm
Body weight: 0.036 µg C

Size: 160 µm
Body weight: 0.063 µg C

Size: 185 µm
Body weight: 0.10 µg C

Size: 210 µm
Body weight: 0.16 µg C

Size: 240 µm
Body weight: 0.24 µg C

Size: 410 µm
Body weight: 0.25 µg C

Size: 490 µm
Body weight: 0.44 µg C

Size: 580 µm
Body weight: 0.62 µg C

Size: 690 µm
Body weight: 1.06 µg C

Size: 900 µm
Body weight: 2.16 µg C

Size: 1.1 / 1.4 mm (♂/♀)
Body weight: 2.8 - 3.9 µg C

N1 N2 N3 N4 N5 N6 C1 C2 C3 C4 C5 ADULT

Time

9-12 days

The size of the mouth opening of the fish larvae determines which stage copepod should be when used as live feed.

x-xmm

* Size is given as total length for nauplii, copepodid and adult stages.
* Scale bars are 100 µm for N1 - N6 and C1 - C3, and 500 µm for C4 - Adult.

Figure 1. An excerpt of the *A. tonsa* Dana production manual.
The complete version can be acquired by contacting SINTEF at andreas.hagemann@sintef.no

Rotifers in aquaculture - Production Manual

Gunvor Øie

INTRODUCTION

Rotifers are used as live feed in the aquaculture of marine fish and crustacean larvae because they are easy to cultivate in high densities on a year-round basis. The size of the rotifers fit the mouth size of most species of fish larvae, and the swimming speed is slow and hence they can easily be captured by the fish larvae. The rotifers tolerate pronounced variations in environmental conditions such as salinity, pH and temperature. Their nutritional value is easy to manipulate, and can thus be influenced to fit the requirement of the fish larval species (Reitan et al. 1993; Øie et al. 1997; Mæhre et al. 2013; Li et al. In press). The first weeks of exogenous feeding are pivotal in the production of marine fish larvae, and rotifers are important live feed for both fish larvae and crustacean species in aquaculture.

A rotifer cultivation manual (in Norwegian) has been developed and recently edited and updated. This manual will be of assistance to new rotifer producers in Norwegian hatcheries.

CONTENT OF MANUAL

The rotifer production manual is divided in the following sections:

1. Introduction
2. Rotifer biology
3. Cultivation of rotifers
4. Nutrition
5. Handling and storage
6. Stock culture
7. Microbial management
8. New technology in cultivation
9. Rotifers in first feeding

Figure 1. *Brachionus plicatilis,* Nevada (Photo: Gunvor Øie).

The complete version can be acquired by downloading at www.rensefisk.no or by contacting gunvor.oie@sintef.no.

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Rotifers (*Proales similis*) for use in first feeding of goldsinny wrasse

Gunvor Øie, Roman Schlehe and Andreas Hagemann

Introduction

Several species of the wrasse family (Labridae) can be used as cleaner fish, and one of them is the goldsinny wrasse (*Ctenolabrus rupestris L.*) (Figure 1). This species is smaller than the ballan wrasse (*Labrus bergylta*), having a maximum body length of 18 cm, and has small pelagic eggs. Its color is different shades of red, orange or light brown and itsmost significant characteristic is the dark spot on the tail, distinguishing it from the other small wrasses. Goldsinny wrasse is most frequent on rocky shores with seaweed to hide among at depths between the subtidal zone and down to 50 meters. The goldsinny wrasse can be found in the Black Sea, the Mediterranean Sea and the Atlantic coasts from Morocco to Norway.

Figure 1. Goldsinny wrasse (*Ctenolabrus rupestris L.*) at Nordland Leppefisk AS, Norway.
Photo: Elin Eidsvik.

One of the challenges in the cultivation of goldsinny wrasse is the small larvae (0.7-1.0 mm). Small fish larvae need small feed particles, and the rotifers used in aquaculture today (different *Brachionus* species) are too big for goldsinny wrasse larvae during the first days of exogenous feeding. To find a new live prey for goldsinny wrasse, the rotifer *Proales similis* was imported from Japan. *Proales similis* is 38% smaller and 60% narrower than *Brachionus rotundiformis*. Today, goldsinny wrasse is harvested from wild populations but, in the future, this species might be produced in marine hatcheries.

Experimental Set-up

The rotifer was imported from the Nagasaki University in Japan, and cultivated in small volumes (initially 1 l, later increased to 4 l) at SINTEF SeaLab (Figure 2). The rotifers were cultured at two different salinities (4 and 20 ppt) at an initial density of 2 ind^{-1} ml^{-1}, water temperature of 22°C and oxygen saturation of 90 - 95%.
The rotifers were fed once a day with *Nannochloropis occulata*.

Results and Discussion

In order to implement *P. similis* in live feed production for marine fish larvae, more testing and experience is needed. The production should be conducted at low salinities and high temperatures (2 ppt and ≤35°C, respectively, Wullur et al., 2009), although the rotifer can also be cultivated at 20 ppt and 20°C with satisfactory results. This study confirms that high culture densities can be achieved within a short period of time despite a low initial culture density, where the highest culture density was reached after 17 days (960 ind. ml^{-1}, 2 ppt salinity). However, ciliates seemed to thrive in the cultures, ousting the rotifers for food resources hence eventually leading to the culture collapsing. We were notsuccessful in removing the ciliates from the cultures by rinsing in a 30 μm sieve, as the rotifers passed through the mesh or were killed by the handling. Due to the similar size range of the observed ciliates and *P. similis*, removing ciliates by filtration was unsuccessful. The best solution for removing ciliates seemed to be freshwater treatment for at least 30 minutes. Also, ciliates seemed to cause most problems at a salinity of 20 ppt. *P. similis* should be cultivated on live microalgae, where both *Nannochloropsis* sp. and *Chlorella* were successfully tested. The body length and width of *P. similis* was measured to be 82.65 μm and 42.85 μm, respectively.

P. similis were shipped to the Environmental Research Laboratory in Machrihanish, Scotland, for use in first feeding experiments. Although the rotifers were never used for start feeding of goldsinny wrasse, they succeeded in bringing the cultures on (Featherstone, P., pers. comm.).

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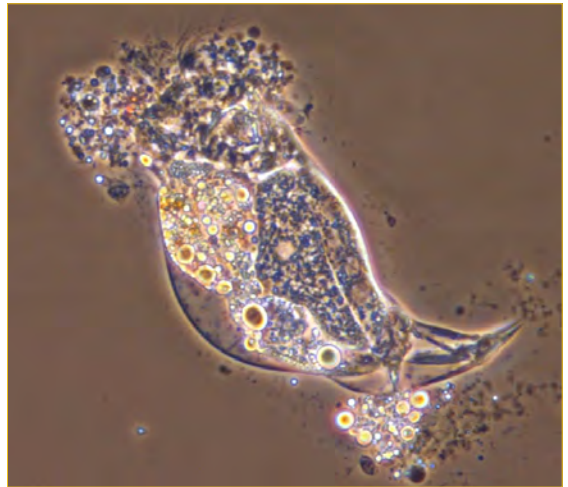
Figure 2. The rotifer *Proales similis.* Photo: Tora Bardal, NTNU.

Recommendations

P. similis should be produced at ≤10 ppt salinity and temperatures between 22–35°C.

Sufficient amounts of *Nannochloropis occulata s*hould be present throughout to avoid culture crash, however, overfeeding should be avoided to sustain an optimal water quality and to avoid contamination of ciliates.

Nutrient composition of rotifers from four different ballan wrasse hatcheries

Kristin Hamre, Erling Otterlei, Espen Grøtan, Helge Ressem and Elin Eidsvik

Introduction

Rotifers are live prey organisms used for first-feeding of cultured marine fish larvae worldwide while, in the wild, fish larvae usually feed on copepods. The reasons for using rotifers for farming purposes are that they can be cultured easily at high densities and that they are suitable as prey. However, fish larvae fed rotifers have lower growth rates, more deformities and become less robust than larvae fed copepods. There are many differences between the two prey organisms in nutrient composition (Hamre et al., 2013) and work is in progress to adjust culture and enrichment protocols for rotifers to make them more similar to copepods. In LeppeProd, we have analysed the nutrient composition of rotifers from the four hatcheries producing ballan wrasse juveniles, in order to monitor the status of rotifer nutrient composition in commercial production and to see if the protocols and culture and enrichment diets can be improved.

Experimental Set-up

Hatchery A: The rotifers were cultured with *Chlorella* (V12, Pacific Trading Co, Ltd.) and enriched for 2-3 hours using a diet composed of an oil blend (50% of the diet) with phospholipids, Croda oil, palmitoyl-l-ascorbic acid, Vitamin A, E, B1, Astaxanthin and a protein blend (50% of the diet): Microfeed (Tromsø fiskeindustri); Orgi Green (Skretting); Sel-plex 2000 (Alltech).

Hatchery B: The rotifers were cultured for 3 days in batch culture, using *Chlorella* (V12, Pacific trading Co, Ltd., 1.8 ml *Chlorella* mill rotifers^{-1} day^{-1}) and then enriched with Multigain (Biomar) for 1.5 hours, the last half hour with antibacterial Pyzece (Novartis).

Hatchery C: The rotifers were cultured on rotifer diet (Reed Mariculture, 1.1 ml mill rotifers^{-1} day^{-1}) and baker's yeast (local supplier, 0.33 g mill rotifers^{-1} day^{-1}) and enriched with Multigain+OriGreen (50/50) 0.35 g mill rotifers^{-1}

Hatchery D: Culture: Monday – Friday with yeast (0.25g mill rotifers^{-1} day^{-1}) and Multigain (0.04g mill rotifers^{-1} day^{-1}), Saturday and Sunday with yeast (0.25g mill rotifers^{-1} day^{-1}) and Rotifer diet (0.2g mill rotifers^{-1} day^{-1}). Enrichment: Overnight alternating between three enrichment diets: S.Presso (INVE) 3x0.12g mill rotifers^{-1}. Multigain 3x0.09g mill rotifers^{-1}. Red pepper (Bernaqua) 3x0.12g mill rotifers^{-1}.

Samples of unenriched and enriched rotifers were taken at the hatcheries A-C. At hatchery D samples were taken only of enriched rotifers, but from rotifers enriched with each of the three different diets. Culture and enrichment diets were also sampled at the hatcheries. The samples were sent to NIFES on a liquid N$_2$ transport tank or on dry ice and analysed, using routine and certified methods.

Results and Discussion

The protein level in rotifers varied between 37 and 45% of DM, in line with previous studies, and was slightly lower (1-2%) in enriched than in unenriched rotifers. The protein concentration in copepods is usually around 60%, and this may be an important reason for the difference between rotifers and copepods in promoting larval growth. Total lipid level varied between 7 and 13%, except at hatchery D, where the lipid level was 16-17%. Total lipid was generally slightly higher (1-2%) in enriched than in unenriched rotifers. The amount of phospholipid was inversely related to the lipid level as shown previously (Hamre et al., 2013), up to 70% at the lowest and around 50% at the highest lipid levels. This is because the fraction of membrane lipids to storage lipids is high in lean rotifers.

Dietary phospholipids are considered important for larval growth (Hamre et al., 2013), and a production of lean rotifers is perhaps the most effective way to increase the dietary phospholipid levels. The fatty acid composition varied between hatcheries. DHA was increased by enrichment with around 10% of total fatty acids, however, the DHA concentration before enrichment was less than 1% at hatchery C and above 10% at hatcheries A and B, affecting the end concentration. Hatchery D had DHA concentrations of 22-35% of total fattyacids. The requirements for fatty acids in cod larvae are not known, but reaching lower copepod levels at around 20% of total fatty acids should be sufficient.

Of the analysed vitamins, vitamin C, D and E were present at levels that are far above the fish requirements (NRC 1993), but the levels are probably not toxic. High doses of vitamin A may give skeletal deformities in fish larvae and the vitamin should be kept between 2.5 and 10 mg kg^{-1} in rotifers and hatchery B and C had concentrations above this range.

The requirement of vitamin K in fish is given as 0.2-2 mg kg^{-1}. All hatcheries except D had levels of vitamin K in the lower range or below this window. The B-vitamins are usually present in rotifers above the levels in copepods and were therefore not analysed in the present study. The bone mineral concentrations (Ca, Mg, P) in rotifers were all above the requirements. The micro-mineral requirements in marine fish larvae have not been studied in detail, except for iodine and selenium. Iodine should be above 3.5 mg kg^{-1} in rotifers to support larval require-ments (Penglase et al., 2013), which was only the case at hatchery D, while selenium was below fish requirements at hatchery B and C and in one of the rotifer batches from hatchery D. At hatchery A they had enriched with sele-nium according to a protocol developed by NIFES and had selenium concentrations well above the requirement. The protein level is clearly very low in rotifers, only about 2/3 of that found in copepods. It is difficult to enrich live feed with protein for reasons described in Hamre et al. (2013). The protein level is a good candidate for explaining the growth promoting effect of copepods. The new knowledge of selenium requirements in cod larvae (Penglase et al., 2010) and enrichment of rotifers with selenium have partly been applied in the industry, while iodine enrichment (Penglase et al., 2013) still awaits implementation. Enrichment with iodine and selenium will probably increase fish larval survivals.

Studies of vitamin K requirements in fish larvae are scarce and should be conducted.

Figure 1. The rotifer *Brachionus* "Caiman". Photo: Scott Mills

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RECOMMENDATIONS

We recommend that the manufacturers of feed for rotifers add iodine and selenium to the diets, but care should be taken not to supplement with too much se-lenium.

The fatty acid composition after culture of rotifers should be given attention in addition to the fatty acid composition of the enrichment diets.

It would simplify rotifer production if one culture diet could be used, which contains all nutrients at sufficient levels to sustain larval growth and development and therefore omit the enrichment step.

It is also recommended to do requirement studies with fatty acids, vitamin K and with micro-minerals other than iodine and selenium.

Start feed quality, feeding regimes and growth of ballan wrasse larvae

This section reports on the effects of different live prey organisms (the rotifer *Brachionus* sp., *Artemia fransiscana* and the copepod *Acartia tonsa* Dana) and a commercial micro-diet, consisting of freeze-dried and preserved calanoid copepods (Planktonic AS), on growth, survival, stress tolerance and feeding preference of ballan wrasse larvae. The start feeding experiments described in the following sections were done at the SeaLab laboratories of NTNU and SINTEF in Trondheim, Norway (Figure 1).

Figure 1. NTNU and SINTEF SeaLab laboratories for start feeding experiments. 18 tanks (160 L) with automatised feeding of live prey (Storvik robot), plankton counter and video surveillance in all tanks. Recirculation or flow-through system. Photo: SINTEF

COMMON REARING CONDITIONS FOR THE EXPERIMENTS IN THIS SECTION

Sea water (34 ppt) was treated with a sand filter and filtered through a 1 μm mesh before being heated and microbially matured, based on descriptions from Skjermo et al. (1997). During the maturation process, the water was continuously treated with a degasser, before being ready to enter the larval rearing tanks. The water temperature was 12°C from the beginning of the experiment and gradually increased to 16°C. The water exchange rate was gradually increased from two times a day at 2 days post-hatch (dph) to eight times a day.

Experiment 1 :
Short term feeding with cultivated copepods and rotifers of different quality

**Gunvor Øie, Stine Wiborg Dahle, Andreas Hagemann,
Maren R. Gagnat, Maria O. Sørøy, Martin Berg,
Kari J. K. Attramadal, Jan Ove Evjemo, Tora Bardal and Elin Kjørsvik**

Introduction

The aim of this study was to examine the effects of different live prey organisms and feeding regimes on the growth, survival and stress tolerance of ballan wrasse larvae.

Experimental setup

Two feeding regimes with short-term feeding with copepod nauplii (*Acartia tonsa Dana*) and two feeding regimes with high and low quality rotifers. (*Brachionus ibericus* (Cayman)) were used for this experiment, and the larvae received the same diet afterwards (enriched *Artemia* nauplii and a formulated diet). Larval growth (dry weight, standard length and myotome height), survival and handling stress tolerance were measured to evaluate the dietary effects. The larvae were fed four different feeding regimes in triplicates from 4 to 30 dph:

- "Copepod": copepod nauplii from 4 to 30 dph.
- "Cop7": copepod nauplii from 4 to 10 dph, thereafter enriched rotifers until 30 dph.
- "RotMG": rotifers enriched with Multigain until 30 dph.
- "RotChl": unenriched rotifers until 30 dph.

The copepods were cultured on the live microalgae (*Rhodomonas baltica*), and the rotifers were cultured on *Chlorella*. The larvae were fed enriched *Artemia fransiscana* nauplii (EG® INVE Aquaculture, Belgium) from 24 to 50 dph, and were then weaned to dry feed (Nofima) from 40 to 61 dph.

Figure 2. Ballan wrasse larvae at start feeding, 4 dph. Photo: Maren R. Gagnat.

Results and discussion

Growth

The results showed that dry weight, standard length and myotome height was significantly higher up till 30 dph for the group fed copepod nauplii for the first 7 days (Cop7) and the group fed copepod nauplii (Copepod) compared to the groups fed rotifers. At 61 dph, the larvae of the copepod groups had a significantly higher dry weight than the larvae fed rotifers (Figure 3B).

The results indicate that the early implementation of a copepod diet, as shown for Atlantic cod (*Gadus morhua*) larvae (Imsland et al., 2006; Øie et al., submitted), has a positive effect on growth in ballan wrasse larvae throughout the larval period. Moreover, the dietary requirements for optimal growth in ballan wrasse larvae were not accommodated by using enriched or unenriched rotifers (Gagnat, 2012; Sørøy, 2012). A period of lower growth was observed for the Cop7 larvae after the diet was changed from copepods to rotifers (12 to 21 dph) (Figure 3A) and copepods to *Artemia* (33 to 47 dph) (Figure 3B).

This was not observed for the other groups which indicate that larvae which were initially fed copepods responded more negatively to a sudden change of live prey types. It was also notable that larvae originally fed low quality rotifers tolerated weaning to a formulated diet more poorly than the other larval groups, as illustrated by their slower growth at the end of the experiment (Figure 3B). Based on the results from this trial it was hypothesized that the ballan wrasse larvae should be fed copepods during the whole live feed period to optimize growth and to avoid a decline in growth when switching live feed (see Experiment 2, follows).
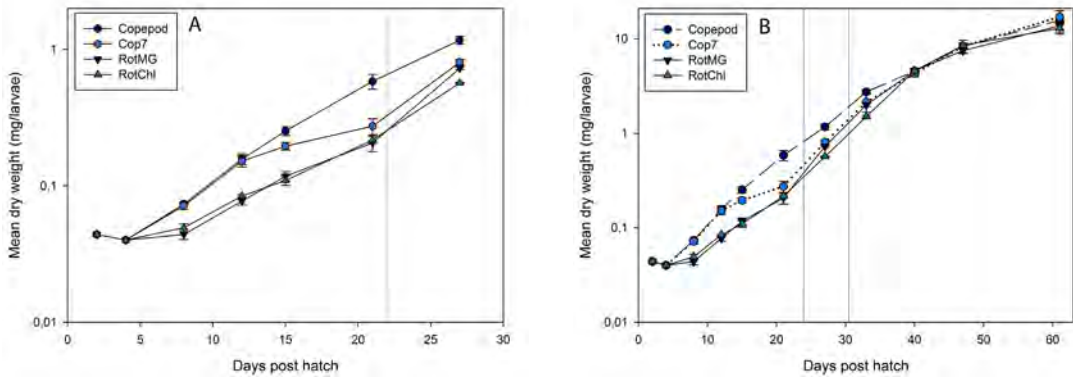
Figure 3. Mean dry weight (mg/larvae ± SE) of ballan wrasse larvae from 2-27 dph (A) and the whole period, from 2-61 dph (B). Logarithmic y-axis. Lines on 24 and 31 dph is the start and end of co-feeding with rotifers/copepods and *Artemia*. Between 31-40 dph the larvae was fed only *Artemia*. The dashed lines on 40 and 51 dph is the start and end of co-feeding with *Artemia* and formulated feed. Exclusively formulated feed from 51 dph.

SURVIVAL

At the end of the experiment (61 dph), the survival was significantly lower in larvae fed unenriched rotifers, compared to the other larval groups (Table 1). The highest mortality was observed during the first 13 days after hatching.

Table 1. Mean survival (% ± SE) for all treatments on 13 and 61 dph. n=3 tanks treatment^{-1}. Significant differences indicated by different letters.

Dph	Copepod	Cop7	RotMG	RotChl
13	16±	24±5.3	17±2.7	12±0.9
61	11±0.5[a]	12±1.4[a]	10±1.3[a]	5±0.6[b]

TOLERANCE TO STRESS

The larval tolerance to handling stress at 29 dph (see page 52) showed that the Copepod treatment had a significantly lower mortality than the RotChl treatment after 24 hours, and there were a significant linear correlation between handling stress mortality and diet quality (Figure 4). There were large differences between the mortality of the larvae from the different replicate tanks, especially in the rotifer fed larvae. Whether a larva survived the stress or not was also significantly correlated to the larval SL, and variance in size accounted for 15.2% of the variance in mortality.
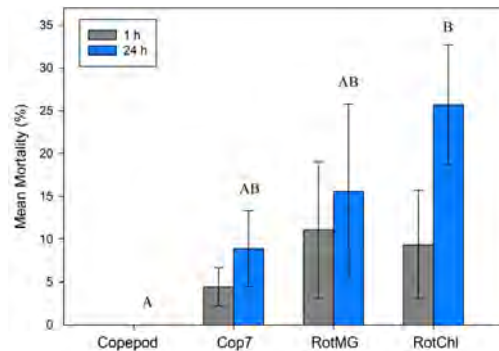
Figure 4. Mean mortality (% ± SE) registered 1 and 24 hours after stress test at 29 dph. AB=Significant differences.

Experiment 2 :
Long term feeding with copepods

Stine Wiborg Dahle, Andreas Hagemann, Yngve Attramadal, Maria G. Stavrakaki, Jan Ove Evjemo and Elin Kjørsvik

INTRODUCTION

Based on the promising results gained from short-term feeding with copepod nauplii (Experiment 1), a start feeding experiment where copepods were used as live prey for a longer period of 41 days was conducted.

EXPERIMENTAL SETUP

The larvae were fed two different feeding regimes from 4 to 45 dph:

1. "Rot": Multigain enriched rotifers (*Brachionus* sp.) from 4 to 40 dph, Multigain enriched *Artemia franciscana* nauplii from 30 to 45 dph.

2. "Cop": Cultivated copepods (*Acartia tonsa Dana*) produced on live *Rhodomonas baltica* from 4 to 45 dph. Nauplii from 4 to 9 dph, copepodites from 6 to 10 dph and adults from 11 to 45 dph.

The present study thus reports on the effects of two different feeding regimes on growth and survival of ballan wrasse larvae; enriched rotifers/*Artemia* and cultivated copepods. Larval growth (dry weight, standard length and myotome height) and survival were evaluated to assess the dietary effects.

RESULTS AND DISCUSSION

GROWTH

First feeding with copepods resulted in a significantly higher growth for the Cop larvae compared to the Rot larvae throughout the experimental period. Dry weight (Figure 5), standard length (Figure 6A) and myotome height (Figure 6B) were significantly higher.
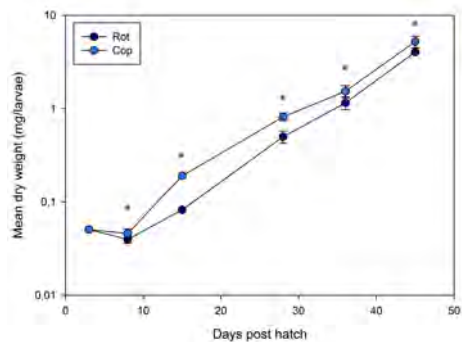
Figure 5. Mean dry weight (mg/larvae ± SE) of larvae fed rotifers/*Artemia* (Rot) or copepods exclusively (Cop) from 3 to 45 dph. *= significant difference. Logarithmic y-axis.
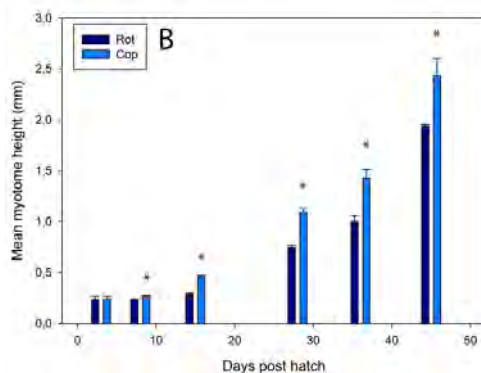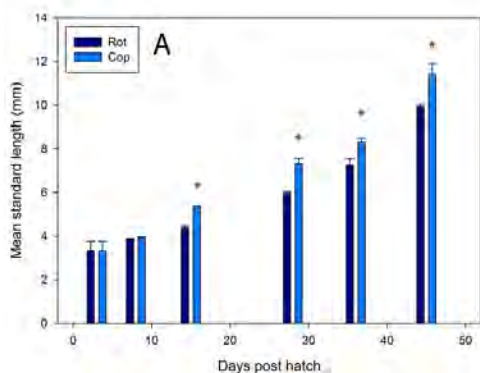
Figure 6. Mean standard length (A) and myotome height (B) of ballan wrasse larvae fed rotifers/*Artemia* (Rot) or copepods (Cop) from 3 to 45 dph. Data= mean ± SE, *= significant difference.

In Experiment 1, the larvae displayed a reduced growth rate when copepods were replaced with rotifers and *Artemia*. In the present experiment, the copepod fed larvae showed a consistent growth that was significantly higher than the larvae fed rotifers/*Artemia* throughout the experiment (Stavrakaki, 2013).

This demonstrated that intensively produced copepods are nutritionally adequate to support growth for ballan wrasse larvae during the live feed period, and that use of copepods was more optimal as first feed than both rotifers and *Artemia*.

In addition to higher growth, the Cop group larvae were perceived to have a more yellow pigmentation on the skin and eyes (Figure 7).

Figure 7. Differences in size and pigmentation of ballan wrasse larvae (45 dph) fed rotifers/ *Artemia* (bottom two individuals "Rot") and copepods (upper two individuals "Cop"). Photo: Maren R. Gagnat.

SURVIVAL

The copepod fed larvae had a somewhat higher survival than the rotifer/*Artemia* fed larvae at the end of the experiment, although the difference was not significant (Tab. 2).

Table 2. Survival (%) of ballan wrasse larvae at 15 and 45 dph, fed rotifers till 40 dph and *Artemia* from 30 dph (Rot), or copepods of continuously increasing size (Cop). Data are mean ± SE.

Dph	Rot	Cop
15	15 ± 1	15 ± 1
45	9 ± 1	11 ± 2

Experiment 3 :
Effect of Planktonic diet on growth, survival and stress tolerance in ballan wrasse larvae

Stine Wiborg Dahle, Kari K. Attramadal, Gunvor Øie, Elin Kjørsvik and Jan Ove Evjemo

INTRODUCTION

The previous start feeding experiments have shown the benefits of feeding ballan wrasse larvae with copepods. The Planktonic diet (Planktonic AS, Norway) consists of preserved and frozen copepods harvested from Norwegian waters (Figure 8). This product has proven adequate as first feed for several species of marine fish larvae and was therefore included in a trial that aimed to assess the effects of different feeding regimes and concentration on the growth, stress tolerance and survival of ballan wrasse larvae.

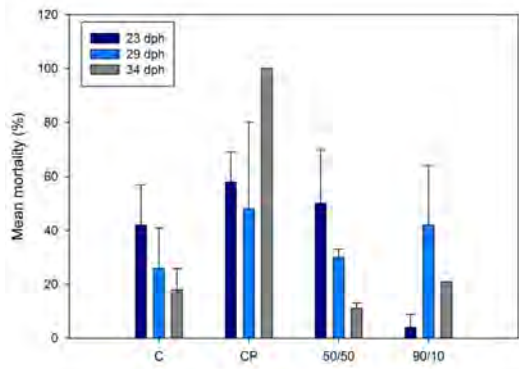**Figure 8.** The Planktonic diet from Planktonic AS, Norway Photo: Tora Bardal, NTNU

EXPERIMENTAL SETUP

The larvae were fed four different feeding regimes in triplicates from 4 to 40 dph:

1. "Control": enriched rotifers from 4 dph to 30 dph, and from 24 to 40 dph enriched *Artemia* nauplii.
2. "ControlP": enriched rotifers (*Brachionus ibericus* Cayman) from 4 dph to 30 dph, and from 24 to 40 dph Planktonic feed (300-400 µm).
3. "50/50": 50 % rotifers and 50 % Planktonic feed (100-300 µm) from 4 dph to 30 dph, and from 24 to 40 dph enriched *Artemia* nauplii.
4. "90/10": 90 % Planktonic feed (100-300 µm) and 10 % enriched rotifers (*Brachionus* sp.) from 4 dph to 30 dph.

Rotifers were grown on the microalgae DHA *Chlorella* and enriched with Multigain. From 24 to 40 dph the larvae were fed enriched *Artemia fransiscana* nauplii (EG® INVE Aquaculture, Belgium). Larval growth (dry weight, standard length and myotome height), survival and handling stress tolerance were measured to evaluate the dietary effects.

RESULTS AND DISCUSSION

GROWTH

The dry weight of larvae in 50/50 and Control were generally similar and increased exponentially from 6 dph, and where at 34 and 40 dph significantly higher than ControlP and 90/10. ControlP had the same growth pattern until they were weaned to Planktonic feed at 24 dph, after which a decline in growth occurred (Figure 9). It was evident that the larvae from 90/10 group showed less than optimal increase in dry weight from the beginning. The general pattern of standard length and myotome height was the same as for the dry weight (data not shown).
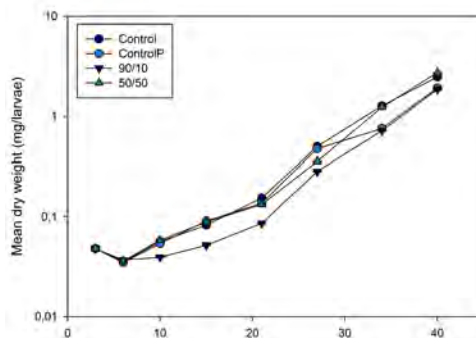
Figure 9. Mean dry weight (mg/larvae ± SE) of ballan wrasse larvae from 3-40 dph. Logarithmic y-axis.

The conditions for optimal growth and survival for the Planktonic treatments were not appropriate since the diet was added too early. Also, horizontal water circulation must be applied in rearing tanks for the diet to be kept suspended in the upper water column and thereby more accessible for the larvae.

TOLERANCE TO STRESS

At 23 dph the 90/10 group showed significantly higher tolerance to handling stress compared to the other treatments. Control group had the lowest mortality at 29 dph, but there were no significant differences in the mortality. At 34 dph the ControlP group showed significant higher mortality compared to the other treatments (Figure 10).

Figure 10. Mean mortality (% ± SE) registered 24 hours after stress test at 23, 29 and 34 dph.

SURVIVAL

The 90/10 group showed significantly lower survival at 24 dph compared to the other groups. The Control and 50/50 group showed a significantly higher survival at 40 dph compared to the ControlP and the 90/10 group (Table 3).

Table 3. Survival (%) at 24 and 40 dph from the four different treatments. Data are mean ± SE.

Dph	Control	ControlP	50/50	90/10
24	26	15	22	13
40	17.5	0.5	14	1

Experiment 4 :
Feeding preference of ballan wrasse larvae at first feeding

Stine Wiborg Dahle and Andreas Hagemann

Introduction

A feeding preference experiment for ballan wrasse larvae was conducted to study live prey preference of larvae offered rotifers (*Brachionus* sp.) and copepods (*Acartia tonsa*) as first live feed (Figure 11). If the larvae have a choice between rotifers and copepods, what will it choose?
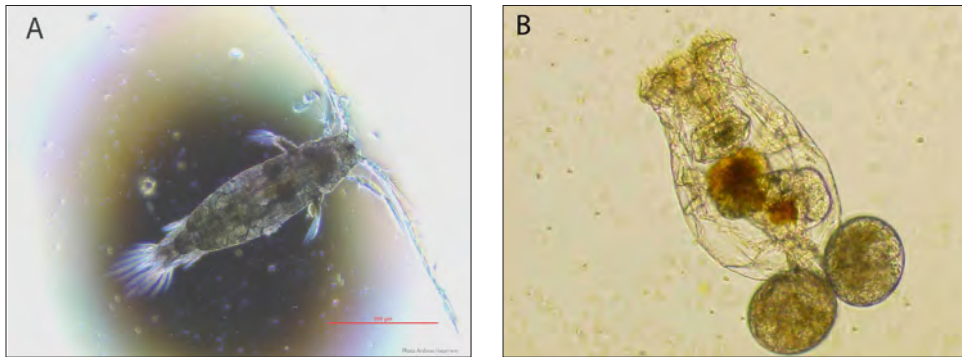
Figure 11. The copepod *Acartia tonsa* (A) and rotifer *Brachionus Nevada* (B). Photos: SINTEF.

Experimental setup

Ballan wrasse larvae (7 dph) were picked randomly from different start feeding tanks and transferred to transparent plastic beakers filled with seawater (1 l, 12°C, 34 ppt). The larvae had seemingly not begun exogenous feeding on rotifers when the trial was initiated. The experiment was conducted with 20 ballan wrasse larvae per beaker and three different treatments in triplicates:

- "Rot": Rotifers (12 000 ind^{-1} l^{-1}).
- "Rot/Cop": An equal mix of rotifers (6 000 ind-1 l^{-1}) and copepod nauplii (6 000 ind^{-1} l^{-1}, 20% 180-240 μm / 80 % 400 μm).
- "Cop": Copepod nauplii (12 000 ind^{-1} l^{-1}, 20% 180-240 μm / 80% 400 μm).

After 1.5 hours, the larvae were killed by an overdose of MS-222 (Tricaine methanesulfonate) before the gut content was evaluated.

Results and discussion

For the group offered just rotifers, 12% of the larvae had rotifers in the gut. For the groups offered copepods, or a mix of copepods and rotifers, 80 % had consumed live prey (Figure 12). Seemingly, only copepods were preyed upon in the group offered a mix of rotifers and copepods as rotifers were not identified in the gut upon dissection. The results thus indicate a pronounced preference for copepods over rotifers as a first live feed, although rotifers are slower swimmers than copepods, and thus should be easier to capture (Kjørsvik, E., pers. comm.). This may be caused by other attributes of the live feed, such as palatability and movement (Sørøy, 2012).
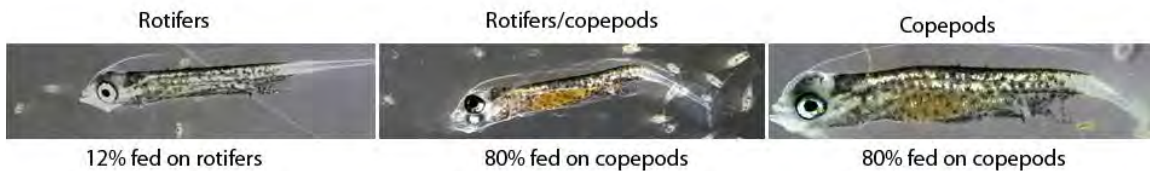
Figure 12. Ballan wrasse larvae from three different treatments of live feed: rotifers (left), a mix of copepods and rotifers (center) and copepods (right). The pictures show a higher gut filling for larvae fed a mix of copepods/rotifers and copepods. The percentage of larvae that had ingested prey for the three different treatments is shown. Photos: SINTEF.

Summarised discussion

The start feeding experiments shows that intensively reared copepods of the species *A. tonsa* Dana is a superior live feed organism for ballan wrasse larvae. Compared to enriched rotifers and *Artemia,* a copepod diet was shown to improve growth, survival and handling stress tolerance for the larvae. Further on, the feeding preference trial showed that ballan wrasse larvae prefer copepods over rotifers as a first live feed. The Planktonic diet was not compared to intensively produced copepods, but it was evident that the time window for the introduction of the Planktonic diet must be changed, and introduced at 10-15 dph for ballan wrasse. In addition, better methods for keeping the Planktonic particles in the upper water column need to be developed, for the diet to be more accessible for the larvae, and to ensure better water quality.

The improved growth, survival and stress tolerance when feeding with copepods could be related to the nutrient composition of copepods (see Table 1 in Appendix 1, page 130), where these components satisfy the marine fish larvae's requirements for optimal growth and development (Bell et al., 2003; van der Meeren et al., 2008). In addition the ballan wrasse larvae seems to have a pronounced preference for copepods over rotifers, that mat may be caused by differenced in the attributes of the live feed, such as palatability and movement.

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Recommendations

The studies highlight the importance of a high quality diet, especially during the earliest days of the ballan wrasse life. Intensively produced copepods should be used as live prey for ballan wrasse larvae until weaning onto dry feed, and should ideally replace both rotifers and *Artemia.* However, using copepods for only the first 7 or 27 days was sufficient to positively affect the growth and handling stress tolerance of the larvae.

Today, the volume of intensively produced copepods, or copepod eggs, is not large enough to supply the demand for live prey organisms in commercial production plants for ballan wrasse. Large scale production plants for copepod eggs or live copepods could solve this problem in the near future, however there is still some work left to do in order to make this production a cost-effective alternative to traditional live feed organisms.

A commercial production of superior live feed such as copepods would contribute to more reproducible production of high quality juveniles in marine aquaculture species such as ballan wrasse, cod, halibut, turbot, sea bass and sea bream.

Moreover, an improved first feed diet could contribute to a higher profitability in the juvenile production phase.

Handling stress tolerance test – sampling & analysis

Elin Kjørsvik, Gunvor Øie, Marit H. Hansen and Maria O. Sørøy

INTRODUCTION

Differences in live feed quality can affect stress tolerance in marine larvae. Dietary DHA and arachidonic acid (ARA) are beneficial for vitality and tolerance to stressors such as handling, decreased dissolved oxygen and increased water temperature in marine larvae (Watanabe and Kiron, 1994; Kanazawa, 1997; Tago et al., 1999; Koven et al., 2001).

Common ways to assess the stress tolerance in fish larvae includes altering the temperature or salinity, or exposing the larvae to air, and recording the effect of the stressor (e.g. mortality, cortisol levels). Our aim has been to introduce a test for larval tolerance to stress, that can be performed in a hatchery. Older larvae and juveniles will be subject to handling treatments, such as netting or exposure to air, and we therefore chose handling stress (air exposure) (Sørøy, 2012). This test has shown consistent results in several experiments with ballan wrasse larvae, and we consider the air exposure test to be a reliable tool for comparative handling stress resistance in larval experiments for ballan wrasse and in the hatcheries.

PROCEDURES FOR SAMPLING

At 29 dph, a total air exposure of 40 seconds was found to be a suitable exposure time for ballan wrasse larvae at this stage. The test should not be used for ballan wrasse larvae older than 30 dph.

The tolerance to handling stress is assessed by a standardised method for netting, followed by air-exposure, according to Øie et al. (submitted). Fifteen larvae from each replicate tank is a common sample size.

METHODOLOGY

1. Larvae (29 dph) are sampled in a flat bottomed sieve (a plastic cylinder with a mesh bottom) placed in sea water.

2. The sieve is placed on paper tissues for 15 seconds to get rid of excess water, before holding it in air for 25 seconds (to mimic standardised handling by netting).

3. Transfer the larvae carefully into a 3L aquarium, with the same sea water and temperature as in the tanks, without feeding.

4. Count dead larvae at 1 and 24 hours after air-exposure

5. Calculate percent mortality (For an example, see Figure 1).
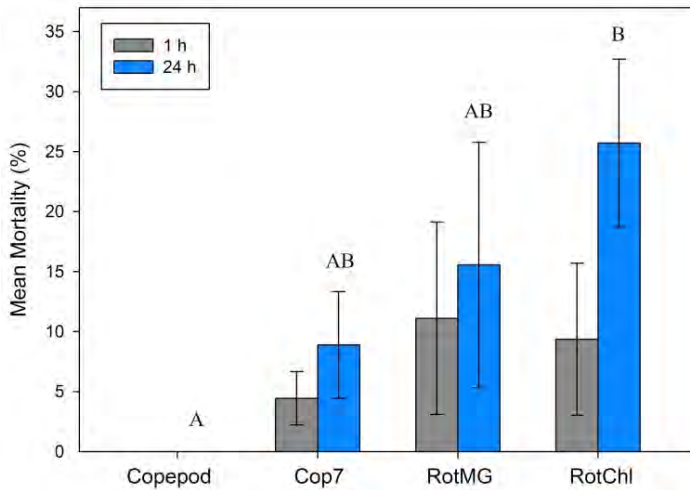
Figure 1. Mean mortality (percentage) of 29 dph ballan wrasse larvae, 1 and 24 hours after handling stress (air exposure for 40 seconds). Significant differences between treatments are indicated by different letters. Error bars are + SE, n=3.

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Functional development of ballan wrasse & effects of start feed quality

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Introduction

The intensive production of ballan wrasse *(Labrus bergylta)* juveniles is a response to the increasing demands for wrasse as cleaner fish in salmon and trout farming. The nutritional quality of the live feed commonly used in the farming of marine larvae today, rotifers and *Artemia* sp., may be suboptimal to the dietary need of developing ballan wrasse larvae. This may therefore contribute to the problems with growth, survival and skeletal anomalies currently observed in the rearing of this species.

Farming of ballan wrasse implies to produce a fish that should function optimally to capture sea lice in an unfamiliar territory. It is thus important to assess the effect of different live feed-types and -quality on the larval quality. Examples of common indicators of differences in larval quality include growth, survival, tolerance to stress, analysis of skeletal anomalies (deformities), ossification processes, shape variations and behaviour (e.g. activity, or ability to catch prey).

Copepods are the natural feed for pelagic marine larvae. In our studies of larval functional development, we used intensively cultivated *Acartia tonsa* copepod nauplii as short-term live feed in the start feeding of the ballan wrasse larvae, either as a supplement to, or instead of rotifers, and we found improved larval growth, as described for the start feeding experiment 1 in "Start feed quality, feeding regimes and growth of ballan wrasse larvae". For the functional development and effects of start feed quality in these ballan wrasse larvae, we studied their swimming activity and prey capture success (Sørøy, 2012), bone development and deformities (Sørøy, 2012), their organ tissue growth and development (Gagnat, 2012; Gagnat et al., submitted), as well as their digestive system development and metabolism (Almli, 2012). The histological studies of the larval muscle development (Berg, 2012), functional digestive system (Romundstad, in progress) and cellular metabolism (Stavrakaki, 2013) are still continuing, and will not be presented.

Swimming activity and prey capture

Fish larval activity and the ability to catch prey will affect growth and ultimately the ability to survive. Measurements of larval quality can hence be based on swimming activity and the larval success in prey capture during a limited time span (Puvanendran & Brown, 1999). Cod larvae fed intensively cultivated copepods or rotifers with a high lipid content had a higher activity level and were more effective predators than larvae fed rotifers of a lower quality in regard to lipid content (O'Brien-MacDonald et al., 2006; Kjørsvik et al., manuscript).

The aim of these studies was to evaluate possible effects of different live feed quality on the functional development of ballan wrasse larvae, by estimating their swimming activity and prey capture success at selected stages.

Experimental setup

The ballan wrasse larval swimming and feeding behaviour was assessed on 28 dph (end copepod/rotifer feeding period). A focal animal technique was used, where a randomly selected larva was observed for 2 minutes, and selected parameters of the swimming behaviour were recorded ("Swimming duration" and "Prey capture", after Puvanendran & Brown, 1999).
3 litre transparent plastic aquariums were used for the observations, and 10 random larvae from each replicate tank were observed individually (30 larvae treatment^{-1}). The larvae were unfed for 3 hours, after which *Artemia fransiscana* nauplii (3000 L^{-1}) was added. Observation of the larvae started 1 minute after prey addition, and lasted for 2 minutes per larva. The larvae were removed from the tank immediately after observation.

RESULTS AND DISCUSSION

The larvae that were fed copepods instead of rotifers were more effective predators when observed at 28 dph, and there were significant differences in the mean larval activity between the treatments (Figure 1). The larvae fed copepod nauplii swam significantly less per minute than the rotifer fed larvae. However, the larvae fed copepods (both Copepod and Cop7) caught significantly more prey than the larvae fed rotifers (RotMG and RotChl).

The larvae swam by beating the tail fin rapidly, and moving the pectoral fins at 28 dph. A distinct hunting behaviour was also detected. This behaviour was most pronounced in the copepod fed larvae, as they appeared to be more actively searching for prey.

The mean duration of swimming, and the number of prey caught were significantly correlated to both treatment and larval standard length (SL). There were significant differences in size (SL and MH) between the observed larvae from the different treatments at 28 dph.
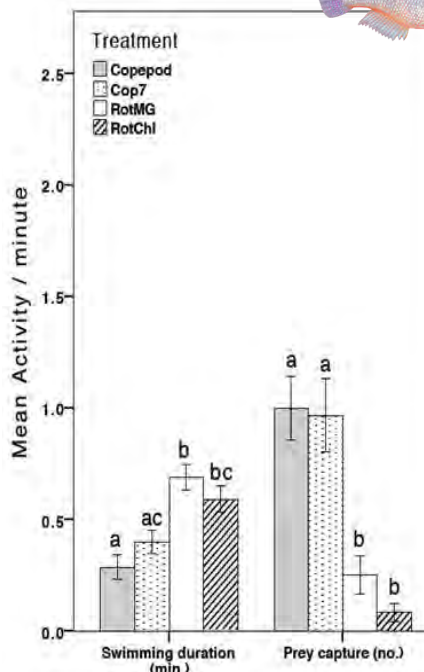
Figure 1. Observed mean ballan wrasse larval swimming activity and *Artemia* prey capture success per minute at 28 dph. The copepod fed larvae were less active than the rotifer fed ones, and they had a significantly higher prey capture success (no. of *Artemia* captured per minute). Significant differences between the treatments are given by different letters. Error bars are + 1 SE.

BONE DEVELOPMENT AND DEFORMITIES

Problems experienced in rearing of ballan wrasse, using rotifers as first-feed, are often low survival, poor growth, and various skeletal anomalies (Grøntvedt, 2010). The process of ossification is affected by nutritional factors (Cahu et al., 2003b; Kjørsvik et al., 2009; Power, 2009). The causes for skeletal anomalies in fish larvae are numerous, and may also be related to suboptimal physical or chemical rearing conditions such as too high water current, or suboptimal light, salinity or temperature conditions. A number of different skeletal anomalies have been described in hatchery reared fish such as cod, sea bass, sea bream and milkfish, the majority of which are related to spinal malformations (for review, see Boglione et al., 2013).

We investigated the development of skeletal ossification and the dietary effects of copepods versus enriched rotifers as start feed, by examining bone development and occurrence of skeletal anomalies in developing ballan wrasse larvae (Sørøy, 2012). These parameters should give a good indication of the suitability of the live feed presented to the ballan wrasse larvae.

EXPERIMENTAL SETUP

The larvae were fixed in 4 % formaldehyde in phosphate buffer, and standard length (SL) and myotome height (MH) was measured on fixed larvae. For analysis of skeletal ossification and skeletal anomalies, 10-15 larvae were sampled from each tank (30-45 per treatment). The larvae were stained with alizarin red (Kjørsvik et al., 2009), and were studied with emphasis on development of the vertebrae, fin rays and squamation, and skeletal anomalies.

RESULTS AND DISCUSSION

The larvae fed copepods instead of rotifers showed better growth and earlier onset of ossification of the axial and fin ray skeleton. The degree of ossification was consistently more related to larval size than to age, and there was no difference between the treatments in at which size the vertebral ossification occurred. An overview of the ossification process in the ballan wrasse larvae is shown in Figure 2. The first vertebral segments started ossification from around 6 mm SL, and the formation of the fully ossified vertebrae occurred around 9-10 mm SL. In ossification of the dorsal fin, on the other hand, the copepod fed larvae were significantly smaller than larvae from the other treatments at similar stages of fin development, i.e. copepod feeding induced a size related earlier ossification than rotifers. At 61 dph, most of the larvae were covered with ossified scales.

Twisted arches (Figure 3) were a common and minor anomaly in larvae from all treatments, and were only observed in larvae at 61 dph. When excluding twisted arches from the anomaly count (Figure 4, page 57), the copepod fed larvae had significantly less skeletal anomalies per larva compared to the larvae fed enriched rotifers (RotChl larvae were not analysed). The other observed anomalies observed at 61 dph were fused vertebrae/arches (in the tail region), and weak axis deviations (22 % of larvae fed enriched rotifers compared to 2% of the copepod fed larvae).
In a similar experiment with cod larvae, we found twisted arches as an effect of the rotifer feeding, whereas it was not observed in copepod fed larvae (Hansen, 2011).

This indicates a nutritional effect, and has apparently not been described before. We regard this as a rather minor anomaly, and when it was excluded in the evaluation (thus comparable to other studies), we found that the incident of anomalies in our larvae was very low compared to what has been observed elsewhere in farmed ballan wrasse.

The evaluation of possible skeletal anomalies was also performed on one year old juveniles from this experiment. Preliminary results indicates that the quality of the fish was generally very good, and that the juveniles fed rotifers as live feed had a somewhat higher incident of anomalies in the ossified structures (Grete Bæverfjord, pers. comm).
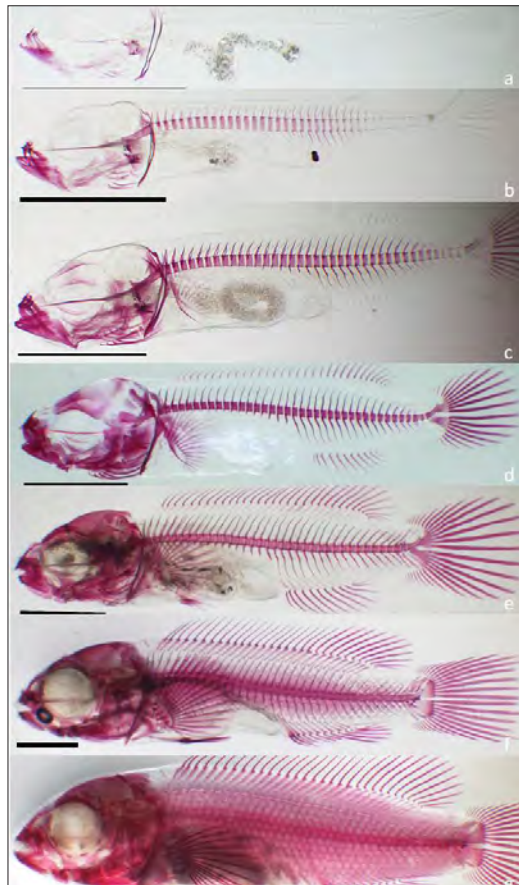

Figure 2. Summary of skeletal development in ballan wrasse larvae, from 15 – 61 dph. A: initial ossification of the head (jaw) structures (5-6 mm SL), B: ossification of the chorda centra of the vertebral column, neural and heamal spines and the tail fin (6-7 mm SL), C: the cranium and fins are ossified (7-8 mm SL), D: continuing ossification of cranium and fins (8-9 mm SL), E: full ossification of dorsal and anal fin (10-11 mm SL), F: formation of scales along the midline (12-14 mm SL), G: scales are fully formed and covers the body (>15 mm SL). Photo: NTNU
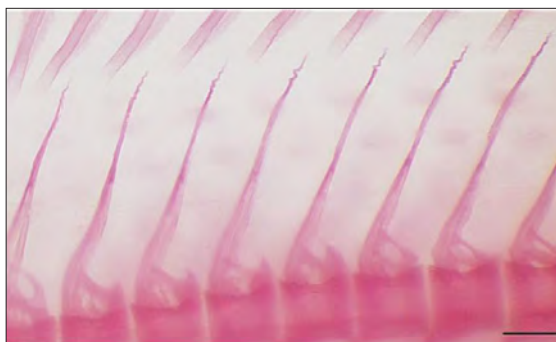
Figure 3. Twisted neural arches in a 61 day old ballan wrasse larva. The bones visible above the neural arches are the pterygiophores. The black bar equals 200 μm. Photo: NTNU
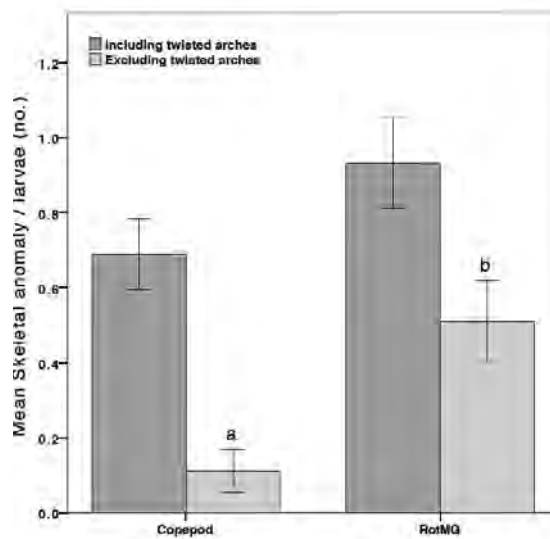
Figure 4. Mean skeletal anomalies per larva in 61 day old ballan wrasse larvae. The graph shows the mean number of skeletal anomalies per larva, when twisted arches (the most common and minor anomaly) were included or excluded. Significant differences between treatments are given by letters. Error bars equal + 1 SE.

ORGAN GROWTH AND DEVELOPMENT

At the time of hatching, marine pelagic fish larvae are supplied with endogenous nutrition through the yolk sac. The main organs and organ systems are immature but become functional by the time of first feeding. As they grow and differentiate during the larval stage, those organs most needed to enhance further growth and survival is prioritised with fastest growth rates. This results in a growth pattern called allometric growth, where some body parts or organs will grow faster than the rest of the body (Alami-Durante, 1990; Osse & van den Boogaart, 2004). Allometric growth occurs especially during the fish larval stages, and typical for the larval period can be a number of sharp morphological changes (Fuiman & Higgs, 1997).

Like many marine fish larvae, the ballan wrasse has an altrical development of the digestive system and immature digestive organs at the onset of exogenous feeding (Osse et al., 1997; Dunaevskaya et al., 2012), and it is generally necessary to use live feed when cultivating marine larvae.

The aim of this investigation was to study the allometric growth pattern of ballan wrasse larvae, and to evaluate possible effects that qualitative differences in nutrition may have on organ tissue growth and development (Gagnat, 2012; Gagnat, submitted). Understanding these processes may help improve the growth, survival and general quality of the fish during commercial production. Also, information on the allometric growth of different organ groups may contribute to an increased understanding of critical points during the larval development.

Experimental setup

Fish larvae were sampled at intervals between 4 – 55 dph, and they were fixed in paraformaldehyde (PFA) in phosphate buffer, embedded in paraffin, cut in 4 μm thick longitudinal sections, and stained with Mayer's hemalun solution and Eosin. For volumetric estimations of tissue volume, serial sections were made through the larvae at fixed intervals.

Tissue volume was estimated by the Cavalieri method (Howard & Reed, 1998) using CAST 2 (Olympus Inc., Denmark), applying a point grid (Michel & Cruzorive, 1988; Mayhew, 1991; Howard & Reed, 1998). Points touching any tissue were registered as hits in its respective category. The volume of the tissue (VT) of ten different organ tissues was determined: intestine, liver, pancreas, heart, gills, muscle, nerve tissue (brain + spinal cord), eye, notochord and "other" tissues, consisting of all tissues not covered by the previous categories (e.g. cartilage, kidney, oesophagus, buccal cavity, and swim bladder).

Together these 10 categories covered all different tissue in the fish larvae, which together made up the total larval reference volume (Howard & Reed, 1998). The number of sections used for tissue estimation for each larva varied between 26 and 34. VT was calculated from the equation $VT = \sum A*(E+C)$, where A represents the summation of the measured area section, E the thickness of the section and C the distance between the measured sections.

Results and discussion

Feeding ballan wrasse larvae with *A. tonsa* nauplii resulted in increased larval somatic growth and faster growth and development of organ systems. The prevalvular intestinal wall of larvae receiving *A. tonsa* appeared thicker and more developed already at 8 dph compared to larvae receiving rotifers, but no other histological differences was observed between the treatments. The copepod fed larvae reached flexion stage at 13 dph (160 day degrees), this was five days earlier than for larvae fed rotifers, where this was observed at 18 dph (225-240 day degrees post hatch). Figure 5 (see p.59) shows the functional histological development from 4 to 55 days, where all major organs have grown in size and complexity.

The increase in total tissue volume correlated exponentially to the larval standard length (SL), and a change in the overall growth pattern was observed around the length of 6.1 mm (Figure 6). While the volume increase previously had been positive allometric, it now became isometric. This could imply the start of metamorphosis around this length.

An especially rapid growth of digestive and respiratory organs occurred from 4 to 8 dph in all groups (Figure 7 for intestine and liver tissue (see p.59)), where a SGR between 30 and 40 % daily was observed for larvae receiving copepods, compared to close to 20 % for the RotChl larvae and even less for the RotMG larvae. Muscle growth was more prioritised later (21 to 33 dph), and had a daily SGR close to 30 % regardless of treatment.

To our knowledge, this is the first study to compare the response of diet on the allometric growth and volume increase of organ tissues. Even though no difference in organ volume and growth was discovered between treatments when comparing at a specific larval size/length, feeding the larvae with copepods resulted in a much faster growth, both in total tissue growth and for the different organs. A. tonsa therefore seems to fit the ballan wrasse nutritional requirements better than rotifers during early larval development.
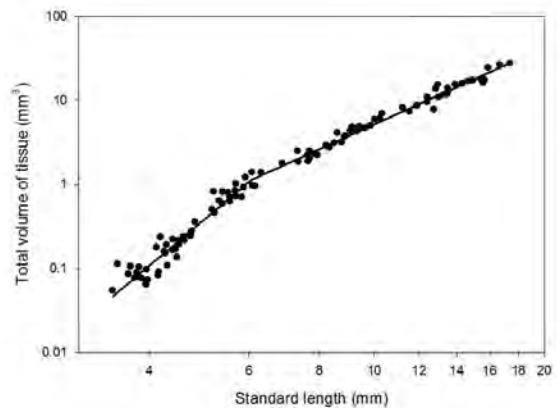
Figure 6. The relationship between standard length and total volume of all tissue (mm3) for ballan wrasse larvae, each point represent values from individual larvae. Data were collected from 4, 8 21, 33 and 55 days old fish larvae. The growth is initially positive allometric (g = 5.66), followed by near isometric growth (g = 3.04) after an inflexion point at 6.1 mm.

Figure 5. Longitudinal sections of ballan wrasse larvae at 4 (A) and 55 (B) dph stained with HE. Letters mean: Intestine = I; liver = L; muscle = M; pancreatic tissue = P. Scale bar = 1 mm. Photo: NTNU
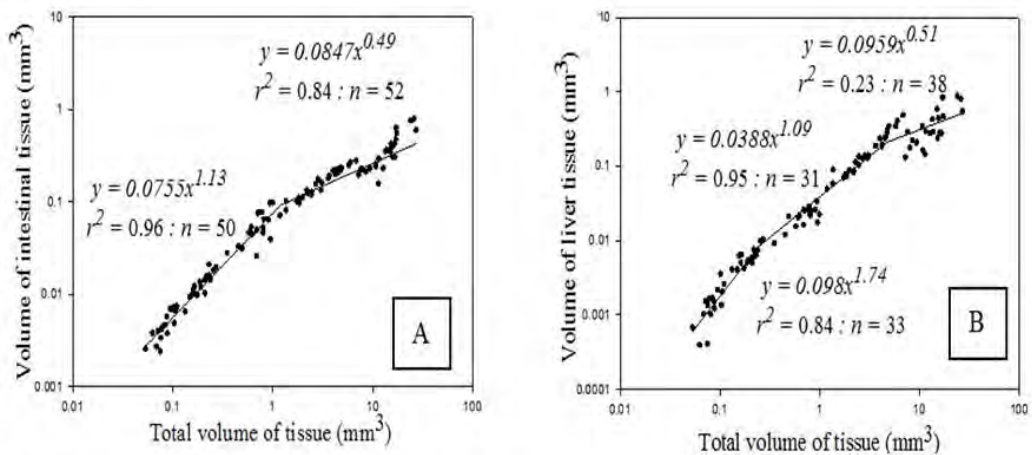
Figure 7. Allometric growth equations and relationship between the total volume of ballan wrasse larvae and the volume of organs associated with digestion (intestine; A, and liver; B) during early stages of development. Each point represents measurements from a single larva.

Larval metabolic composition as a function of different live feed

The metabolome represents the collection of all metabolites in a biological cell, tissue, organ or organism, which are the end product of cellular processes (Bundy et al., 2009). There are different platforms used for the detection of metabolites and one of these is Nuclear Magnetic Resonance spectroscopy (NMR). NMR is a non-destructive, non-targeted fingerprinting technique which can be used to detect a wide range of different classes of low molecular weight metabolites with differing charges, stability or volatility. The use of metabolomics in aquaculture could help develop methods for both better farming of the species and also better processing techniques with quality of the product in mind. As shown by Schock et al. (2012) in a study on cobia (*Rachycentron canadum*), the use of metabolomics can provide valuable insight into the effects of feed manipulation on aquaculture species. In this study 1H-NMR was used to examine the differences in ballan wrasse larval metabolic composition as a function of the different types of live feed (*Acartia tonsa* or rotifers, Almli, 2012).

Experimental setup

Fish larvae (sampled at 8, 12, 15, 21, 27, 33, 40, 47 and 61 dph) were flash frozen in liquid nitrogen and stored at -80°C until they were homogenized in methanol (MeOH, 67%). The supernatant was added chloroform ($CHCl_3$) and distilled water, and the polar phase was vacuum centrifuged at 30°C for 30 minutes and frozen at -80°C before freeze-drying. Twenty-four hours before the NMR analysis, the samples were dissolved in 200 µl solution of D2O in PBS (pH 7.4, 1mM TSP). NMR analysis was performed on a Bruker DRU 600 spectrometer (Bruker BioSpin GmbH, Rheinstetten). For principal component analysis (PCA) multivariate analysis, the NMR data were imported into MATLAB using ProMetab software. A PLS-toolbox (Eigenvector research ltd.) was used for the analyses (for further details, see Almli, 2012).

Results and discussion

Of the 23 different metabolites identified in the ballan wrasse spectrum, the six metabolites with the highest variation in the spectra were selected and investigated. These metabolites were trimethylamine-N-oxide (TMAO), betaine, taurine, lactate, creatine and alanine. The PCA analysis for all groups at all sampling days showed that TMAO was the major metabolite separating the larval groups that were fed the different types of live feed (Figure 8, page 61). The Copepod and the Cop7 group were found to have high values of TMAO when the larvae were fed exclusively with copepods, while in the rotifer groups, betaine was the most abundant metabolite. At 12 dph the Copepod and Cop7 groups showed a higher value of taurine. Later, at sampling days 40 and 47 dph, when groups were fed the same diets, the differences between the groups had faded.

Analysis of live feed

The four different live feed were analyzed to identify the possible content and concentration of the metabolites taurine, betaine and TMAO. Betaine was found in all live feed species, while taurine was found in only copepods and *Artemia*, where copepods had the double amount. TMAO was only identified in the copepods (Tab. 1) and is probably the reason for the high levels of TMAO found in the larvae fed the copepod diet.

Table 1. Concentrations of betaine, taurine and trimethylamine-N-oxide (TMAO) found in the four different live feed organisms. Concentration in nmol mg/dry weight. ND= Not detected.

Treatment	Betaine	Taurine	TMAO
Artemia sp. nauplii	49.97	30.31	ND
Unenriched rotifers	614.10	ND	ND
Enriched rotifers	489.65	ND	ND
Copepod nauplii (*A. tonsa*)	174.20	61.63	166.50
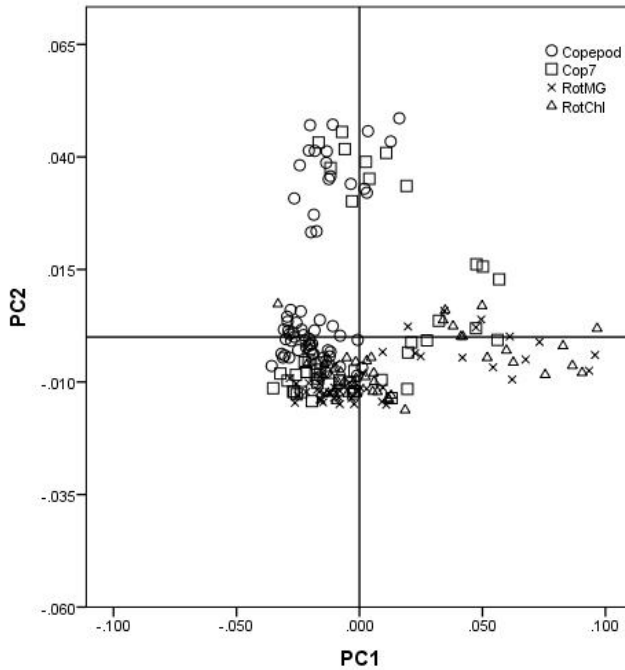
Figure 8. Principal Component Analysis (PCA) score plot of ballan wrasse for all days and all groups (n=203).

Our metabolic analyses of the larvae showed that changes in the levels of certain metabolites can be used as biomarkers for growth and development. The differences seemed to be correlated to feed composition, with TMAO and taurine standing out as reliable biomarkers for growth differences (Almli, 2012).

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RECOMMENDATIONS

Feeding of the ballan wrasse larvae with cultivated copepods *Acartia tonsa* resulted in a more rapid growth and functional development, when compared to feeding with rotifers.

The larvae fed copepods had a faster growth and development of tissue and organ systems, they were also more effective predators, more tolerant to handling stress, and they had less skeletal anomalies than those fed rotifers. Even larvae fed copepod only for the seven first days had an improved growth after 60 days, compared to the rotifer fed larvae.

Also worth noticing is the long-term poor performance of larvae fed low quality rotifers, even though their growth was similar to those fed well enriched rotifers. Ensuring that rotifers have the best possible nutritional enrichment and quality when eaten by the larvae, is thus of major importance. However, using the cultivated copepods *A. tonsa* were highly superior to enriched rotifers, copepods seemed to fit the ballan wrasse nutritional requirements very well during early larval development, and even short early feeding periods with copepods gave a long lasting effect on the ballan wrasse larvae.

Microbial control of the rearing water of larvae

Kari J.K. Attramadal, Giusi Minniti, Gunvor Øie, Elin Kjørsvik, Mari-Ann Østensen, Ingrid Bakke and Olav Vadstein

INTRODUCTION

Most often, marine fish larvae do not develop full immune competence until several weeks after hatching (Magnad-ottir, 2006). Massive mortality of larvae in the first weeks is common in hatcheries, and the reproducibility between replicate fish tanks is typically low. These losses and lack of reproducibility are often attributed to infections from opportunistic bacteria (Vadstein et al., 2004). Microbial control is therefore particularly important during the mouth opening and live feed period. Sources of bacteria in marine hatcheries are the water, live feed and air/aeration (and microalgae if used instead of clay for creating turbidity). Most bacteria in the water are harmless for the fish, and may even be beneficial by competing with the opportunistic bacteria for limiting resources. The species composition of bacteria of the water experienced by the fish is determined by the composition of the intake water and the selection occurring after the water has been pumped in to the system.

There are particularly two processes in intensive hatcheries that may destabilise the microbial environment and select for the dominance of opportunistic bacteria (Figure 1); disinfection, and the sudden increase in the supply of organic matter. Disinfection of intake water is an important barrier against introduction of specific pathogens to the system, but results in a water with a low content of bacteria (few competitors) which favours the subsequent proliferation of the fastest growing (opportunistic) bacteria (Hess-Erga et al., 2010). A sudden increase in the supply of organic matter represents available free niches for the bacteria and a higher potential for bacterial growth, which favours the faster-growing opportunistic types.
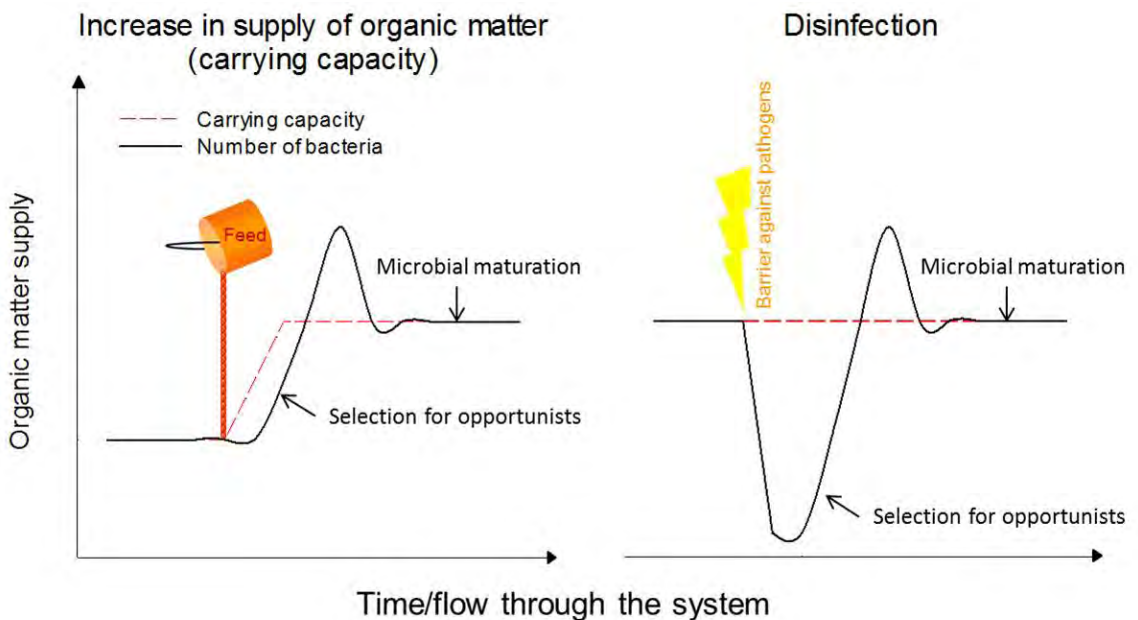
Figure 1. Processes in intensive aquaculture promoting selection for opportunistic bacteria in the rearing water.
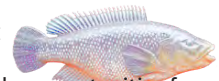
Control of the selection pressure is a key to steer the microbial flora in the system. To reduce the opportunities for proliferation of potentially harmful bacteria the water can be stocked with harmless bacteria outcompeting the opportunists. This can be achieved by letting a high number of bacteria compete for nutrients in a biofilter with a biofilm consisting of slow growing species, so called microbial maturation (Skjermo et al., 1997). Several studies have documented that microbially matured water increase growth, survival and the between-tank reproducibility of growth and survival of marine fish larvae (Vadstein et al., 1993, Skjermo et al., 1997, Salvesen et al., 1999, Attramadal et al., 2012a, b).

Due to processes like hatching and feeding, the water in the fish tanks has a higher supply of organic matter than the intake water, and consequently a higher microbial carrying capacity. Theoretically, the chance of opportunistic proliferation in the fish tank can be minimized by microbial maturation of the incoming water to tanks at a carrying capacity similar to that in the rearing tanks. This can be done by feeding the maturation filter directly with fish feed in a flow through system or through reuse of the rearing water in a recirculation system (Attramadal et al., 2012a).

EXPERIMENTAL SETUP

In a start feeding experiment with Ballan wrasse (*Labrus bergylta*) we compared the bacterial environment in a flow-through system where the water presented to the fish was microbially matured in a biofilter fed with fish feed (FED) and in a similar system where the water was matured without addition of extra organic matter (MMS).

RESULTS AND DISCUSSION

The net growth potential of bacteria in the water of the fish tanks (measured as number of bacteria in sample after 3 days of incubation compared with initial number of bacteria in sample) was significantly higher and more variable in the MMS (Figure 2). This supports the hypothesis that the FED system had strong competition and few free niches available for opportunists compared to the MMS. In addition, the species composition (measured by PCR-DGGE) of the water going in to the fish tanks was more similar to that in the fish tanks in the FED system than in the MMS, supporting the hypothesis that feeding the maturation unit increases the microbial control and stability of the rearing water.
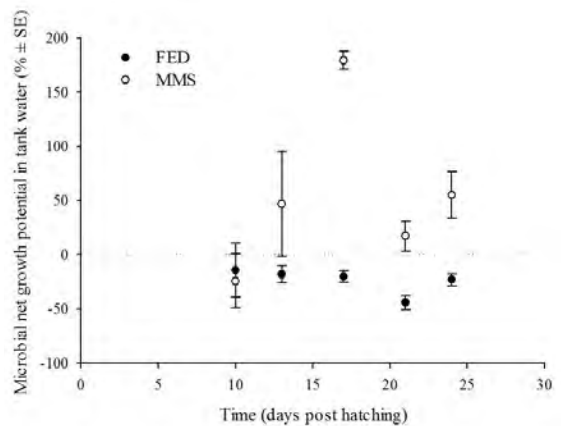
Figure 2. The microbial growth potential in the water of the rearing tanks (number of bacteria in sample after 3 days of incubation as percent of initial number of bacteria in sample).
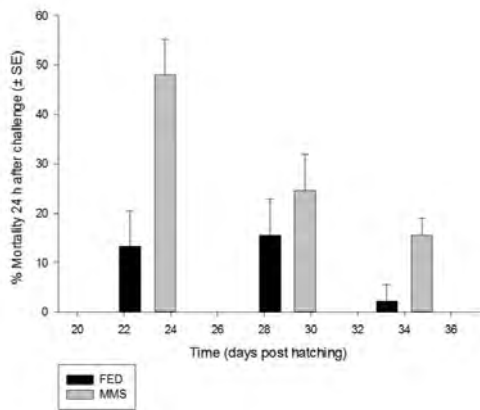
Figure 3. Mortality of larvae following challenge test (catching on sieve and exposure to air).

The microflora of the larvae was significantly different between the larvae reared in the FED and the MMS. Compared to the larvae in the MMS, the larvae in the FED showed higher growth (650 ± 272 and 498 ± 209 mg DW larva-1 (AV ± SD) in the FED and MMS, respectively, 27 dph) and stress tolerance (Figure 3), but lower survival (7 ± 1 and 22 ± 2% (AV ± SE) in the FED and MMS, respectively, 27 dph). It is likely that the feeding of the maturing filter of the FED was exaggerated during this experiment, which may have influenced the larvae negatively, i.e. odour of H2S was observed from the fish tanks during the first days. Optimisation of the dosing of feed to maturation systems remains, but the results from the experiment confirms the possibility of solving parts of the problems related to general infections in commercial culture of marine larvae through competent use of water treatment and microbial selection pressure.

Recommendations

To control the development of the microbial environment in the fish tanks one has to control the microbial composition and substrate supply coming from sources outside the tank. Microbial maturation stabilises the microbial community and selects for a more beneficial microbial environment for the fish. However, increased supply of organic matter (feeding) in the rearing tanks compared to the matured inflowing water opens for blooming of opportunistic bacteria. In flow-through systems this opening for opportunistic proliferation in the fish tanks can be minimised by feeding the maturation filter. The correct dosing of feed to maturation systems remains to be optimised. There is a potential for optimising production by choosing the best water treatment for improving not only physiochemical conditions but also the larvae/microbe interactions (Figure 4).
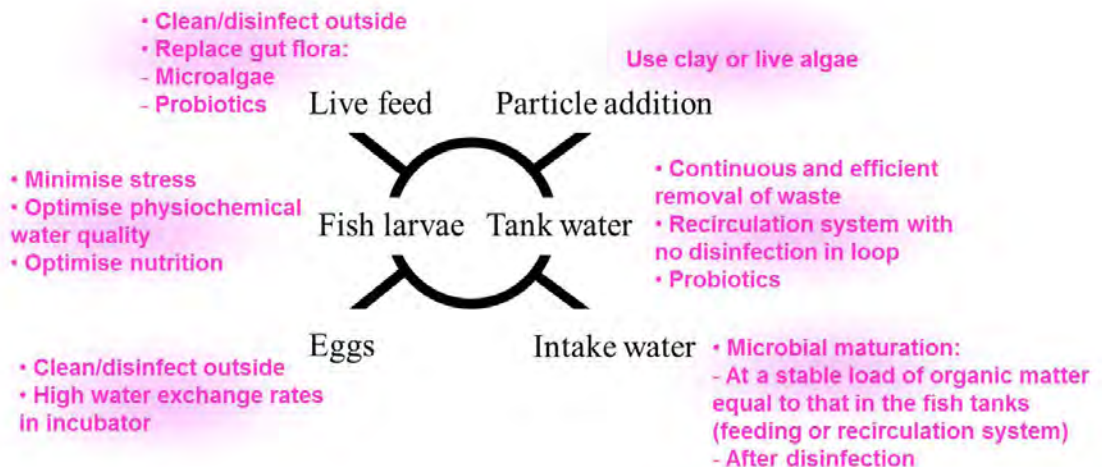
Figure 4. Recommendations to increase microbial control in the culture of cold water marine larvae.

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Light perception of ballan wrasse

Anne Berit Skiftesvik, Ellis Loew (Cornell University, USA), Howard Browman and Reidun Bjelland

INTRODUCTION

CHARACTERISING THE SPECTRAL SENSITIVITY OF BALLAN WRASSE

Microspectrophotometry (MSP) was performed on cultured larval-juvenile and adult ballan wrasse, *Labrus bergylta*. This technique measures the spectral absorbance of the visual pigments present in individual retinal photoreceptor cells, the rods and cones, isolated from the eyes of fish under study. A survey of the photoreceptor cell population in the retina defines the fish's spectral sensitivity.

The retina of the ballan wrasse contains both rods and cones. Rods underlie dim-light, nocturnal (scotopic) vision while the cones are responsible for bright-light (photopic) and color vision. Figures 1 and 2 show the best-fit template curves for the suite of cone visual pigments present in the eyes of larval/juvenile (Figure 1) and adult (Figure 2) ballan wrasse. The visual pigments present in the cells are classified according to the spectral region of the λ_{max}. For example, a λ_{max} at 535nm would make that cell a 'green' cell, etc. The majority of cones fall into three λ_{max} ranges – violet, blue and green. The violet cone is only found in larval/juvenile wrasse, and not in the adults. The loss of short-wavelength-sensitive cones with aging is quite common in fish.
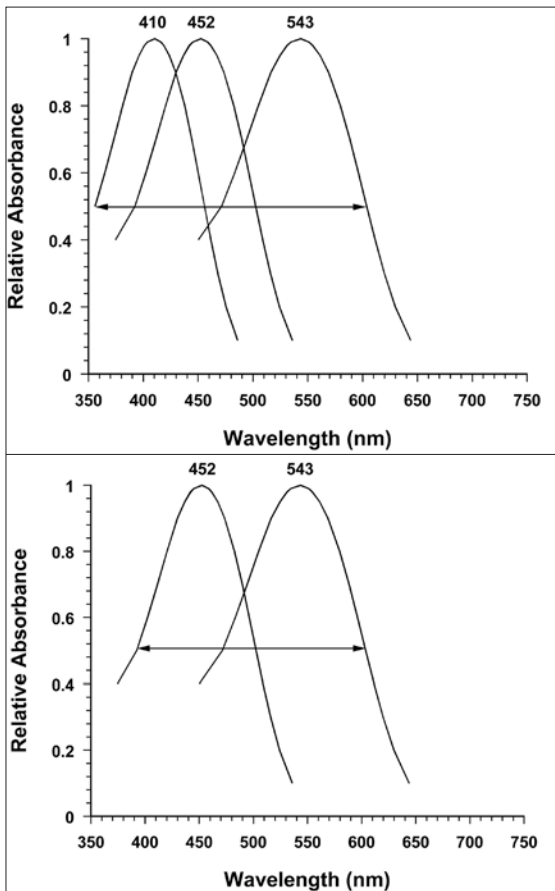
Figure 1. Visual pigments present in cones of larval/juvenile ballan wrasse. The number above each curve is the λ_{max} of the pigment. The horizontal line at the 0.5 relative absorbance level is an indicator of the potential spectral sensitivity range of the eye – that is, the eye of larval-juvenile wrasse will not be sensitive to light at wavelengths that fall below this line. Importantly, these fish have very little sensitivity to red light.

Figure 2. Visual pigments present in cones of adult ballan wrasse. The number above each curve is the λ_{max} of the pigment. The horizontal line at the 0.5 relative absorbance level is an indicator of the potential spectral sensitivity range of the eye – that is, the eye of adult wrasse will not be sensitive to light at wavelengths that fall below this line. Importantly, these fish have very little sensitivity to red light.

PRACTICAL RECOMMENDATIONS FOR LIGHTING AND TANK COLOUR

LED arrays are available consisting of numerous individual LEDs having different spectral outputs. For example, arrays to be used as grow-lights for plants contain UV, blue and red LEDs in a matrix and provide the right spectral output for supporting plant growth. Large white arrays are also available for uniform area illumination. It is certainly feasible to place a number of such arrays containing LEDs of specified spectral output over tanks to replace current sources.

However, it would be more convenient to simply use regular household bulb replacements if they are suitable since these are where all the R&D is being directed.

Comparing the spectral sensitivity of adult ballan wrasse (Figure 2) with the spectral emissions shown in Figure 3, it is clear that the warm white LED would provide suitable stimulation of both cone classes and would be an excellent replacement for current lamps. For larvae and juveniles the 'fit' is not as good given the output of the LEDs in the violet region where the third visual pigment is located. However, there is enough overlap between the LED emission spectrum and the violet pigment absorbance to allow for adequate stimulation of this cone.

As for tank color, black would seem to be the best choice since it would provide maximum possible contrast between 'target' and background when the light source is above the tank (the 'darkfield' situation).
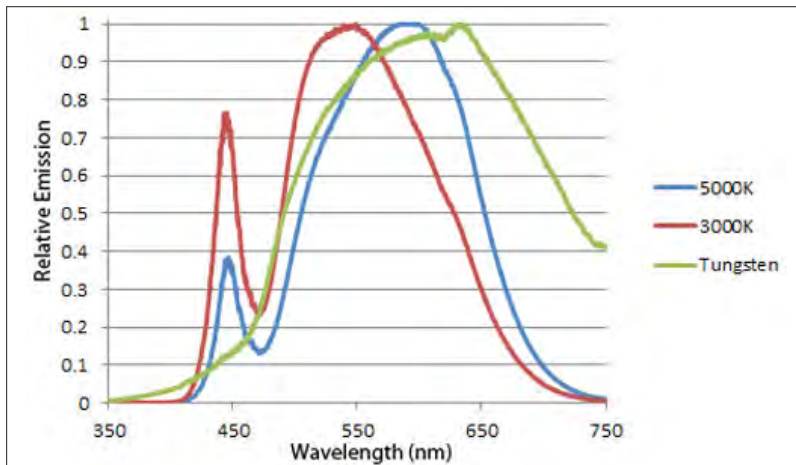
Figure 3. Spectral output of two commercial LED bulbs, warm white (3000° K) and white (5000° K) and a standard 60W incandescent bulb (2800° K).

Understanding and remediating clumping behaviour

Anne Berit Skiftesvik, Caroline Durif and Reidun Bjelland

INTRODUCTION

Clumping behaviour is a major problem in the intensive culture of ballan wrasse. In commercial farming, both larvae and juvenile ballan wrasse often form large dense balls. Approaches to disperse these aggregations have included increased water flow, altered light environment, among others. The goal of this experiment was to improve our understanding of what triggers this behaviour, and to investigate possible approaches to reduce this problem.

EXPERIMENTAL SET-UP

Newly hatched ballan wrasse larvae were distributed evenly into four 1500 L tanks (B1, B2, B3, B4). The light conditions were natural sunlight through skylight windows in the roof of the building. Larvae were start-fed with rotifers from day 4 post-hatch, and offered *Artemia* from day 32 post-hatch. Weaning started at day 76 post-hatch. Clay was added to the water as long as the larvae were fed rotifers to keep larvae away from the tank walls. Water temperature was 12-14°C during the first 3 weeks and between 14-16°C for the rest of the experimental period.

Behaviour of larvae/juveniles was observed directly by filming. Several adjustments to the culture conditions (e.g. light, water movement) were made to test if they affected the frequency of clumping. Differential larval mortality in the culture tanks produced large difference in the number of larvae present in each of the tanks – therefore, the effect of density was also evaluated. At the end of the experiment, length, weight and fin status were recorded for each tank.

RESULTS AND DISCUSSION

Most of the time fish were evenly distributed in the tanks. Clumping behaviour was first observed at day 46 post-hatch. In the early stages of clumping behaviour, light changes or uneven light in the tank triggered clumping, but had less effect later on. Feeding always triggered clumping, and in the tank with highest fish density (B2), the clumping was more intense and the "clump" lasted longer than in tanks with fewer fish. Increased/high water movement always triggered clumping. Disturbances such as unexpected noise, sudden shadows, etc. also triggered clumping behaviour.

Clumping is most likely a natural behaviour that occurs when the fish feel threatened, and is a common reaction in many fish species. In natural environments, they will also be near the bottom where there are seaweed and stones, and under those conditions hiding may be more common than clumping.

Uneven numbers of fish in the experimental tanks provided additional information about possible density effects. Tank B2 had more than 3 times the number fish than the other 3 tanks. There were no significant differences in tail damage between the tanks. The proportions of fin damage differed slightly between tanks as there were no undamaged fish in B2 (high density tank) (Figure 1). However, there were very few seriously damaged fish in any of the tanks. Tank B2 had the highest density and the mean length of those fish was significantly shorter than those in the other tanks (Figure 2). The mean weights of fish were significantly lower in tanks B2 and B3 compared to B1 and B4 (Figure 3), and there was no obvious relationship with density. The condition factor was calculated for fish in each of the four tanks - the fish in B4 had a significantly higher condition factor (Figure 4).

Tank B2 had the highest number of fish - the density in this tank was close to that in commercial rearing of the species. Generally, clumping behavior was stronger here, and lasted longer when it occurred. Although the growth generally was very high, the growth in tank B2 was lower than for the other tanks, perhaps due to less available food for each of the fish or because too little food was provided.
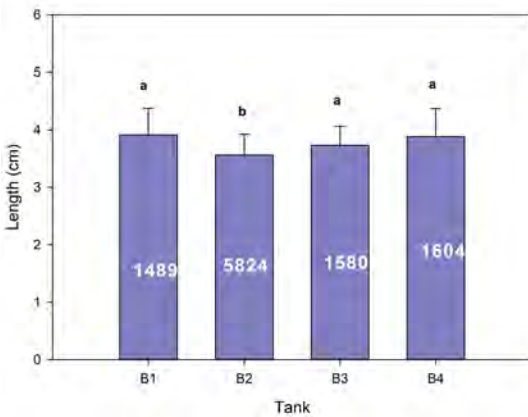
RECOMMENDATIONS

Avoid disturbing the larvae/juveniles in any way.

Keep the water movment and noise levels low, the lighting diffuse and even, since any disturbance will cause the larvae/juveniles to clump.
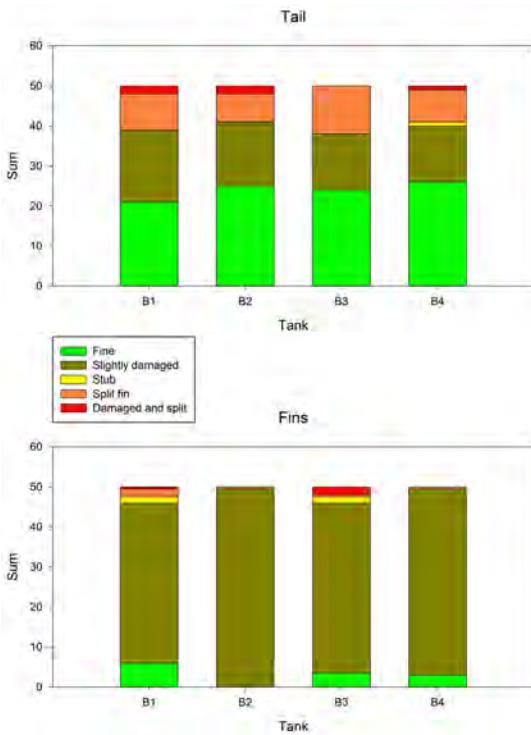
Figure 1. Occurrences of tail and fin damage on the fish in each tank (n=50 per tank).

Figure 2. Average length + sd (n=50 fish per tank) of wrasse in tanks with different densities of fish. The numbers in white are the numbers of fish remaining in each tank at the end of the experiment.
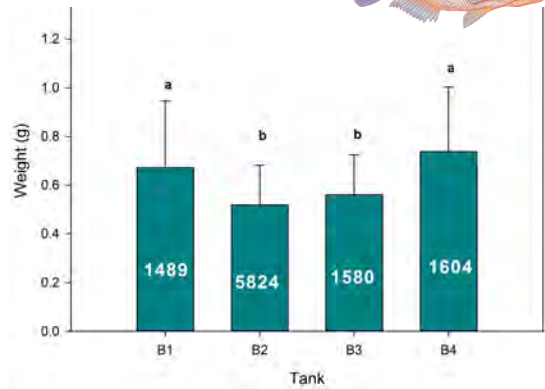
Figure 3. Average weight + sd (n=50 fish per tank) of wrasse in tanks with different densities of fish. The numbers in white are the numbers of fish remaining in each tank at the end of the experiment.
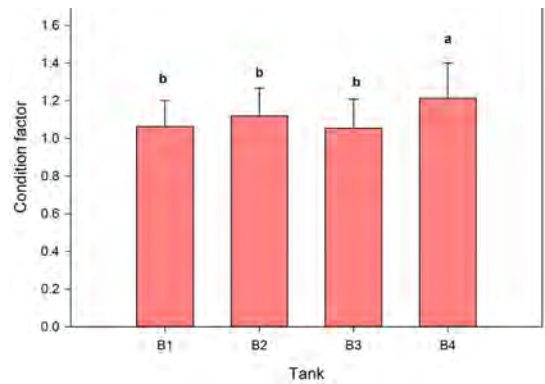
Figure 4. Average condition factor + sd (n=50 fish per tank) of wrasse in tanks with different densities of fish.

Photographic illustration of clumping.
Photo: IMR

The effect of live prey versus a formulated diet on dietary enzymes

Tom W. Hansen, Arild Folkvord, Espen Grøtan and Øystein Sæle

Introduction

Numerous investigations have been made on the activity of digestive enzymes on homogenates of whole fish larvae. There has been some controversy of how much enzymes from live prey in the intestine of the fish larvae have contributed to these measurements. This investigation set out to analyse the effects on the same-size larvae fed either rotifers or pellets.

Experimental set-up

Hatchery-reared *L. bergylta* ballan wrasse were studied from 2-55 dph to examine the molecular basis of digestive ontogeny related to the pancreas. An isolated feeding trial was performed on 27-34 dph larvae to compare the effect of diet on enzyme activity and the possible exogenous contribution by live feed.

The genes coding for key pancreatic enzymes were analyzed by qPCR: trypsin, major enzyme for protein digestion, Cyp7A1, vital for synthesis of bile acids and bile salt, BAL probably the only enzyme for digestion of neutral lipids, sPLA2 1B which digests phospholipases, amylase, digesting carbohydrates and pancreatic chitinase that digest chitin. Enzyme activity was measured on trypsin, neutral lipase, sPLA2, amylase and chitinase in fed and unfed larvae.

Restults & Discussion

We did not observe any effects of diet on enzyme activities of chitinase or sPLA2. However, a probable feed-dependency was observed at a transcriptional level, where rotifers seem to stimulate up-regulation. The regulation of BAL was the only exception, where an up-regulation was observed after weaning both in the ontogeny series and the experimental part. However, this was not reflected in the enzyme activity measurements.

Our data on pancreatic chitinase and amylase mRNA levels (Figure 1) suggest the importance, or at least tolerance of carbohydrates in the diet of early larval and juvenile ballan wrasse. Dietary lipids seem to be important to the late larval stages, as BAL mRNA levels increased significantly after 30 dph.

Consistent indications of a molecular response to the feed given suggest that feed type and/or quality could affect the regulation of several important genes in marine larvae.

Based on measured enzyme activities in rotifer fed and weaned larvae, we suggest that the supply of exogenous digestive enzymes from rotifers to larval ballan wrasse is negligible.

Acknowledgements

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see http://www.sciencedirect.com/science/article/pii/S109649591200190X

Recommendation

We conclude that enzymes in the diet are negligible for digestion in larvae, this aspect should therefore be ignored when assessing the value of live prey versus formulated diets.
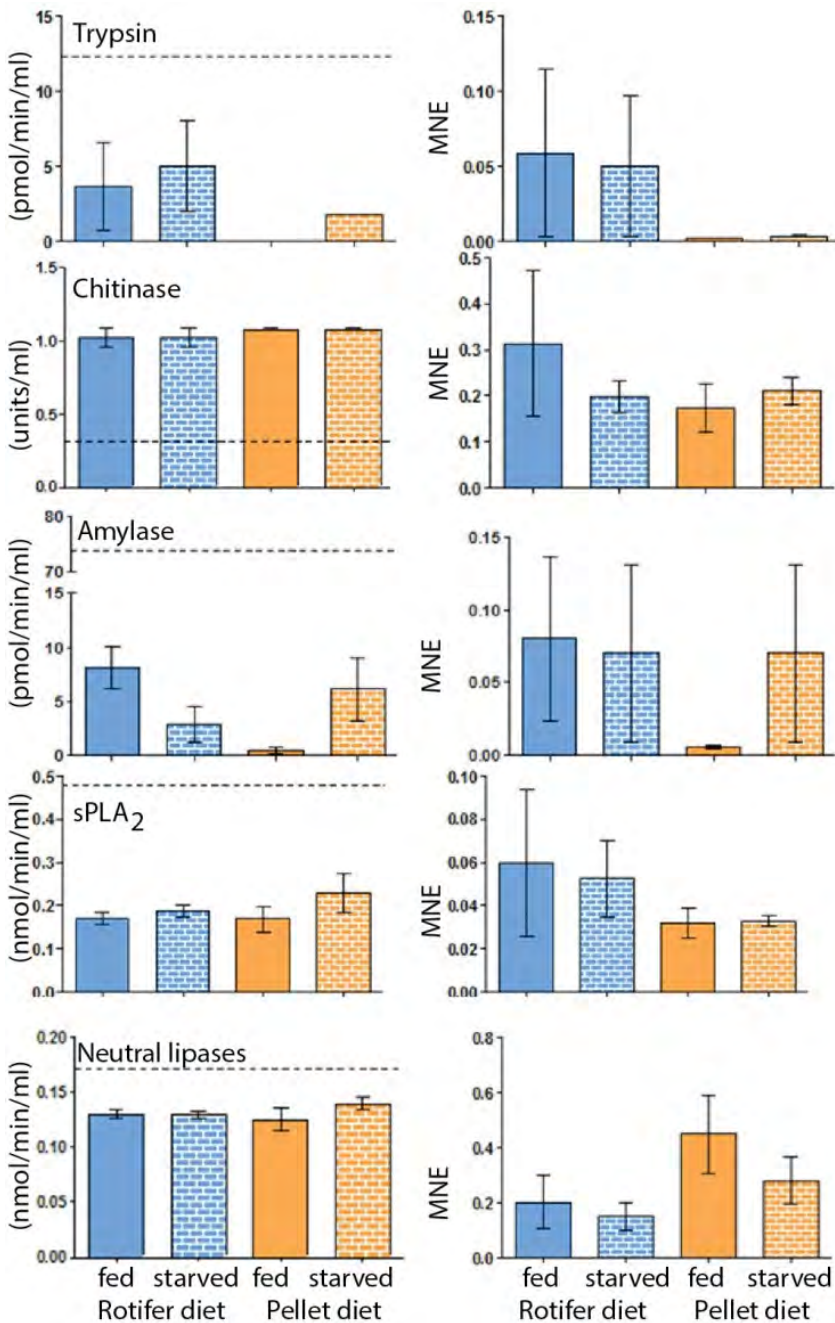
Figure 1. Enzyme activities ± SD (left column) and gene expression, as mean normalized expression (MNE ± SEM) (right column) in the feeding trial, for trypsin, pancreatic chitinase, amylase, sPLA2 1B and neutral lipase (BAL). Stippled line represents enzymatic activity in the rotifers. Blue bars represent rotifer fed larvae and orange bars pellet fed larvae. Filled columns show activity/gene expression of larvae with food in the intestine and bars with pattern show activity/gene expression of larvae that were starved for 12 hours.

CHAPTER 5

Characteristics of the digestive functions in ballan wrasse fed dry and moist diets

Åshild Krogdahl, Øystein Sæle, Kai Lie, Katerina Kousoulaki, Kristin Hamre, Synnøve Helland and Ingrid Lein

INTRODUCTION

Ballan wrasse has a short and simple intestine without stomach and caeca (see Figure 1) whereas most carnivorous fish species at the adult stage have a distinct stomach. To be sure to find the right feed and feeding regime for cultivating ballan wrasse, knowledge of the digestive tract, its organisation, function and response to variation in feed composition and feeding regime is needed. The present investigation was conducted to reveal characteristics of the intestine of ballan wrasse regarding gross and micro anatomy, gut function and effects of dry and moist diet on these.

EXPERIMENTAL SETUP

Ballan wrasse from Profunda, average weight 56g, were distributed among12 tanks, 80 fish per tank, with 350 L water volume, black walls, plastic shelters and overhead light. A 24 hour light regime was applied, and beltfeeders were used for continuous feeding.

The experimental feeds were produced by Nofima. Two extruded feeds (2mm) were made deviating only regarding a marker, i.e. ytterbium and yttrium, for investigation of passage rate and nutrient digestibility along the digestive tract. Both diets contained 51% LT fish meal, 26% shrimp meal and 12% wheat flour, plus vitamins and minerals to satisfy assumed requirements based on knowledge gained in a previous project (NFR 200523/S40: Optimised nutrition and egg quality in the cleaner fish Ballan wrasse (*Labrus bergylta*)). Protein level on dry matter basis averaged 59%, lipid 14% and ash 16%.

Each of the diets were split in two batches of which one was fed in the dry form and the other after moistening with water, 100 g feed plus 80 g water. Each of the resulting four diets was fed to fish in three replicate tanks. The day before sampling feed was withdrawn overnight. The next morning the diets were switched regarding marker, i.e. fish fed dry feed with yttrium was offered feed with ytterbium and vice versa and similarly for fish fed the moistened diets. Sampling started two hours after the switch of diets and samples were taken at five additional times over the next 16 hours. Five fish were sampled from one tank for each diet at each sampling time. The same tank was sampled only two times over the sampling period.

The fish were killed with a blow to the head. The intestine was dissected, cleaned of fat and opened longitudinally, as indicated in Figure 1 which also indicates the localisation of the internal organs surrounding the intestine. The intestinal sections were separated, chyme was collected, the tissues were weighed and samples of tissue taken for histological and functional studies as indicated in the presentations of the results. As the amount of chyme sampled was insufficient for the planned analyses, a follow-up similar trial was conducted, for collection of additional samples of faeces. The samples were preserved in formalin or with liquid nitrogen as appropriate for the various analyses (Froystad et al. 2006; Bakke-McKellep et al. 2000; Bæverfjord and Krogdahl 1996; Chikwati et al. 2012; Penn et al. 2011).

Two-way ANOVA was used for evaluation of all the results except the histological data for which a descriptive evaluation was used.

Since the RNA integrity of the samples from the main study was not satisfactory, gut segments used for gene expression were sampled from a commercial fish farm (Marine Harvest Labrus, Øygarden outside of Bergen, Norway). The fish had been fed Skretting Labrus feed. Intestinal segments from five adult ballan wrasse (58 ± 5 g) were sampled and all excised gut tissues were examined to insure presence of food. Gut content was removed by rinsing each segment in PBS buffered milliq water. Each segment was flash frozen on liquid nitrogen and stored at -80°C waiting further processing. Preparation of cDNA libraries and sequencing was performed by the Norwegian Sequencing Center on an Illumina HiSeq trimmed and filtered were assembled into a *de novo* transcriptome library using Trinity pipeline (Grabherr et al., 2011). Gene expression analyses were performed using a variety of bioinformatic tools; Bowtie, edger, DEseq and Clucore omics explorer. Functional analyses were performed using DAVID Bioinformatics Resources 6.7.

RESULTS AND DISCUSSION

The intestine of the ballan wrasse is short and lies in the abdomen in one loop surrounded by the other internal organs (Figure 1). The histomorphological structure of the ballan wrasse intestine showed the same general organisation from the proximal to the distal end and was similar to that of Atlantic salmon (AS). Moisture content (MC) of the feed did not affect its structure (Figure 2).

Mucosal fold height diminished along the intestine (Figure 2). The distal ¼ showed a different structure than the more proximal, similar to what is observed in AS, indicating a difference in function between the proximal and mid sections compared to the distal most section. Pancreatic tissue was distributed within the adipose and mesenteric tissue along the intestine.

A prominent bile duct connects the gall bladder with the proximal intestine (Figure 3).
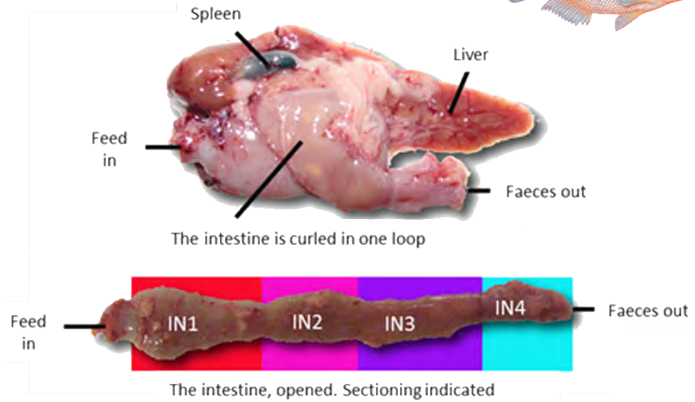
Figure 1. Upper picture: Package of internal organs as dissected from the abdominal cavity. Lower picture: The intestine cleared of fat and opened longitudinally. The coloured background indicates the sectioning of the intestine for structural and functional investigation. Photo: Øystein Sæle, NIFES


Figure 2. Representative samples pictures of mucosa (H&E staining) along the intestine of the fish from the proximal ¼ (IN1) to the distal ¼ (IN4)
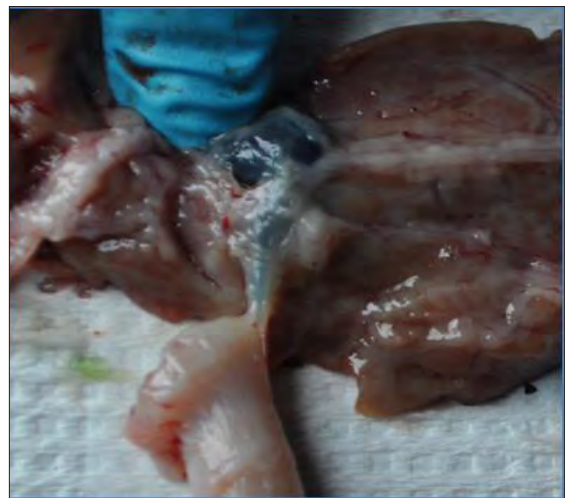
Figure 3. Illustration of gall bladder (black) and bile duct (green) entering the intestine. Photo: Synnøve Helland, Nofima

In ballan wrasse, a species which does not have a stomach, the intestinal tract comprises about 2% of body weight, somewhat less than the gastro-intestinal tract of Atlantic salmon (AS) (Figure 4). The intestinal weight did not differ significantly between fish fed dry and moist diet. In the chyme, the intestinal content pH increased from about 7.7 to 8.2 along the intestine. Feed MC did not affect chyme pH significantly (Figure 4). Chyme dry matter decreased from about 18% in the proximal ¼ of the intestine to about 15% in the distal ¼. Fish fed moist feed showed lower chyme dry matter than fish fed dry feed (Figure 4).
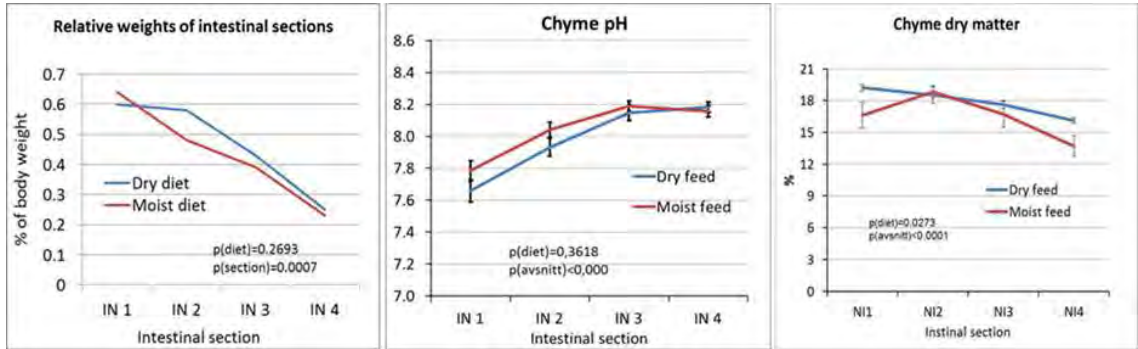
Figure 4. Illustration of results regarding relative weights of intestinal sections (left), pH along the intestine (middle) and chyme dry matter (right) in fish fed the two experimental diets. Error bars indicate +/-SEM.

Activity profile of the pancreatic enzymes trypsin and amylase in the chyme changed along the intestine (Figure 5). Trypsin activity increased through the first ¾ of the intestine, and decreased in the distal ¼ to the level of the proximal most ¼. Amylase activity showed a different picture (Figure 5), with decreasing activity all along the intestine. Neither of the enzyme activities showed effects of diet MC. Secretion of trypsin and amylase seemed to be regulated independently, supposedly according to the level and quality of protein and starch in the diet (Figure 5). Bile salt concentration in the chyme, essential for lipid digestion, was constant in the proximal ¾ of the intestine and decreased to a low level in the distal ¼, indicating efficient recirculation (Figure 5). It appeared somewhat higher in fish fed the moist diet, but was low compared to AS.
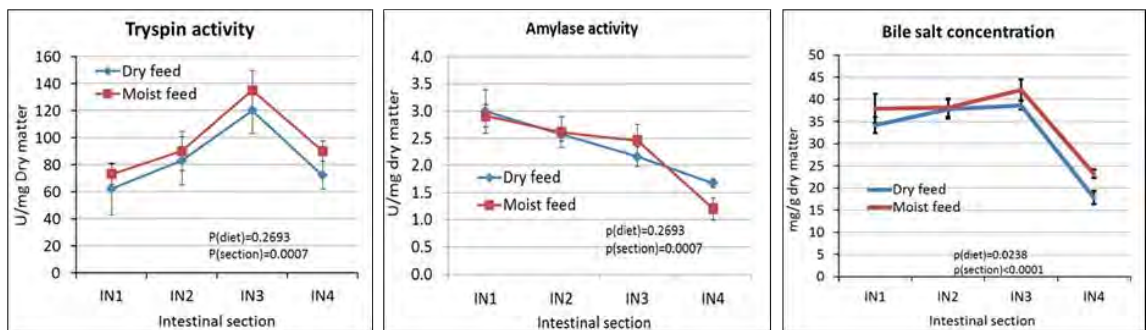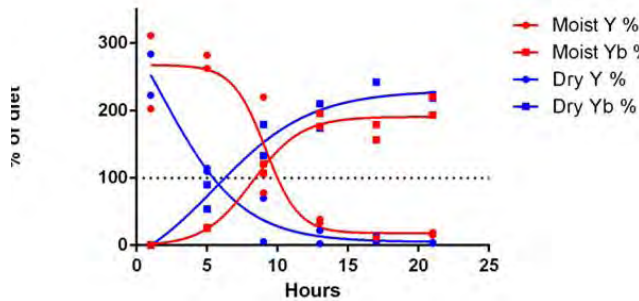

Figure 5. Illustration of results regarding trypsin and amylase activities and of bile salt concentration in chyme along the intestine. Error bars indicate +/-SEM.

Hydrolytic capacity of the brush border enzyme leucine aminopeptidase (LAP), located in the intestinal wall surface membrane, and for which the products of the pancreatic protease hydrolysis are substrates, was low in the proximal ¼, high in the second ¼ and decreased somewhat towards the distal end (Figure 6). No effect of diet MC was apparent. The profile of hydrolytic capacity of maltase (Figure 6), hydrolysing maltose, the product of amylase hydrolysis, differed from that of LAP, high in the first and second ¼'s of the intestine, lower in the distal two ¼'s.

Passage rate of feed through the digestive tract in fish on continuous feeding was found to be 9 to 13 hours. Dry feed seemed to pass somewhat faster than moist feed (Figure 7).
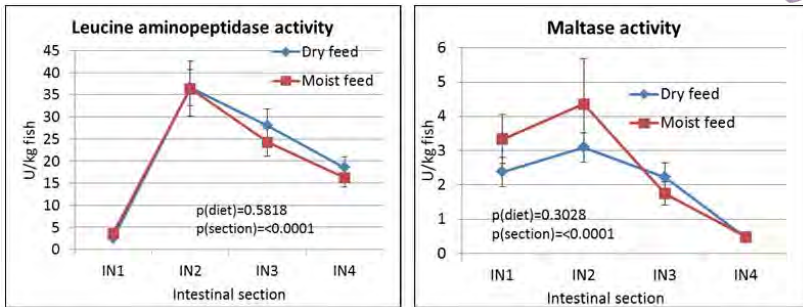
Figure 6. Illustration of results regarding activity of the brush border membrane enzymes leucine aminopeptidase (left) and maltase (right) along the intestinal tract. Error bars indicate +/-SEM.


Figure 7. Illustration of results regarding intestinal passage of diets as estimated from faecal evacuation of the markers. Dry=dry diet, Moist=moist diet, Y=yttrium, Yb= ytterbium.

The proximal intestinal segments seemed to absorb the major part of the nutrients (Figure 8). At the level of the second ¼ of the ballan wrasse intestine apparent digestibility ranged between 55 and 74 for the different macronutrients, reaching a maximum in the third ¼, showing the range 75 to 90%. Highest digestibility was observed for polyunsaturated fatty acids, followed by starch and protein (Figure 8). The monounsaturated and saturated fatty acids showed the lowest digestibilities. The results seem to indicate that the nutrients of the moist feed were absorbed more efficient in the proximal intestinal segment and, in total, show higher digestibility than the dry diet.
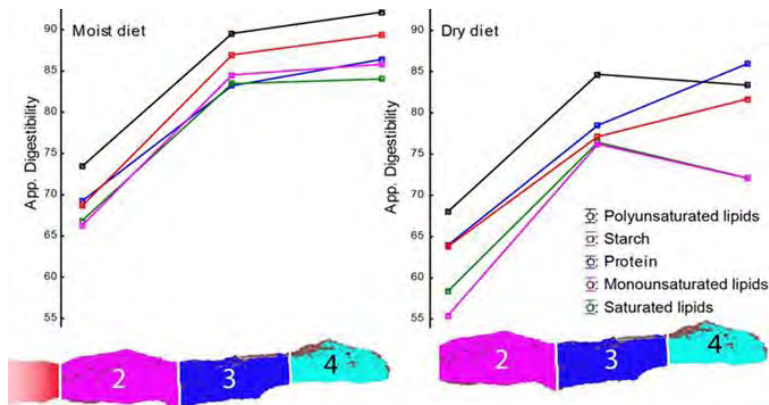
Figure 8. Apparent digestibility of macronutrinents in the three distal most segments of the wrasse intestine.

Some fish showed severe inflammation of the gut wall indicating that gut health is an issue also in ballan wrasse (Figure 9, on following page). However, no effects of diet MC were observed.
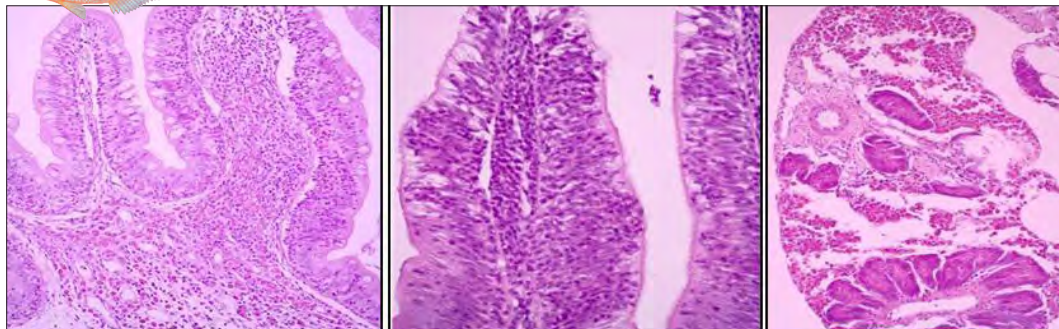
Figure 9. Illustration of inflammation in intestinal mucosal (left and middle) and pancreatic tissue. The high number of small dark cells indicates severe inflammation.

DIFFERENTIAL EXPRESSION OF GENES ALONG THE GUT

The transcriptome of the intestine indicates that, regardless of being short and in lack of a stomach in the front, the distribution of intestinal functions is fairly similar to other fish species. The transcriptome analysis using RNA seq showed that the anterior part (segment 1) and the anterior mid gut (segment 2) generally have similar functions. This is different to what we originally anticipated based on gross morphology of the intestinal tract. The transcriptomic analysis also showed that especially the hind gut (segment 4) distinguished itself from the other intestinal segments analysed.

Deep sequencing and assembly of 270 mill reads verified the absence of genes coding for gastric proteins such as gastric lipase, pepsin and gastrin. Funtional analysis using the DAVID Bioinformatics Resources revealed that the anterior part (bulbus) and anterior mid gut had the highest expressions of genes involved in nutrient digestion and uptake like lipid metabolism and transport, protein digestion and catabolic processing of carbohydrates (Figure 10). These two sections of the intestine had also a higher mitochondrial activity than further posterior.

The dominating role of segment 1 and 2 in digestion and uptake of nutrients is similar to what we observed by apparent digestibility of lipids, starch and protein (Figure 8).

The functional analysis of sequencing data suggests that, similar to salmon, the hind gut is important for immune related functions such as antigen recognition and leucocyte/neutrophil activation and differentiation. Endocytosis and lysosomal processes were also more abundant in the hind gut than in the anterior part. This is most probably linked to the high presence of neutrophils in this part of the intestine.

Besides the immune related functions of the hind gut, several key genes coding for proteins involved in active uptake of bile and vitamin B12 were highly expressed in the hind gut. This indicates that the hind gut is important for the sodium-dependent reabsorption of bile acids and vitamin B12 from the lumen. The enriched presence of immune relevant genes, key bile acid transporters and vitamin B12 transporters suggests that the hind gut of wrasse has similar functions as the ileum of mammalian small intestine.
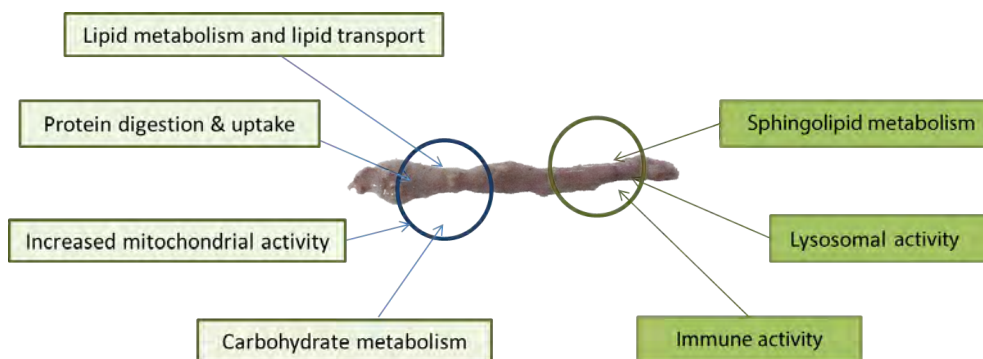
Figure 10. Functional gene expression analysis of different segments of wrasse intestine. The figure shows enriched biological processes in the different part of the intestine.

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RECOMMENDATIONS

Based on the present results the moist feed seemed to suit the digestive functions better than the dry feed: the results indicate that moist feed passes more slowly through the intestine in parallel with higher bile salt concentration and activities of most digestive enzymes and a seemingly better nutrient digestibility.

Our conclusion is that moist feed should be used by commercial producers until more optimal diets are developed.

However, further studies are needed with other dietary ingredients to be able to find optimal diet composition for optimal feed utilisation and intestinal health.

The digestive system of juvenile ballan wrasse

Elin Kjørsvik, Steinar Flaten, Tora Bardal and Per-Arvid Wold

Introduction

The ballan wrasse (*Labrus bergylta*) is successfully used as a cleaner fish in Norwegian salmon (Salmo salar) farms in order to remove the salmon louse (*Lepeophtheirus salmonis*). Nearly all cleaner fish in Norwegian salmon cages today are caught from the wild and farming of the ballan wrasse seems necessary to replace wild catches, for this species to become a more sustainable and environmentally-friendly alternative to chemical treatments. There is also a need for a more constant supply of suitable sized fish. However, this species seems to have specific nutritional requirements and is sensitive to diet quality. The ballan wrasse has a relative short digestive tract and lacks an acidic stomach and pyloric caeca. Understanding the morphology and the functionality of the digestive capacities and mechanisms is therefore important to obtain optimal nutrition of these fish (Hamre and Sæle, 2011). The main aim of this study was to describe the digestive system of the ballan wrasse by histological and stereological methods.

Experimental set-up

Five one-year old ballan wrasse juveniles were dissected, and the whole digestive system including liver, pancreas, and intestine was fixed in phosphate-buffered formalin. The tissues were embedded whole in paraffin, cut in transverse, serial sections (Figure1), and stained with H & E. The sections were analysed both through a microscope and a stereo microscope, and photographed. Volumes of the digestive tissues, and the internal surface area of the intestinal villi, were estimated by the Cavalieri method (Howard & Reed, 1998), using CAST 2 (Olympus Inc., Denmark). Estimates of microvilli height and density were done on electron micrographs from three different parts of the intestine, in order to estimate the total intestinal surface area. No calibration to shrinkage due to fixation was calculated.

The intestinal numbers of goblet cells were estimated from three intestinal parts of each of the five sample fish, one from the anterior intestine, one from the midgut, and one from the posterior intestine.

Results and discussion

The one year old juvenile fish varied in size between 10 and 14 cm total length (TL). The gut was Z-shaped, and the total gut length was estimated to vary between 2.4 and 4.1 cm (Figure 1). The total gut length compared to the TL of the fish, and the gut length thus seems much shorter than for other investigated fish species without a stomach and with similar feeding patterns (Karachle and Stergio, 2010).

The intestinal epithelium was characterised by many, very long, slender and heavily branched villi (Figure 2) in the anterior and midgut areas. The villi gradually became shorter and less branched towards the posterior intestine. In teleost fish, the pancreatic exocrine tissue can be extrahepatic, or it can penetrate into the liver parenchyma, depending on the species. In ballan wrasse, pancreatic tissue was partly interspersed within the liver tissue (socalled hepatopancreas), and extrahepatic pancreatic tissue was also mixed with adipose tissue and was observed along the whole intestinal tract (Figure 2). The intrahepatic exocrine pancreatic tissue was separated from the hepatocyte tissue by thin septa of connective tissue. The total organ tissue volumes were strongly correlated to fish size (Figure 3).
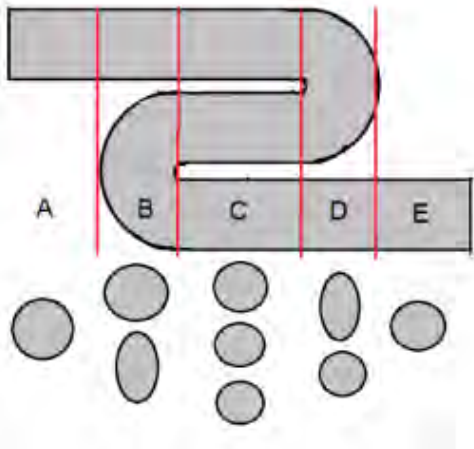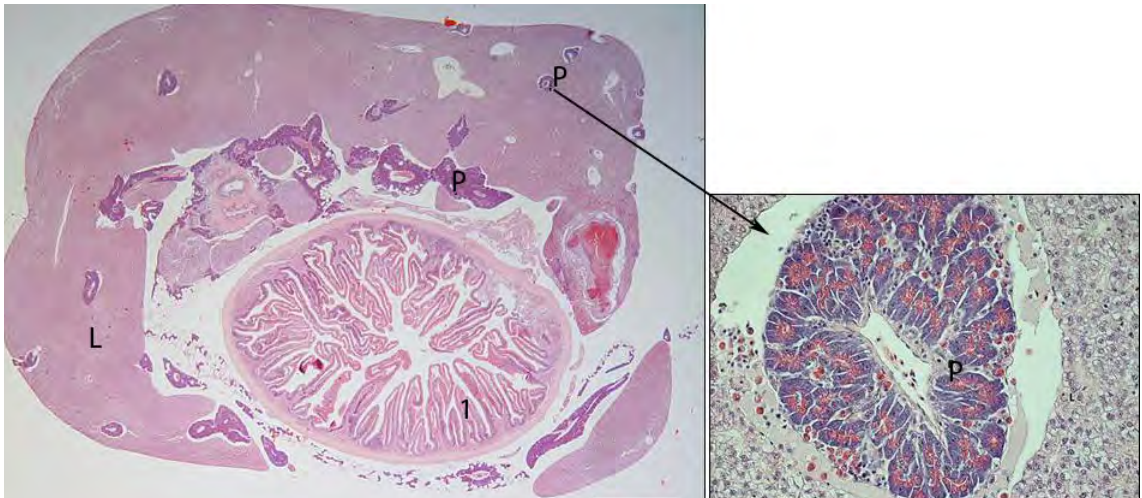
Figure 1. Overview of the typical layout of the Z-shaped intestine and sectioning areas.
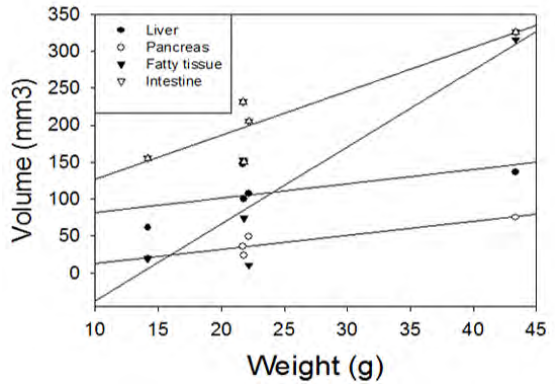
Figure 3. The organ tissue volumes of ballan wrasse juveniles were strongly correlated to fish size.

Figure 2. The anterior part of the digestive system in the ballan wrasse was dominated by liver tissue (L) containing intrahepatic pancreatic tissue (P). The anterior part of the intestine (1) is visible, and the villi were long and heavily branched. Adipose tissue (empty vacuoles between intestine and liver) was present interspersed with the extrahepatic pancreatic tissue (P).

The highest density of mucous-producing goblet cells was found in the posterior part of the gut. The frequency of goblet cells in the posterior intestine was between 10–15 goblet cells per mm gut tissue, and there was no significant difference between four of the fish. One fish had very high density of goblet cells in the posterior gut, but the mucosal epithelium was very thin and was loosened from the lamina propria, possibly due to inflammation.

Intestinal villi will increase the surface area of the intestine to several times what it would be if it was just a simple tube. In addition, the surface of all the absorptive epithelial cells throughout the intestine is covered with microvilli (also known as a "brush border"). Because of these, the total surface area of the intestine (including the surface area of microvilli) varied from 1127 cm^2 to 2962 cm^2, and the intestinal surface area was positively correlated to fish total length (Figure 4, see following page).

To our knowledge, this is the first quantitative description of digestive tissue volume and total intestinal epithelial surface area in a teleost. Compared to a small mammal, the mean gut length of adult mice (33-41 g) would be about 55.5 cm, with a total intestinal surface area of 1.41 m² (Casteleyn et al., 2010). However, if we compare the intestinal surface area per cm of intestinal length, the ballan wrasse gut had 2.7 times the total intestinal surface per area of a mouse gut. The ballan wrasse intestine thus seems like a highly efficient digestive tube. Intestinal length and morphology can be influenced by many factors. Knowledge about how the intestinal surface area and gut morphology may be affected by fish age, different diets and feeding regimes, could shed more light onto the digestive functionality and nutritional requirements of this species.

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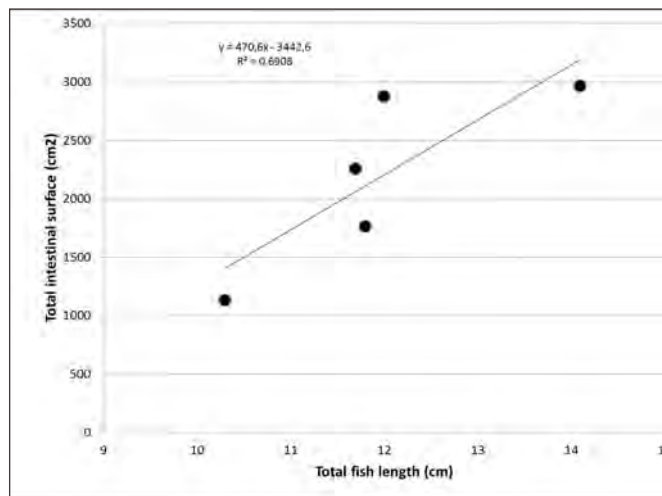
Figure 4. Total intestinal surface area (including microvilli) plotted against total length in ballan wrasse juveniles.

Effects of feeding frequency
on growth and gut health of ballan wrasse juveniles

**Synnøve Helland, Ingrid Lein, Øystein Sæle, Kai Kristoffer Lie,
Katerina Kousoulaki, Stephanie C.M. van Dalen, Peter H. M. Klaren,
Anne Marie Bakke and Åshild Krogdahl**

Introduction

Ballan wrasse (*Labrus bergylta*) is a slow-growing species both in the wild and under farming conditions. As cultivation of this species is new, many aspects of basic knowledge of importance for optimal production are lacking. Optimal feeding of any animal requires knowledge in areas such as nutrient requirements, preferred nutrient sources, and natural feeding rhythms. The present work was conducted to gain understanding of effects of variation in feed delivery on performance and digestive function. Some fish species have persistent diurnal rhythms for feed intake, while others grow best with continuous feeding. No information is available regarding the feeding habits of the ballan wrasse. In a previous study, initial work was done to describe structural and functional characteristics of the digestive tract and the digestive processes in this fish species, which has no stomach and the intestine is short without intestinal caeca. The present work provides more basic information on the digestive function with a focus on the effects of feeding regime. Three feeding regimes were tested: demand feeding using self-feeders, continuous feeding and batch feeding. A fourth treatment was included, i.e. dry feed versus moist feed, to follow up questions raised in our previous study regarding suitable moisture content of the ballan wrasse diet. The results were evaluated by survival, growth, physiological characteristics and intestinal health.

Experimental set-up

The feeding experiment ran for 4 months (Nov. 9 2012-March 5 2013). Twelve tanks (volume 350 L, black wall, shelters and overhead light) were used of which three were equipped with self-feeders while the remaining 9 tanks were equipped with belt feeders. Each tank was stocked with 90 ballan wrasse produced by a commercial hatchery (Profunda, generation 2011). All fish were kept under 24 hours light which is the standard procedure in commercial farming of ballan wrasse. The water temperature and oxygen level in the tanks were measured daily and were 14.5°C ±0.4 (min 13.7, max 15.4) and 92.7% ± 4.9 (min 83, max 108), respectively (average ± SD). Fish weight was recorded four times, and samples for investigation of effects on gut function and health and on appetite were taken at the end of the experiment.

One batch of experimental feed was produced from dry ingredients by Nofima. The feed was delivered to the fish either dry or moistened by adding 80g of water per 100g of diet for 30 minutes to allow the water to be fully absorbed into the material before feeding. The production procedure allowed the moist diet to maintain its pelleted structure.

Four different feeding regimes were tested:

- Demand-feeding with dry feed, using automated self-feeders

- Continuous feeding (delivered every 10 min.) with dry feed, using belt feeders

- Continuous feeding (delivered every 10 min.) with moist feed, using belt feeders

- Two portions dry feed per day (delivered at 08:00-09:00 and 14:00-15:00), using belt feeders

At termination of the experiment the fish were euthanized and weight measured before the intestines were exposed and cut in four sections. The intestinal sections were numbered from the proximal to the distal end and identified as IN1 (segment 1), IN2 (segment 2), IN3 (segment 3) and IN4 (segment 4) (See "Characteristics of the digestive functions in ballan wrasse fed dry and moist diets"). All samples were routinely processed and evaluated separately. Samples were taken for investigation of structural and physiological characteristics of the intestinal tissue as well as the characteristics of the intestinal content, such as pH, pancreatic trypsin activity and bile salt concentration as described by Krogdahl and Bakke, 2005.
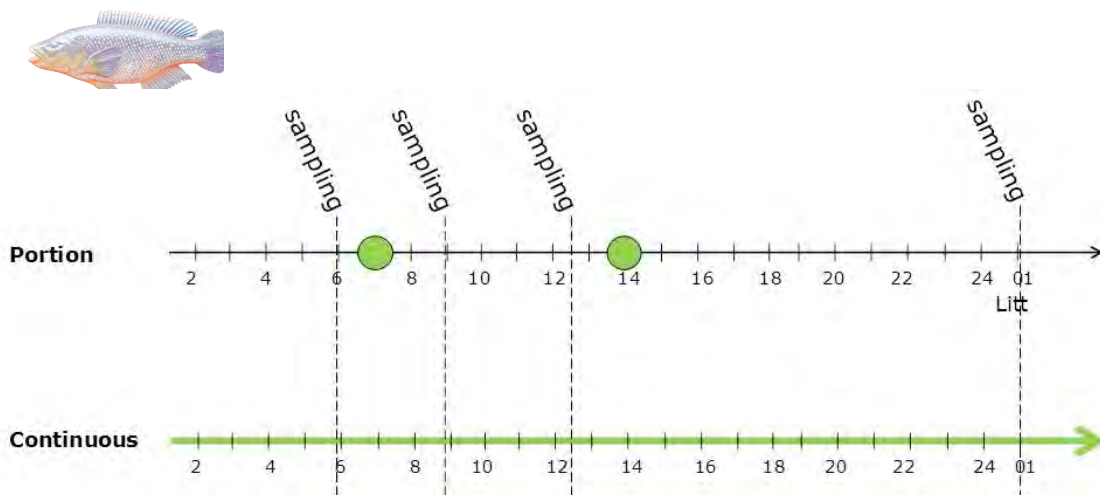
Figure 1. Feeding (green) and sampling time points for gene expression analysis. Green circles indicates continuous feeding.
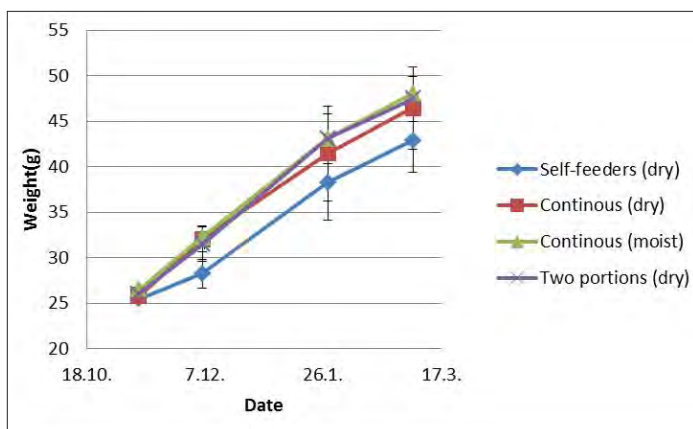
Molecular gene expression analysis was conducted using NSG deep sequencing technology. Whole intestines from a separate sample of fish were excised for gene expression analysis, frozen in liquid nitrogen and homogenised. Six fish from three time points were analysed for each of the two feeding regimes; 1) before feeding 06:00, 2) right after a feeding at 09:00 and 3) 11 hours after the last feeding at 1:00 (Figure 1). Batch-fed and continuously-fed fish were sampled in parallel.

Global gene expression was analysed using the "next generation sequencing methods" on an Illumina HI Seq sequencer. Sample preparation and sequencing were carried out by the Norwegian Sequencing Center in collaboration with CEES. Differentially expressed genes were analysed using one-way ANOVA implemented in Qluecore Omix Explorer package. Functional analysis (biological function) for significantly regulated genes was performed using the DAVID Bioinformatics Resources 6.7 (Huang and Lempicki 2009, Nature Protocols, 4 44-57).

Results and discussion

From start until the first weighing, wrasse fed by self-feeders grew slower than the wrasse on the other three feeding regimes. However, after the first weighing, the self-feeding group caught up with the other three groups with regard to growth rate (Figure 2, p=0.41). The slower initial growth of the fish fed by demand-feeding auto-mats is probably due to a learning period during which the animals learnt to operate the automats. According to the originl plan, the fish should have been adapted to the self-feeding system for two weeks before start of the experiment, but this turned out not to be possible due to technical problems with the self-feeding system.

Specific growth rates were calculated for each of the three periods following weighing (Figure 3) and show a lower SGR in period 1 for the demand-feeding treatment compaared to the other groups. The SGR was similar for all treatments in period 2 and 3.

For the whole experimental period the SGR was 0.44 for the self-feeding regime, and 0.52 for continuous dry and 0.51 for the continuous moist and the two portion regimes.

Until day 50 of the experiment, there was little or no mortality, but the mortality increased towords the end of the experiment. Highest survival was found for the two continuous feeding regimes, although no significant differences were detected (Figure 4, p=0.427).
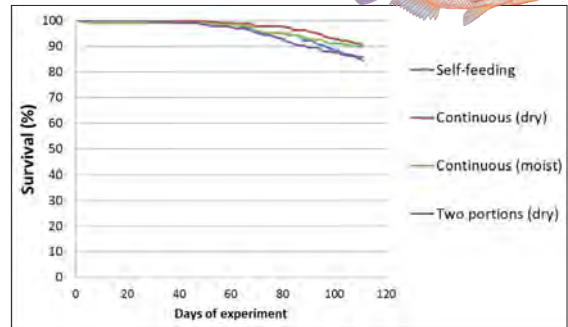

Figure 2. Weight (gram) of ballan wrasse fed either dry feed from a self-feeder, dry feed continously, moist feed continuously, or dry feed in two meals (average ± SD, n=3).
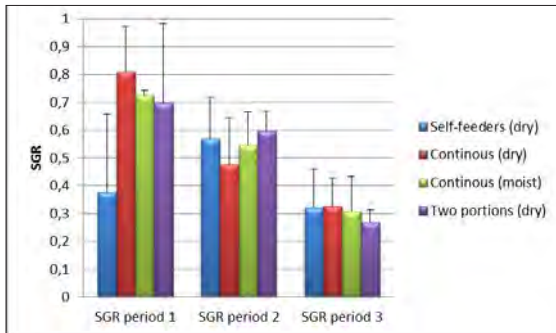
Figure 3. Specific growth rate (SGR) of wrasse fed either dry feed from a self-feeder, dry feed continuously, moist feed continuously, or dry feed in two meals by belt feeders (average ± SD, n=3). The periods 1, 2 and 3 refers to the time between 9 Nov. 2012, 6 Dec. 2012, 28 Jan. 2013 and 5 March 2013, respectively.


Figure 4. Survival (%) of ballan wrasse fed either dry feed from a self-feeder, dry feed continuously, moist feed continuously, or dry feed in two meals by belt feeders (average ± SD, n=3).

Registration of meal requests from the three tanks with self-feeders showed no clear diurnal rhythm, but a slight increase between 08:00 and 11:00 (Figure 5). This may indicate that the ballan wrasse does not have diurnal feeding rhythms, or that the continuous light regime may have interfered with possible diurnal feeding rhythms. The frequency of meal requests from the self-feeders increased during the experimental period (Figure 5).

Together with the slower growth of this group during the first period of the experiment, this indicates that the fish need an adaptation period to the self-feeding system lasting longer than the one week used in the present study. Registration of meal requests from the three tanks with self-feeders showed no clear diurnal rhythm, but a slight increase between 08:00 and 11:00 (Figure 5). This may indicate that the ballan wrasse does not have diurnal feeding rhythms, or that the continuous light regime may have interfered with possible diurnal feeding rhythms.

The frequency of meal requests from the self-feeders increased during the experimental period (Figure 6). Together with the slower growth of this group during the first period of the experiment, this indicates that the fish need an acclimation period to the self-feeding system lasting longer than the one week used in the present study.
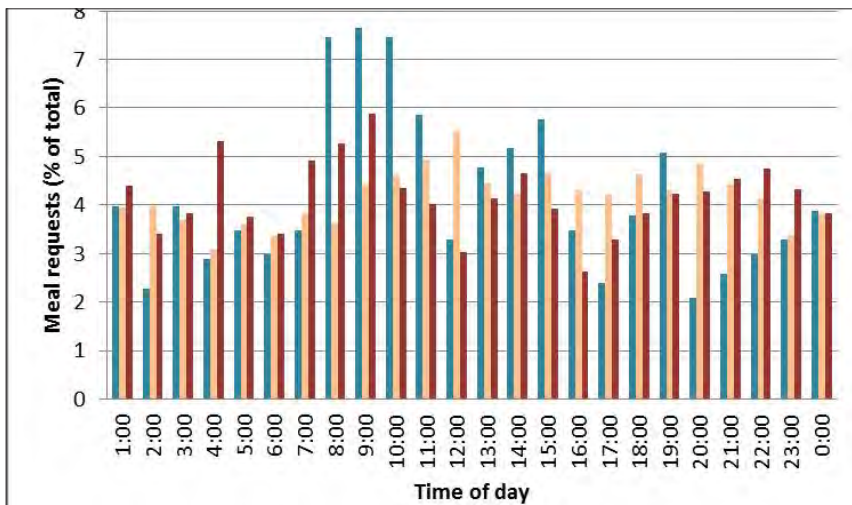
Figure 5. Number of meals requested (% of total requests) registered for each of the three tanks (each colour is one tank) with self-feeding during the 24 hours of the day.
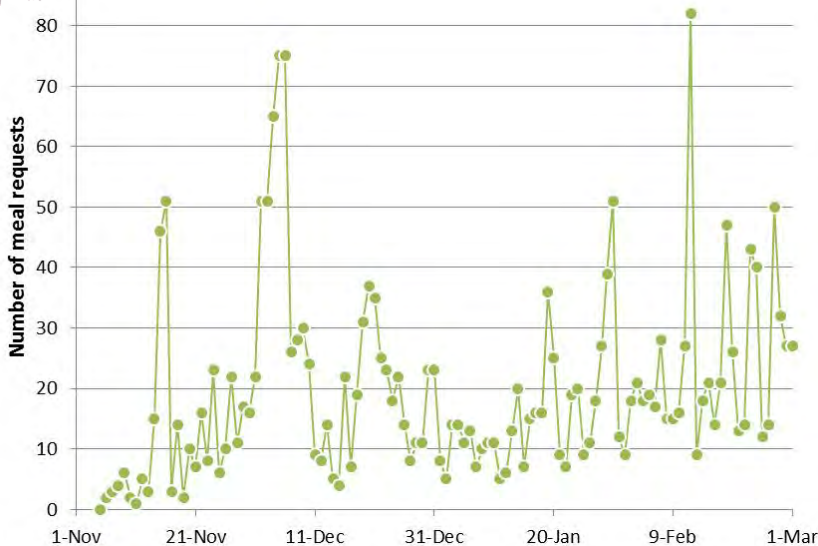
Figure 6. Number of meals requested by self-feeder in one of the tanks during the experimental period.

Histological findings

The results regarding intestinal health status of the fish, as indicated by histological examination, are summarised in Table 1. Regardless of feeding regime, infiltration of immune cells was apparent between pancreatic cells and in the surrounding adipose tissue. An increase in the abundance of IELs (intraepithelial lymphocytes) in the epithelial layer of the intestine, without or with a moderate cell infiltration in *lamina propria* was also noticed. Small single-celled parasites (possibly cryptosporidia) located among the enterocytes were observed in some samples. However, no distinct differences in the pancreatic or intestinal tissue were observed between the experimental groups. Compared to fish examined in our earlier study, the intestines of the present fish appeared clearly healthier. Figure 7 shows representative pictures from intestinal samples. Inflammatory changes were more commonly observed in the distal-most intestinal region (IN4).

Table 1. Summary of evaluation of inflammation indicators of tissue along the intestinal tract. Numbers indicate mean number of individuals from each feeding regime for each intestinal region IN_{1-4} (n=12-15) with each respective score of 0 = no signs of inflammation, and 1 = mild, 2 = moderate and 3 = severe inflammatory changes.

	IN_1				IN_2				IN_3				IN_4			
SCORE	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**
Feeding regime																
Self-feeding	15	0	0	0	15	0	0	0	8	6	1	0	6	3	4	2
Cont. dry	13	1	1	0	13	0	0	0	9	5	1	0	6	4	4	0
Cont. moist	12	2	0	1	11	2	2	0	6	5	3	1	6	3	2	1
Two meals	13	2	0	0	14	1	0	0	11	3	1	0	7	4	1	1

This experiment generally confirmed previous findings regarding structure of the intestinal wall and that the structure in the ballan wrasse does not deviate importantly from other species, such as salmonids.

INDICATORS OF INTESTINAL FUNCTION

The intestines of the wrasse constituted about 1.5% of the body weight, in accordance with our previous study. Feeding regime affected intestinal weight, and was highest in fish fed the self-feeding regime, followed by fish fed two meals (Figure 8). Fish coninuously fed showed the lowest weight regardless of the water content in the feed. A continuous feeding will supposedly reduce the average amount of feed present in the intestine at a given time. It may be suggested that with a lower rate of uptake of nutrient per unit of time, less capacity is needed and therefore the tissue weight is less.
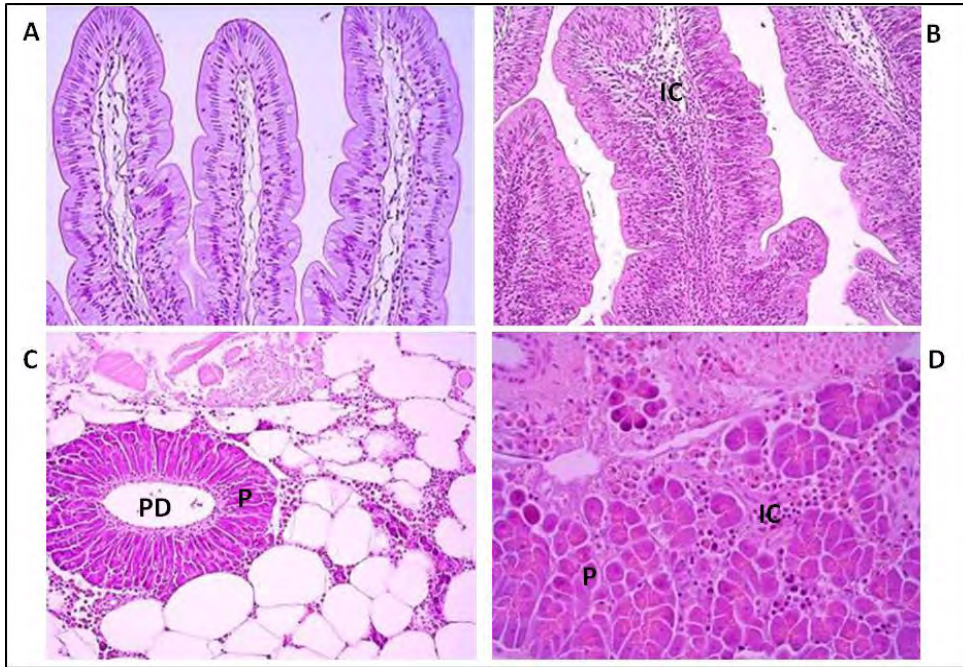
Figure 7. Representative pictures of histological sections from the intestines (A and B) and pancreatic tissue (C and D) of ballan wrasse from the present experiment. No clear difference was observed between the feeding regimes. A: Normal mucosal folds. B: Mucosal folds with moderate immune cell (IC) infiltration. C: Pancreatic tissue (P) with a pancreatic duct (PD). D: Pancreatic cells (PC) with scattered IC infiltration.

In the intestinal content pH was affected by both feeding regime and feed water content. The values were substantially higher in fish fed continuously with dry feed than other fish (Figure 8). The mechanism behind this difference is not clear.
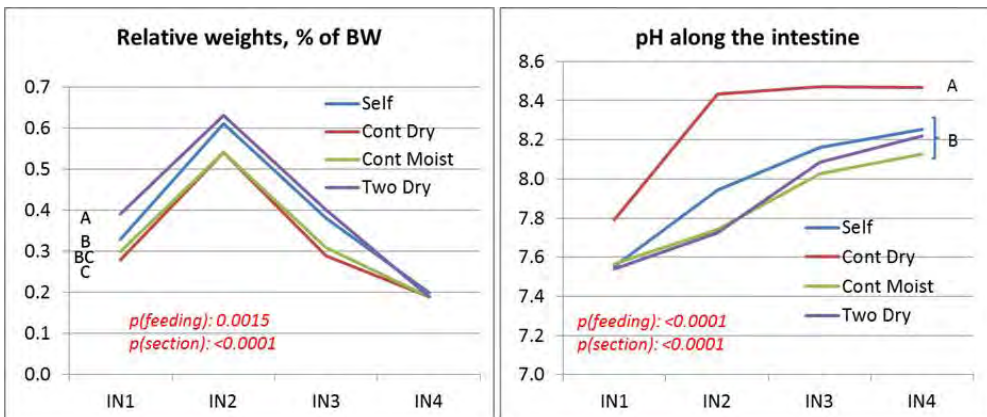
Figure 8. Relative weight (% of body weight) of and pH in each intestinal section of fish on the different feeding regimes: Self=Self feeding; Cont Dry=Continuous dry feed; Cont Moist=continuous feeding with moistened diet; Two Dry=two meals of dry diet a day. Significant differences are indicated by different letters next to the curves.

The content of dry matter (not shown) and activity of trypsin (Figure 9) in the intestinal contents were not significantly affected either by feeding regime or water content in the feed. The content of bile in the intestinal content, however, differed between the treatments. Fish fed two meals dry diet per day stood out with higher levels than other fish (Figure 9), most notably in the distal-most IN4. Bile salts are necessary for efficient lipid digestion and for maintaining a healthy intestine. Several factors may affect body pool of bile salts, of which a major part is present in the intestinal content. The current findings suggest that a reduced number of meals may negatively affect reabsorption and recirculation, and thus may result in increased de novo production of bile salts in the body. To understand the mechanism behind and consequences of this finding, further studies are needed.
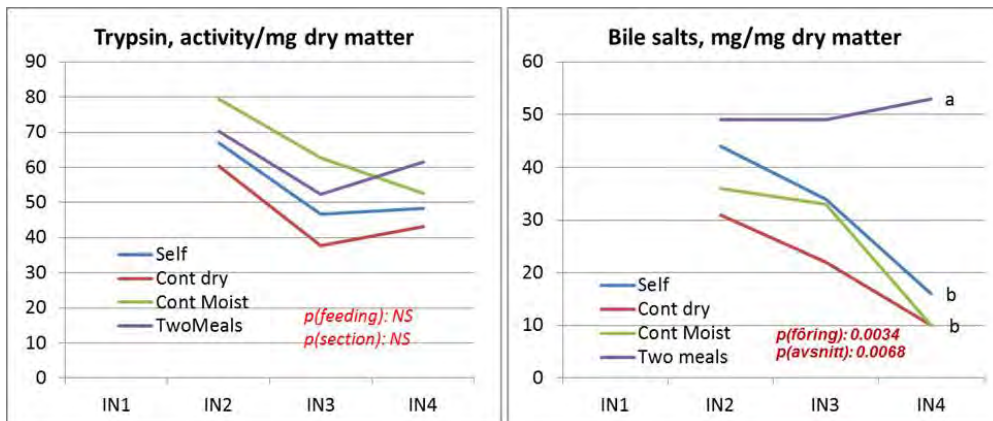
Figure 9. Trypsin activity and bile salt concentration along the intestinal tract in fish on the different feeding regimes (See Figure 6 for explanation of abbreviations).

The capacity of enzymes that hydrolyse disaccharides and peptides, here represented by maltase and leucine aminopeptidase, varied with feeding regime and was highest in the fish that were fed two meals (Figure 10). A possible explanation is that a larger amounts of feed, such as after a large meal, will trigger the intestine to hold more enzymes than fish on continuous feeding.
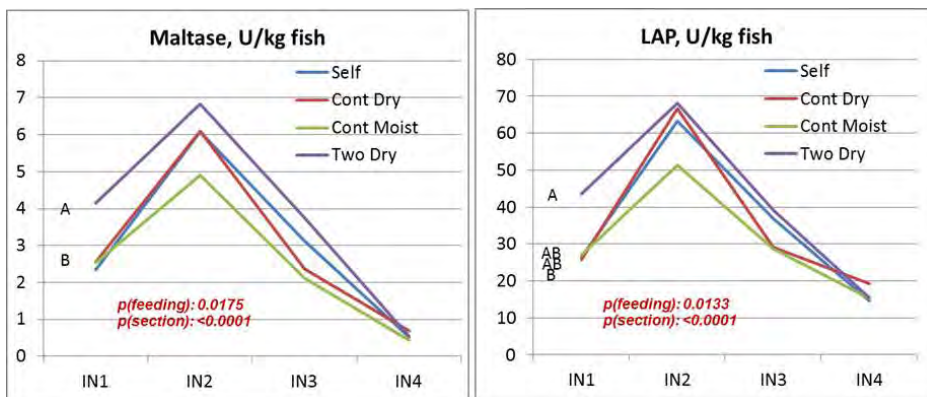
Figure 10. Capacity of leucine aminopeptidase (LAP) and maltase along the intestinal tract in fish on the different feeding regimes (See Figure 6 for explanation of abbreviations).

In summary, the observations on gut structure and function showed that feeding regime and moisture of the diet clearly affect ballan wrasse intestines. The differences may be related to the amount of feed present in the gut at a given time, i.e. that continuous feeding, resulting in lower average amount of feed consumed at each feeding, make the gut adapt to this situation, seemingly in a useful manner. The gut therefore seems flexible and may be expected to be able to handle diets with different composition and moisture content quite well.
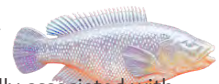
Gene expression analysis

30 million reads in each sample were sequenced giving a good coverage and a representative picture of what happens in the gut at the time of sampling. Analysis of gene expression of the intestine indicates that 16 hours after the last feeding (06:00) and one hour before the next feeding, there was an increased abundance of genes involved in processes such as cell death, cell division, transcription and increased degradation of cellular proteins compared to after feeding (09:00) (Table 2). This is similar to what we have seen previously in cod larvae that have been starved. This could indicate that the fish 16 hours after feeding had started a breakdown of the gut as has previously seen in fasting fish.

However these processes are usually associated with autophagy. We found no evidence of autophagy in the form of different expression of the classical "autophagy-related" ATG markers.

As expected, there were no major differences in gene expression between 06:00 and 09:00 in the fish that were continuously fed. However, it appeared that continuously fed fish sampled at 01:00 stopped eating somewhere between 14:00 and 17:00. The estimate is based on previously determined feed passage time for ballan wrasse which was between 9 and 13 hours. Lack of food in the gut at this time point was also reflected in the gene expression which showed a down regulation of processes involved in energy metabolism at this time point. This could indicate that the fish had developed a feeding rhythm despite continuous access to food.

Table 2 Selection of dominating biological processes up-regulated in ballan wrasse 16 hours after last meal compared to two hours after last meal.

Biological processes (GO termer)	No. of genes	Pvalue	FDR
RNA biosynthetic process	39	4.93E-10	8.60E-07
Transcription	134	1.39E-07	2.42E-04
Regulation of transcription	158	1.60E-07	2.78E-04
Cellular macromolecule catabolic process	54	3.82E-05	0.06649515
Macromolecule catabolic process	56	7.39E-05	0.12880436
Protein catabolic process	47	9.47E-05	0.16487868
Proteolysis involved in cellular protein catabolic process	45	1.62E-04	0.28200805
Regulation of cell cycle	28	6.12E-04	1.06159346
Cell death	46	0.00363908	6.15728303

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Acknowledgements

Recommendations

The ballan wrasse seems to be dynamic and adaptable to different feeding regimes, and neither growth nor intestinal health was much affected by feeding regime. The survival was slightly higher when the fish were offered feed continuously. Based on our data, a continuous feeding of dry alternatively moist feed seems to be a good feeding regime.

Nutritional requirements of juvenile ballan wrasse

Kristin Hamre, Andreas H. Nordgreen, Espen Grøtan and Olav Breck

Introduction

In classical nutritional studies it is common to measure the requirements of one or a few nutrients at the time. Here we have used screening methods to quickly obtain an overview over ballan wrasse nutritional requirements. Firstly we ran a multivariate feeding experiment to estimate the optimum dietary composition of protein, lipid and carbohydrate. Secondly, we screened the nutritional status of wild and farmed ballan wrasse by analysing the whole body nutrient profiles. Assuming that wild fish have a good nutrient status, differences between farmed and wild fish would indicate deficiency or over-supplementation in farmed fish. The data have been published in Hamre et al. (2013).

Experimental setup

A three-component mixture design was used for the feeding experiment. 13 different diets were produced, varying from 34-74% in protein, 10-35% in lipid and 5-25% in carbohydrate (on dry wt). Twelve diets were administered to fish in single tanks and one diet was fed to fish in 3 tanks to obtain a measure of tank variation. The fish (1.27±0.19g at start) were held in 100 L tanks, at 50 fish per tank, with flow-through water at 16 °C, continuous light and feeding. The experiment lasted for 56 days. Weight, length and macronutrient composition of whole body and liver were measured at the end of the experiment. Three groups of cultured juvenile fish from triplicate or duplicate tanks (mean weight 3-13g) were sampled for analyses of whole body nutritional composition from the production line of MHL. The fish were held in 25 m³ tanks with continuous fluorescent light, temperature 12 – 14°C, oxygen 7.5 – 8.0   mg L-1 and had been fed either the Labrus diet (Skretting) or the broodstock diet described under "Broodstock nutrition" (75% Vitalis (Skretting), 25% shrimp) for more than three months. Wild fish (430±138 g) were caught in August 2011 in fish traps near Austevoll Aquaculture Research Station. Samples of 30-50 pooled cultured fish or individual wild fish were homogenised and analysed at NIFES, using routine and certified methods.

Results and discussion

The optimum dietary macronutrient composition for juvenile ballan wrasse, according to the present study, is 65% protein, 12% lipid and 16% carbohydrate, based on maximum lengthwise growth (Figure 1). The high optimum protein content may have been affected by the quality of the dietary ingredients since shrimp was used as a fixed part of the protein source and may have functioned as an attractant. Furthermore, later studies have shown that the lipid class composition of added lipids has a large effect on growth, and lipid quality will probably affect the optimum macronutrient composition.

Of the nutrients analysed in juveniles, the least need for further investigation is in water-soluble vitamins, vitamin E, selenium and copper (Table 1). The high levels of Na in cultured fish were probably due to wounds and fin erosion and are of minor interest when it comes to nutrition. Iron was present at approximately 60% in farmed fish compared to wild fish. Furthermore, the level of taurine was lower in fish fed the Vitalis+shrimp diet than in wild fish, in line with the results on broodstock (Chapter 2), while the Labrus diet supplied sufficient amounts of taurine. The levels of bone minerals in wild and cultured fish were not significantly different, but the mean value in fish fed the Vitalis+shrimp diet, but not the Labrus diet, was substantially lower than in wild fish (large variation).
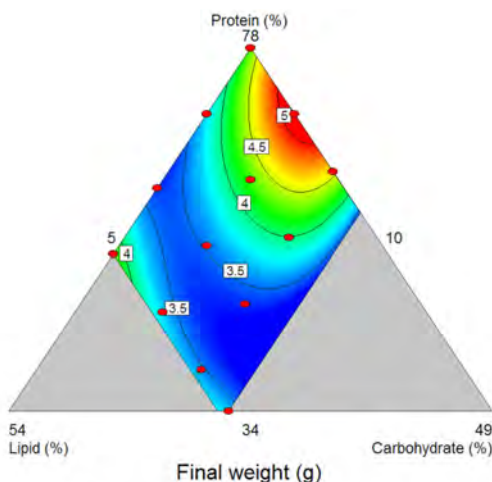
Figure 1. Final weight of ballan wrasse juveniles fed different combinations of protein, lipid and carbohydrate in the diet. The triangle represents all possible combinations of the three macronutrients, the red dots are the diets and the gradient shows the distributions of final weight in grams after fitting the data to a cubic model (R2=0.96).
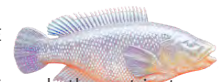
Table 1. Nutrients present at different levels in whole body of farmed and wild ballan wrasse (mean±SD). Several other nutrients were measured and similar. The farmed fish was produced by Marine Harvest Labrus and fed the given diets for at least 3 months in triplicate or in duplicate (G01-02- 2010). The group labels refer to generation no (G) and year (G02-2011: generation 2, 2011). (TFA, total fatty acids).

Fish origin		G02-2011	G01-2011	G01-02-2010	Wild fish[1]	P	P
Feed		Labrus[2]	Labrus[2]	Vitalis+shrimp[3]	-	ANOVA	Kruskal Wallis
Taurine	g kg^{-1}	12.9±1.2 [a]	13.2±1.2 [a]	7.9±0.2 [b]	11.4±0.9 [a]	0.0001	0.019
Lipid	g 100g^{-1}	6.9±0.4 [a]	9.4±1.9 [a]	22±1[b]	12.8±5.7 [ab]	0.002	ns
Sum vitamin A	mg kg^{-1}	0.9±0.2 [a]	1.7±0.8 [a]	2.1±0.1 [ab]	17±8 [b]	0.002	0.003
Vitamin D	mg kg^{-1}	0.25±0.09 [a]	0.11±0.03 [a]	0.10±0.03 [a]	0.94±0.19 [b]	$<10^{-5}$	0.003
Sum vit K	µg kg^{-1}	46±8	18±11	13±0	81±49	na	0.01
Ca	g kg^{-1}	38±3	43±1	30±5	41±12	ns	ns
Mg	g kg^{-1}	2.3±0.3 [a]	2.0±0.2 [ab]	1.4±0.0 [c]	1.8±0.2 [bc]	0.0002	0.01
P	g kg^{-1}	33±6	32±4	23±5	29±7	ns	ns
Fe	mg kg^{-1}	56±7	52±13	49±14	90±24	0.015	0.018
Zn	mg kg^{-1}	86±2 [a]	73±5 [ab]	62±4 [bc]	57±10 [c]	0.0005	0.001
20:4n-6 ARA	%TFA	0.6±0.1	0.5±0.0	0.7±0.1	2.4±0.9	na	0.004
20:5n-3 EPA	%TFA	8.4±0.1 [a]	7.5±0.4 [a]	15.1±0.1 [b]	9.0±1.1 [a]	10^{-6}	0.02
22:6n-3 DHA	%TFA	19.9±0.5	16.2±1.9	10.9±0.0	15.9±3.1	na	0.03
DHA:EPA		2.36±0.05 [a]	2.13±0.16 [a]	0.72±0.00 [b]	1.81±0.43 [a]	0.001	0.02
ARA:EPA		0.07±0.01	0.07±0.00	0.05±0.01	0.27±0.13	na	0.005

1 The wild fish were caught by fish traps near Austevoll Aquaculture Research Centre in Western Norway
2 Labrus feed is an ongrowing diet produced for ballan wrasse by Skretting AS. Stavanger. Norway. The feed had been fed for 3 months and 4-5 months before sampling
3 Vitalis is a broodstock diet produced for marine fish by Skretting AS, Stavanger, Norway. It was blended with shrimp at the rearing facility. The feed had been fed for 4-5 months before sampling.

Vitamin A, D and K were all higher in wild fish than in cultured fish. These vitamins do not usually show a clear relationship between body level and requirement, so it is unknown if the requirements were covered in the cultured fish. Moreover, there are several different forms of each of these vitamins, and there are relatively few studies on the requirements in fish, at least for vitamin D and K. These are all reasons that the requirements for these vitamins in ballan wrasse deserve further studies.

ACKNOWLEDGEMENTS

REFERENCES

Hamre, K., Nordgeen, A.H., Grøtan, E., Breck, O. (2013). A holistic approach to development of diets for ballan wrasse (*Labrus bergylta*) – a new species in aquaculture. PeerJ 1:e99; DOI 107717/peerj.99

RECOMMENDATIONS

Based on these early results, the macronutrient composition of diets for juvenile ballan wrasse should be in the area of 65% protein, 12% lipid and 16% carbohydrate. However changing the quality of the protein and lipid sources may affect the optimum composition of the diet.

The levels of nutrients in the ongrowing diets should be adjusted so that the nutrient composition of farmed fish matches that of wild fish. This may be difficult to achieve for vitamin A, D and K and requirements may be far lower than what is indicated by the wild fish levels. Requirement studies should be performed for these vitamins.

Need for phospholipids
Description of the digestion, turnover and metabolism of lipids

Øystein Sæle, Kristin Hamre, Andreas Nordgreen, Anne-Berit Skiftesvik and Ingegjerd Opstad

INTRODUCTION

It is well established, for several fish species, that to increase the dietary phospholipid (PL) with triacylglycerol (TAG) may increase growth. This investigation was done to find the optimal PL/TAG ratio for ballan wrasse.

EXPERIMENTAL SETUP

Four experimental diets were prepared with an increasing amount of soy lecithin (0, 2%, 4% and 6% wet weight in diets 1 to 4 accordingly). To balance the fatty acid profile 6, 4, 2 and 0% wet weight soy oil were added to diets 1 to 4. This produced diets with ~17% total lipid and 29, 41, 47 and 60% of the total lipid were phospholipids (PL). Most of the lipids came from raw materials making up the rest of the diets (10% W.W.). In addition to growth measurements, the intestine was dissected out to look at genes that regulate lipid uptake, production of new lipids and transport of lipids to the body. The trial lasted for 8 weeks.

RESULTS AND DISCUSSION

There was an increase in growth when the PL fraction of the total lipids increased from 41 to 60%. The increase of PL did also increase survival, 80 ± 4, 89 ± 5 and 94 ± 6% (SEM) accordingly for the fish fed diets 29, 41 and 47% PL. The group fed the 60% PL diet however had a survival of 81 ± 11% (Figure 1).

To see if lipid class (neutral lipid v.s. PL) influenced the production of active transport into the cells in the intestine, we looked at the expression of three important genes for lipid transport. None of these were regulated by the PL content in the diets. Perilipin is a protein that "coats" the lipid droplet of the cells, thus is the gene for this protein up-regulated when lipid droplets are formed. However, the perilipin expression was the same in all diet groups, indicating no difference in lipid droplet formation.

Dietary lipids are packed in the intestinal cells into chylomicrons (or VLDLs) for transport to the body. These particles are built up of large proteins called apolipoproteins (ApoB and ApoA-IV), that together with PLs at the surface, hold neutral lipids inside the particle. It has been demonstrated in marine fish larvae that high levels of dietary PL are not only beneficial but essential. The most accepted theory is that the intestinal tissue in the larvae has a very low capacity to produce new PL. Partial digestion of PL in the intestinal lumen produces one fatty acid (FA) and one lyso-phospholipid (lysoPL).
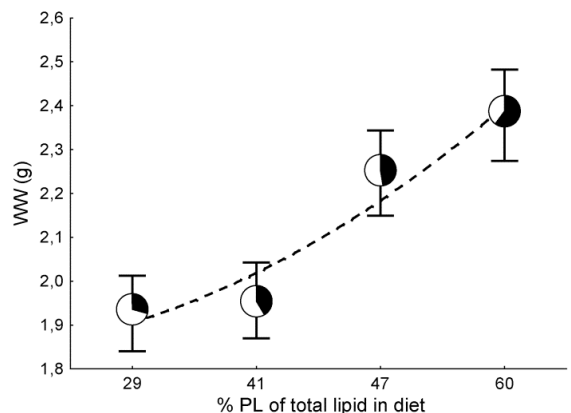
Figure 1: Growth of ballan wrasse juveniles after 8 weeks on trial diets.

When absorbed into the intestinal cells, enzymes (LPCATs) can re-attach a FA to the lysoPL to produce a PL. This way the cell does not have to produce PL from scratch, via the pathway using the enzymes AGPAT and GHPT1 (see Figure 2). When PL was increased in the diets, genes regulating new production of PL were not affected. Genes that re-model lysoPL, however, were up-regulated when more PL was introduced in the diet. This demonstrates that the intestine can increase its capacity to maintain dietary PL, but not increase the production of new PL.

Figure 2. Schematic figure of intestinal cell (enterocyte).
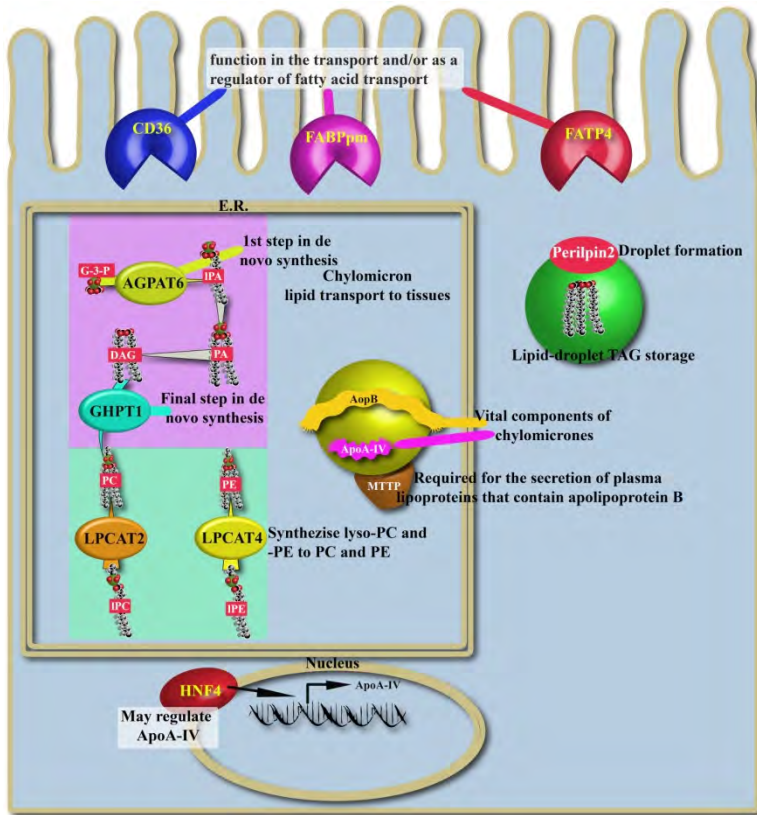
Protein	Function
CD 36	lipid transport
FABPpm	lipid transport
FATP4	lipid transport
Perilipin	Lipid droplet formation
AGPAT	PL synthesis
GHPT1	PL synthesis
LPCAT2 & 4	PL remodeling
HNF4	Induce ApoA-IV
ApoA-IV	Chylomicron
ApoB	Chylomicron
MTTP	Chylomicron transport

As for the genes involved in lipid transport, there was no dietary effect on the transport of lipid particles (MTTP). When 60 % of the dietary lipids were PL, there was a lower production of ApoA-IV. This is hard to interpret but one could speculate that PL can be transported in alternative ways to ApoA-IV particles.

Main conclusion and recommendations

The intestine of ballan wrasse cannot up-regulate the new production of PL but can take care of an increase of PL in the diet.

In a diet of 15 to 20% total lipid about 50% of this should be PLs.

Effects of transfer from continuous light to different light regimes on growth, survival, and fin and skin health

Synnøve Helland, Espen Grøtan and Chris Noble

INTRODUCTION

Different light regimes affects feeding and behaviour of fish in the grow-out phase. Long day lengths normally have large and positive effects on growth for both for marine and salmonid fish species (Boeuf and Le Bail, 1999). Observations on the catching of wrasse in the wild show that the fish is most active during dusk and dawn. Wrasse are also known for their sleeping behaviour during prolonged periods of the day. The need for regeneration of taps during dark hours has been a concern since continuous light is used in commercial farming of wrasse in Norway. Because farmed wrasse has shown low growth rates and are prone to fin damage, the potential effects of different light regimes on growth, survival, and fin and skin health were investigated.

EXPERIMENTAL SETUP

The experiment was done with farmed ballan wrasse from Marine Harvest (G2012), 523 fish (mean 8.4 g) per tank (circular, 350 L, no shelter), fed equal amounts of Otohime feed during light hours. Each tank was sheltered and separated by black tarpaulin. Four different light regimes in triplicate were tested; 24 h L (90 lux), 24 h L (264 lux), L:D 16:8 h (90 lux: 0 lux),), L:D 16:8 h (90 lux: 1 lux).

RESULTS AND DISCUSSION

A clear negative effect of transfer from continuous to discontinuous light was observed from the first weighing until the end of the experiment (Figure 1).

Specific growth rate (SGR) was calculated for each of three periods during the experiment. During the first period the SGR was approximately 50% of what was found for fish experiencing continuous light. During the second and third period, SGR was more similar for all light regimes (Figure 2).
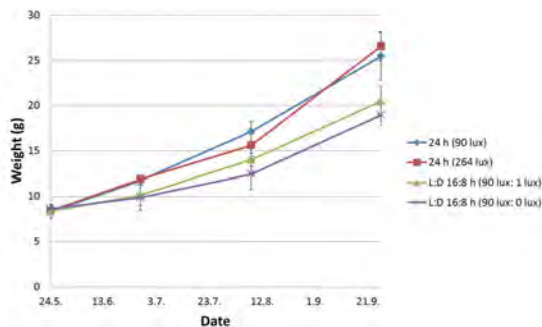

Figure 1. Weight of ballan wrasse farmed under different light regimes, average ±SD, n=3.
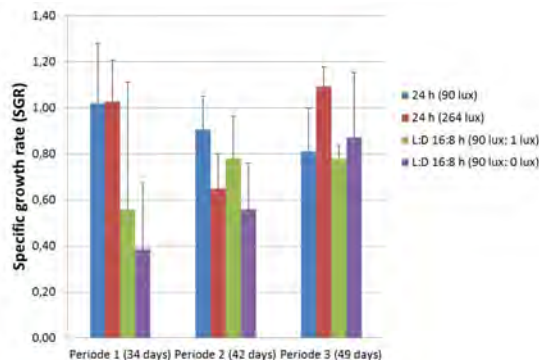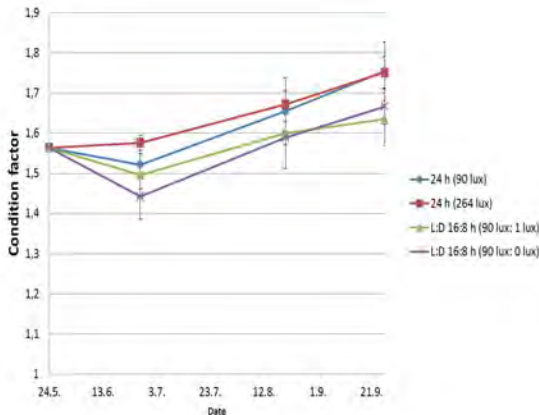

Figure 2. Specific growth rate (SGR) for ballan wrasse farmed under different light regimes, average±SD, n=3.

REFERENCES

Boeuf, G. and Bail, P.Y. 1999. 'Does light have an influence on fish growth?' Aquaculture, 177: 129-152.
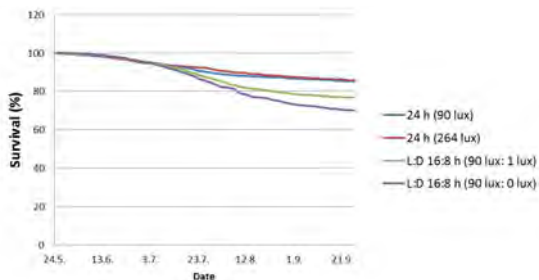
A reduced condition factor was found at the first weighing for wrasse transferred from continuous light regime (before the start of the experiment) to discontinuous light regimes (Figure 3). However, at the second and final weighing wrasse from all treatments had a condition factors similar or higher than at start of the experiment.

The numbers of split fins increased during the first period, thereafter reduced during the second period, and were almost stable during the last period, except for L:D 16:8 (90 lux:0 lux). (Figure 5). However, no significant effect of light regime on number of split fins was found.

Figure 3. Condition factor of ballan wrasse reared under different light regimes, average±SD, n=3.

Figure 5. Average number of split fins on ballan wrasse reared under different light regimes, average±SD, n=3.

The survival was similar, 96% for all light regimes until the first weighing (Figure 4). After that a lower mortality was found in the continuous than the discontinuous light regimes, with lowest survival (70%) in the L:D 16:8 h (90 lux: 1 lux) group.

The numbers of eroded fins were higher than the average number of split fins, but a similar trend was found with an initial increase and then stabilisation at a lower level (Figure 6). No effect of light regime was found except a higher degree of erosion of the dorsal fin of wrasse from the continuous light regimes.

Figure 4. Survival (%) for ballan wrasse reared under different light regimes, average, n=3.
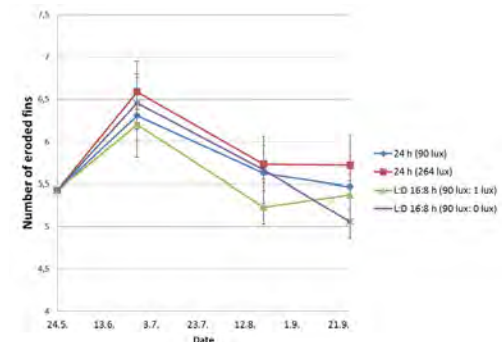
Figure 6. Average number of eroded fins on ballan wrasse reared under different light regimes, average±SD, n=3.
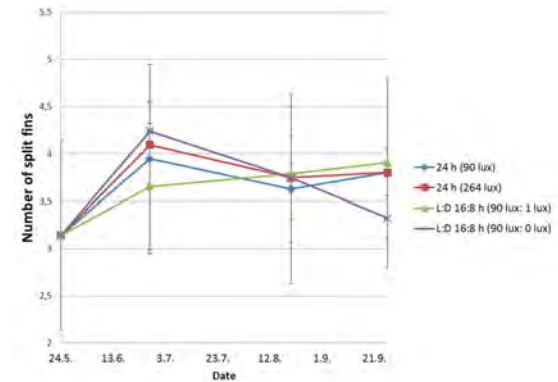
Wrasse from the two continuous light regimes positioned themselves in the upper water level of the tanks and they were orange-red in skin colour. This was in contrast to wrasse from the discontinuous light regimes that positioned themselves closer to the bottom and which had a more brownish skin colour.

The study clearly demonstrated a negative effect of transfer from a continuous light regime prior to the experiment to discontinuous light regimes in the experiment. This was shown as reduced growth, SGR, and condition factor. Negative effects on survival were first observed during the second and third periods. No clear effects of light regimes on skin and fin health were found, except for some increased erosion on the dorsal fin of wrasse from continuous light regimes.

RECOMMENDATIONS

An abrupt transfer of ballan wrasse from continuous to discontinuous light regime should be avoided. When a transfer is needed, e.g. light regime of brood fish, or transfer to salmon net pens a more gradual transfer should be tested.

Protein quality, commodity options and ballan wrasse weaning performance

Katerina Kousoulaki, Espen Grøtan, Karen Kvalheim, Øistein Høstmark, Marianne Klinge and André Bogevik

INTRODUCTION

The success of ballan wrasse larvae weaning appears to be largely dependent on specific dietary raw materials related to crustaceans, such as shrimp or krill (Kousoulaki et al., 2011; Kousoulaki et al., 2014; Bogevik et al., 2014). If the given weaning feed does not have adequate attractant properties, a significant number of the larvae population will literally starve to death. This behaviour continues also through the on-growing stages of this species. Effective weaning feeds that secure satisfactory feeding response, good growth and low levels of skeletal deformities for ballan wrasse need to be developed. During first-feeding of fish larvae, dietary fish protein hydrolysates and other marine water soluble dietary ingredients are commonly used as feeding attractants (Berge & Storebakken, 1996; Yilmaz, 2005). Within the scope of this work, a series of weaning trials were performed in collaboration between Marine Harvest Labrus and Nofima with the scope of developing appropriate weaning diets and protocols.

EXPERIMENTAL SETUP

Two experimental weaning trials of ballan wrasse larvae were performed in 100 L tanks at the research facilities of Marine Harvest Labrus at Øygarden, Norway. In the first trial, we tested the effect of water solubles of fish (stickwater) and krill origin (krill hydrolysate) in diets devoid of fish meal and with shrimp meal added as an attractant (Table 1). In the second weaning trial, we compared the weaning performance of ballan wrasse larvae fed the Nofima standard weaning diet added, or not, with whole fish meal using different protocols.

A new weaning diet, further enriched with marine hydrolysates (krill and squid), phospholipids (krill oil) and 2 functional dietary components: SP1 and Aquate (Alltech Inc; Produs Aqua AS) compared to the standard was also tested (Table 2). The mean water temperature throughout the trials was 16.5°C and the oxygen levels at 90-100 % saturation. Continuous light and feeding was practised. Automatic feeders were used giving 2 meals per minute throughout the day. Final weaning body weight and length, deformity incidences, fin damage and survival rates were studied.

Table 1. Trial 1 weaning diet formulations (Feed 1: Nofima standard weaning (W) feed; Feed 2: Nofima standard weaning feed supplemented with hydrolysates (H); trial duration 43 days).

Diet formulation*	Nofima standard W	Nofima standard W + H
Feed type	extruded	agglomerated
Cod muscle meal (%)	52.18	37.64
Shrimp meal (%)	28.50	29.0
Krill hydrolysate (%)		10.00
Stick water of fresh frozen herring (%)		12.00
Fish oil (%)	3.54	6.20
Wheat (%)	10.00	
Vitamin premix (%)	2.00	2.00
Mineral premix (%)	0.55	0.55
Soya lecithin (%)	2.00	1.00
Carop. Pink (10%) (%)	0.05	0.05
NaH_2PO_4 (MSP) (%)	1.00	1.00
stay C (%)	0.18	0.37
VitD3 (%)		0.19
Sum	100.00	100.00
Feed sizes used	0.3mm-0.6mm and 0.6mm-0.8mm successively	

Table 2. Trial 2 experimental weaning (W) diets' main ingredients (FM: fish meal) (duration 72 days).

Diet formulation*	Otohime	Nofima Std W+FM	Nofima Std W	Nofima new W
Fish meal	X	X		
Cod muscle meal		X	X	X
Shrimp meal		X	X	X
Krill meal	X			
Krill hydrolysate				
Fish hydrolysate				
Squid meal	X			X
Soy phospholipids	X	X	X	
Krill phospholipids				X
Functional components (Aquate+SP1)	?			X

Table 3. Weaning trial 2 protocols. Co-feeding of the larvae with *Artemia* and the weaning feeds lasted for 1 day. As control in both trials the fish were fed the commercial OTOHIME diet. In the experimental weaning protocol (Exp. WP) 1 the fish were fed for 6 days the Nofima Std W+FM and then changed to Otohime. In the Exp. WPs 2 and 3 the fish were fed during 3 days either the Nofima Std W or the Nofima new W diet and then changed to the Nofima Std W+FM which was given for another 3 days and then changed to Otohime. In the Exp. WPs 4 and 5 the fish were given either the Nofima new W or Nofima Std W, respectively, throughout the whole feeding experiment.

Weaning protocol	control	Exp. WP1	Exp. WP2	Exp. WP3	Exp. WP4	Exp. WP5
Live feed	*Artemia*	*Artemia*	*Artemia*	*Artemia*	*Artemia*	*Artemia*
Weaning feed (s)	Otohime B2	Nofima Std W+FM	Nofima Std W	Nofima new W	Nofima new W	Nofima Std W
		Otohime B2	Nofima Std W+FM	Nofima Std W+FM		
			Otohime B2	Otohime B2		

Results and discussion

Good growth rates (SGR~ 5) and weaning survival ranging from 31 to 62% were achieved. All fish fed the Nofima standard W diet for the whole weaning period developed head deformities but had the highest survival rates and no fin damages. The fish fed diet Nofima std. W+H containing the marine soluble ingredients for the whole weaning period, or any of the two diets for 10 days and then OTOHIME, developed significantly lower levels of deformities (Tables 4 & 5 and Figure 1).

In a number of consecutive industrial weaning trials at Marine Harvest Labrus using diets prepared by Nofima and containing higher amounts of soluble phosphorus and phospholipids than in the present weaning trial 1, when fish were given weaning diets devoid of fish meal or fish hydrolysate for periods longer than 10 days, developed head deformities at nearly 100% rate.

This result is not observed when the fish are weaned with diets that contain fish meal.

In the second experimental weaning trial presented here, fish survival was highest when ballan wrasse larvae were weaned from *Artemia* to the Nofima new W diet (up to 95%) (Table 6). As this diet contained several different ingredients than the standard Nofima std W diet, we cannot say from the results of this experiment whether the improved survival was due to higher feed intake due to the presence of higher amounts of marine hydrolysates that acted as attractants or due to the lower amounts of plant phospholipids and the higher amounts of marine phospholipids, or the presence of the functional additives. However, in this experiment too, despite good feed intake rates, severe head deformities were induced in the larvae that were fed over longer periods with the diets that did not contain fishmeal.

Nonetheless, the transition from a non-fishmeal diet to a fishmeal diet resulted in lower feeding responses and higher mortality rates.

Recent, still unpublished work (Bogevik et al., 2014; Kousoulaki et al., 2014), provide possible explanations why ballan wrasse show lower feed intake rates but good growth when fed fishmeal rich diets. The authors focus mainly on the attractant properties of the free amino acids of the water soluble fraction of crustacean raw materials and also the potential feeding repellent effect of lipid oxidation metabolites or chemical antioxidants in commercial fishmeals. The stickwater fraction of herring fish meal has been previously shown to have growth promoting effects in on-grown Atlantic salmon fed low fish meal diets (Kousoulaki et al., 2009). The present results, where we saw that ballan wrasse larvae fed diets without whole fishmeal or stickwater develop severe head deformities, reveal the even more crucial role that fishmeal and its water soluble fraction play in early fish development. However, possible mechanisms behind this effect are not described since larval diets traditionally contain high levels of fish meal.
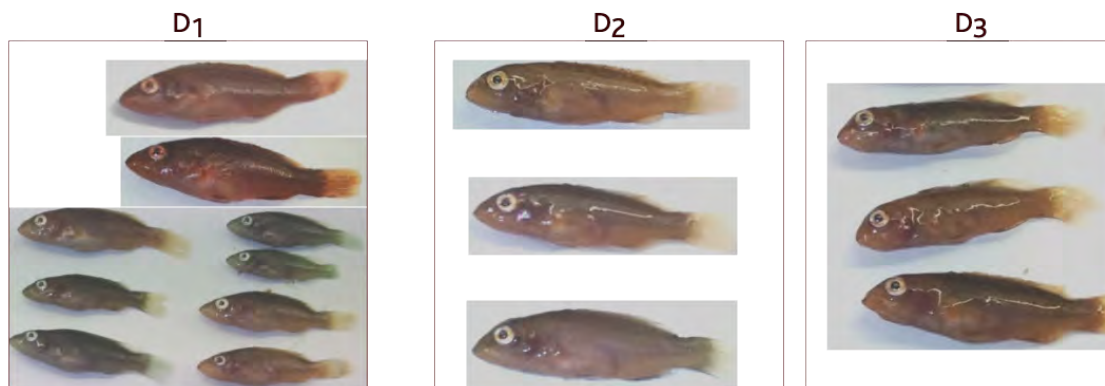
Figure 1. Ballan wrasse larvae with no (D1), mild (D2) or severe (D3) head deformities. Photo: Katerina Kousoulaki, Nofima

Table 4. Weaning trial 1: Ballan wrasse weaning performance. Values are mean ± standard deviation (n=3).

Weaning protocol	Nofima std W +H 43 days	Nofima std W 43 days	Nofima std W +H 10	Nofima std W 10	One-way ANOVA (P)
Start fish number/tank	300	300	300	300	300
Survival (%)	35±19.6	62±9.2	31±21.4	46±1.9	ns*
Start weight (g)	0.11	0.11	0.11	0.11	
Final weight (g)	0.89[a]±0.04	0.97[ab]±0.03	1.05[b]±0.04	0.98[ab]±0.01	0.046
SGR	4.9[a] ±0.10	5.1[ab]±0.06	5.2[b]±0.08	5.1[ab]±0.01	0.006
Final standard length (cm)	3.37±0.10	3.46±0.08	3.49±0.01	3.57±0.08	ns
% Total head deformities (D)	22a±11.5	100[b]±0.0	5[a]±1.4	8[a]±5.0	0.000
% Severe head deformities (D3)	9[a]±3.3	95b±2.4	2a±3.0	4[a]±1.2	0.000
% Mild head deformities (D2)	13±8.8	5±2.4	3±1.6	4±3.8	ns
% No head deformities (D1)	78[b]±11.5	0[a]±0.0	95[b]±1.4	92[b]±5.0	0.000
% Good fish (survival*D1)	29±19.5	0±0.0	29±20.7	42±4.0	ns
% Fish of acceptable quality (suvival*(D1+D2))	32±19.3	3±1.2	31±21.9	44±2.4	ns
*ns: non-significant. Means with different superscript letter are significantly different by Duncan test (P<0.05).					

Table 5. Weaning trial 1: fin damage observations

Nofima std W+H 43 days	Damaged pectoral fins but not destroyed, some damaged caudal fins
Nofima std W 43 days	No pectoral fin damages, some damaged caudal fins
Nofima std W+H 10 days + OTOHIME 33 days	No pectoral fin damages, some damaged caudal fins
Nofima std W 10 days + OTOHIME 33 days	Damaged pectoral fins but not destroyed

Table 6. Weaning trial 2: Ballan wrasse weaning performance (n= 1 or 2).

	control	Exp. FR 1	Exp. FR 2	Exp. FR 3	Exp. FR 4	Exp. FR 5
	Otohime B2	Nofima std W+FM --> Otohime B2	Nofima std.W-> Nofima std. W+FM -> Otohime B2	Nofima new W->Nofima std.W+FM -> Otohime B2	Nofima new W	Nofima std. W
Start fish number/tank	112	112	112	112	112	112
Start weight (g)	0.21	0.21	0.21	0.21	0.21	0.21
Final weight (g)	3.7	2.92	2.47	2.51	2.64	2.68
SGR	4.07	3.72	3.49	3.51	3.58	3.6
Survival (%)	8.93	32.6	58.9	72.8	94.6	83.9
Losers (%)	0.9	4.0	12.1	14.3	0.0	0.0
Head deformities	low	low	low	low	severe	severe

RECOMMENDATIONS

In conclusion, it seems that, in the weaning phase, ballan wrasse larvae will have a significantly higher initial feeding response when the formulated diet contains shrimp meal and no fishmeal, irrespective of the nutritional quality of the feed. It appears moreover that, in the first days of weaning, shrimp meal cannot be substituted by krill meal without a significant loss of feeding response and a consequent loss of fish. Nevertheless, in the absence of fish meal, or whole fishmeal hydrolysate, ballan wrasse larvae may wean to formulated diets with high survival rates and good growth but develop severe head deformities. This fact presents both a welfare and a quality issue, rendering the fish of suboptimal quality in terms of their potential for cleaning salmon of lice in the cages. Start feeding with weaning diets without fishmeal, shortly afterwards changed to a fishmeal, or fishmeal stickwater-rich diet, containing either krill meal or shrimp meal, can safeguard good larval quality, growth and survival rates.

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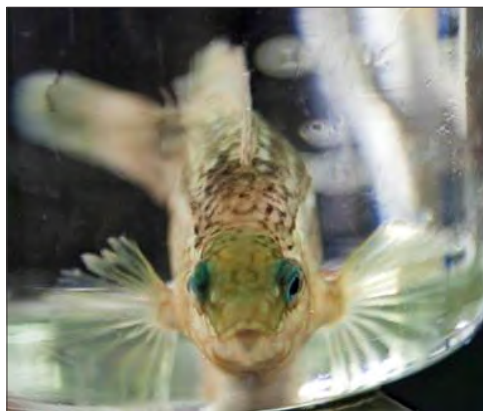
Figure 2. Ballan wrasse juvenile. Photo: Tor Nielsen, SINTEF

Protein quality and feed technical quality effects on ballan wrasse on-growing performance

Katerina Kousoulaki, Espen Grøtan, Reidun Bjelland, Karen Kvalheim, André Bogevik and Anne Berit Skiftesvik

INTRODUCTION

Ballan wrasse juveniles have poor performance during on-growing when given extruded diets, even in the presence of dietary krill or shrimp. The nutritional requirements of this species remain largely unknown. Moreover, this species is stomach-less and has only a short intestine, possibly unable to optimally digest conventional extruded feeds (Figure 1). In order to test this hypothesis, one on-growing trial was performed testing extruded and non-extruded diets, water-stable and water-unstable extruded pellets, containing variable amounts of water soluble compounds.

Figure 1. Ballan wrasse and its gastrointestinal tract. Photo: IMR

EXPERIMENTAL SETUP

Four experimental ballan wrasse on-growing diets were tested in an on-growing trial performed during autumn 2013, in 500l tanks at IMR research station in Austevoll, Norway. The scope of this trial was to benchmark the performance of diets of different technical quality, and different high quality marine ingredients content (Table 1). The experimental tanks were equipped with a cleaning arm and automatic feeding systems which were filled daily. The water salinity throughout the experiment was 35.2 ppt and the temperature 15.5°C. Juveniles had been fed with Othohime prior to the start of the experiment. At the start, 250 juveniles of an age of 179 days post hatching, with an average size of 5 grams were placed in each experimental tank.
The feeding trial lasted for 64 days.

The overall hypothesis was that ballan wrasse on-growing performance is affected by:

1. The feeds' technical quality (hardness and water stability of pellets)

   a) Moist vs dry feeds

   b) Warm extruded vs cold extruded

2. The feeds' content in attractants and readily digestible components (level and source of water soluble proteins)

   a) Krill meal vs. krill hydrolysate

   b) Fish meal vs. fish hydrolysate

Table 1. Feed type and marine protein ingredient content of grower experimental ballan wrasse diets and feeding methods. Moist feeds meant that 15% water was added to the dry feeds before feeding.

Diet formulation*	OTOHIME	Nofima Std G*	Skretting new	Nofima new G
Feed type	Cold extruded	Warm extruded	Warm extruded	Cold extruded
Feeding method	Dry	Moist	Moist	Moist => Dry
Fish meal	x	x	x	x
Shrimp meal		x		
Krill meal	x			x
Krill hydrolysate			x	x
Fish hydrolysate				x
Squid meal	x			x

*Produced by Skretting.

Results and discussion

The on-growing trial results showed good growth performance and stable condition factor of ballan wrasse using the four experimental diets. Growth rates for the four diets used in decreasing order were Otohime > Standard Nofima ≥ Skretting new ≥ Nofima new G (Table 2). The commercial diet Otohime was, as also previously observed (MHL industrial trials) the best performing feed. However, this type of Otohime comes only in small sizes suitable for weaning and small fry, representing thus no real solution for the on-growing stages of farmed ballan wrasse. The fish fed the extruded diets containing fishmeal and either krill hydrolysate (Skretting new) or shrimp meal (Nofima standard G) had better performance compared to the fish fed the Nofima new G diet. The Nofima new G diet, which was cold extruded with low hardness and water stability and contained the highest amounts of marine water soluble compounds, induced highest feeding response but the fish in this treatment grew less and unevenly. All experimental fish showed mild or worse caudal and pectoral fin damages irrespective of dietary treatment. In general, fish appetite was strongest when the feeds were added water prior to feeding, except for the Otohime feed which was given dry. The diet Nofima new G was given wet for half of the trial period and then switched to dry, when we observed a dramatic reduction in feeding response and increasing mortality rate probably due to starvation (Figure 2).
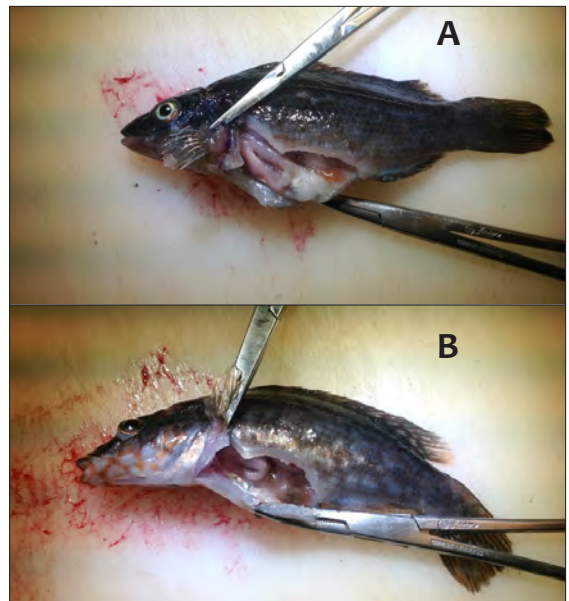
Figure 2. Well fed (A) and starved (B) ballan wrasse juveniles. Photo IMR

Table 2. Ballan wrasse on-growing performance fed diets with different marine ingredients and/or technical qualities. Values are mean ± standard deviation (n=3). Age of juvenile at start-173 dph, intermediate-209 dph and final-237 dph.

FEED	OTOHIME	Nofima Std G*	Skretting new	Nofima new G	ONE-WAY ANOVA (P)
Feed type	Cold extruded	Warm extruded	Warm extruded	Cold extruded	
Feeding method	Dry	Moist	Moist	Moist => Dry	
Start weight (g)	5.2±1.32				
Start standard length (cm)	7.1±0.69				
Condition factor start	1.4±0.48				
Intermediate weight (g)	9.0[a]±0.3	7.7[b]±0.6	7.7[b]±0.4	6.6[c]±0.2	0.001
Final weight (g)	13.8[a]±0.50	10.7[b]±1.05	10.1[bc]±0.45	8.4[c]±1.62	0.001
Final standard length (cm)	9.7[a]±0.14	9.0[b]±0.29	8.9[b]±0.09	8.4[c]±0.34	0.001
Mortality (%)	0.3[a]±0.2	0.9[a]±0.6	1.9[a]±0.8	19.3[b]±11.1	0.008
Condition factor end	1.48±0.02	1.40±0.03	1.40±0.02	1.33±0.14	ns
SGR total	1.69[a]±0.06	1.24[b]±0.17	1.15[bc]±0.08	0.81[c]±0.32	0.003
Pectoral fin damage score[1]	1.85±0.09	1.81±0.18	1.79±0.15	1.87±0.04	ns
Caudal fin damage score[1]	1.57[ab]±0.06	1.45[a]±0.05	1.43[a]±0.01	1.63[b]±0.13	0.038

[1]Subjective scoring of scale from 1 to 3, 1 being no or little damage, to 3 being severe damage of fins. Means with different superscript letter are significantly different by Duncan test (P<0.05).

RECOMMENDATIONS

Ballan wrasse performance in the on-growing phase is affected by protein, raw material quality as well as the feeds' technical properties. Dietary krill meal, krill hydrolysate and shrimp meal efficiently stimulate feeding response in ballan wrasse juveniles during the on-growing phase. High levels of dietary water soluble components in moist feeds may give higher feeding response, but do not necessarily result in better growth rate. Cold extrusion may give better ballan wrasse on-growing results but there is need for further relevant documentation studies. To date, the on-growing performance of ballan wrasse given the available commercial diets is not yet satisfactory. There is a need to develop nutritionally complete diets of appropriate technical quality that suit the stomach-less, short and of basic pH digestive system of ballan wrasse.

ACKNOWLEDGEMENTS

Anti-fouling surface materials for ballan wrasse shelters

Stine Wiborg Dahle, Andreas Hagemann and Per Stenstad

INTRODUCTION

The ballan wrasse is a coastal species, usually found amongst cliffs, rocks and kelp. During the night, when threatened or stressed, it resides in natural crevices for protection. In commercial hatcheries, deviant behaviour in terms of clumping is commonly observed from 40-50 dph when hides are absent (Grøtan, E., Marine Harvest, pers. comm.). Stress is a major contributing factor to fish disease and mortality (Barton, 2002). Various types of shelters have been implemented in hatcheries without any notable effect on stress reduction (Grøtan, E., Marine Harvest; Overrein, I., former Nordland Leppefisk, pers. comm.). Tested designs include artificial kelp forest, shreds of tarpaulin, branched pines (Figure 1) and PVC tubes of different shapes and sizes. Moreover, implementing hides in production tanks can impair water circulation and increase fouling and bacterial load. The industry requests shelters of a functional design and anti-fouling surfaces that can ensure good fish welfare by providing a less stressful tank environment.

Figure 1. Ballan wrasse juveniles seeking shelter. Photo: Thor Nielsen.

Anti-fouling is the ability of specific coatings to prevent buildup and deposition of marine organisms and bacterial biofilm formation on surfaces. In the present study, the anti-fouling surfaces were developed by SINTEF Materials and Chemistry (not yet commercially available), and applied to ballan wrasse shelters to study the effects on fouling and biofilm formation. The main focus was to produce non-toxic and environmentally-friendly surfaces. This was achieved by using highly hydrophilic coatings that prevent the attachment of microorganisms. An antimicrobial compound is chemically immobilised to the surface, which may give a long-lasting antimicrobial surface, with no leakage of the antimicrobial compounds to the surroundings.

EXPERIMENTAL SETUP

Three different anti-fouling surfaces were developed:
1. SB7: 2-hydroxyethyl methacrylate (HEMA) grafted on polyethylene (PE).
2. SB9: HEMA and an antimicrobial compound (quaternary amine) chemically bound to PE.
3. SB13: A HEMA / polyester (50/50) composite with fibreglass as reinforcement
4. Control/PE: PE without anti-fouling surface

The anti-fouling surfaces were exposed in three different environments (Figure 2), with three replicates and untreated PE as a control. The anti-fouling effects were assessed visually and the sampling of biofilm was conducted by swabbing with Transwab® and plating on agar for recordings of opportunistic bacteria.

Brood Stock (Nofima)
8 weeks exposure

Start feeding (MH Labrus)
2 weeks and 2 months exposure

**Live feed production (SINTEF)**
6 days exposure

Figure 2. Anti-fouling surfaces exposed in: brood stock, start feeding and live feed production tanks (copepod production). Photos from left to right: Helge Ressem, Thor Nielsen, Andreas Hagemann.

Results and discussion

The SB13 material showed reduced fouling in live feed production tanks (Figure 3) and a significant lower numbers of opportunistic *Vibrio* sp. bacteria in start feeding tanks (Figure 4), compared to the other materials. *Vibrio* sp. can be pathogenic to ballan wrasse, e.g. *Vibrio logei* and *Vibrio splendidus* (Johansen, 2013), and should hence be minimised. However, the material curled up in contact with seawater (Figure 3), and was therefore improved by using double layers of fibreglass reinforcement. The second trial with the improved SB13 material was conducted in broodstock tanks. Due to the low organic loading in tanks, there was no bio-fouling on the materials after 8 weeks exposure.

SB7　　SB9　　SB13　　Control/PE
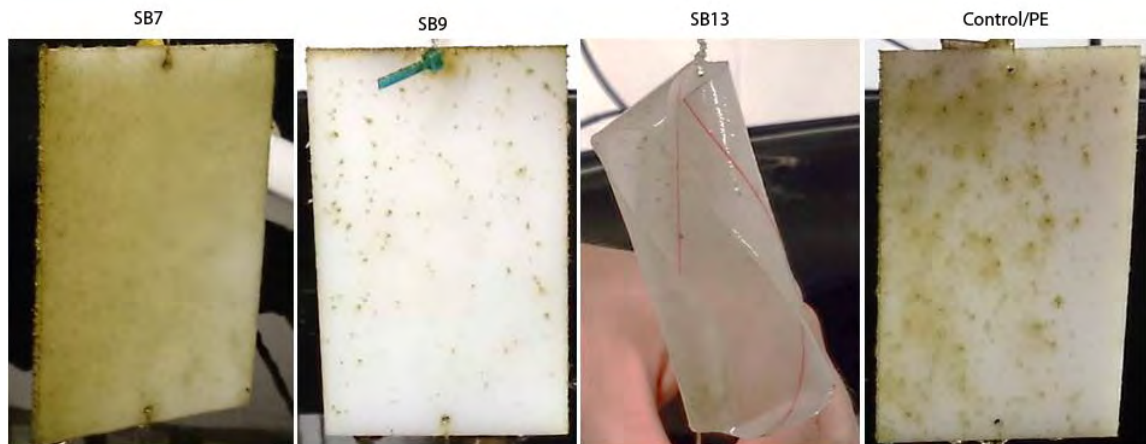
Figure 3. Fouling after six days exposure in live feed production tanks at SINTEF (high organic load), displaying less fouling on SB13 compared to control/PE (only PE). Photos: SINTEF.
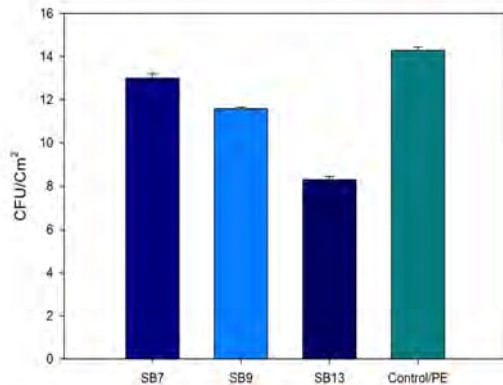
Figure 4. Opportunistic *Vibrio* sp. bacteria (CFU/cm2) from anti-fouling materials and control/PE (only PE), in start feeding tanks at Marine Harvest Labrus after two weeks exposure. Data: Mean ± SE. CFU= Colony forming units.

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RECOMMENDATIONS

It is highly recommended to use shelters from 40-50 dph in ballan wrasse production tanks to ensure good fish welfare by reducing levels of environmental stress. To reduce fouling and biofilm formation, a non-toxic anti-fouling surface can be applied to the shelters. So far, the HEMA/polyester 50/50 polymerised on fibreglass shows the most promising anti-fouling capacity. This can be further improved by increasing the amount of HEMA, and the introduction of quaternary amines on the surface may prevent bacterial growth. This surface should last for several years without a reduction in anti-fouling effectiveness. Polyester can be prepared in different shapes and thereby manipulate and optimise the shelter design. Reduced fouling and biofilm formation will ensure a reduction of microbes hence improve water quality, reducing the manual labour spent on maintaining clean shelters. Designing optimal shelters should be a future priority to ensure good fish welfare by reducing environmental stressors causing diseases and deviant behaviour.

Chapter 6
Delousing of Atlantic salmon
with two different sizes of farmed ballan wrasse

Ingrid Lein, Chris Noble, Sissel Nergaard and Synnøve Helland

Introduction

Until now, mainly wild wrasse have been used as cleaner fish in Norwegian salmon farming. Ballan wrasse farming is a new production activity and it has been questioned whether farmed wrasse, previously fed dry feeds, will graze on salmon lice as efficiently as wild-caught wrasse. There has also been some uncertainty about what is the optimal size relationship between wrasse and salmon. Wild wrasse varies considerably in size while farmed wrasse can be sorted for less size variation before transfer to salmon cages. The present study had two main objectives; to document the efficiency of farmed wrasse as lice eaters in cages with large salmon (>2 kg), and to test the effect of wrasse size on their grazing efficiency in cages with large salmon.

Experimental setup

The experiment was done in a total of 12 research cages (5x5x5 m, 125 m^3), four replicates per treatment at the Nofima research station at Ekkilsøy. The experiment was planned to start in June 2012, but was delayed until late September due to insufficient quantities of sexually mature salmon lice available for the collection of egg strings used for hatching of lice nauplii.

Treatments

- Control, salmon only, no addition of wrasse

- Large salmon + small wrasse (45-55 g, 5% of salmon number)

- Large salmon + larger wrasse (70-80 g, 5% of salmon number)

The ratio of wrasse/salmon is within the range used in commercial salmon farming.

Salmon lice

Egg strings of sexually mature lice (*L. salmonis*) were harvested from lice found on salmon at the research station at Ekkilsøy September 26th. The lice eggs were incubated in small containers (1 L) at 14.5°C and the eggs hatched immediately after incubation. After hatching the salmon lice larvae were held in small tanks (25 L) at 13°C until they reached the copepod stage.

Atlantic salmon

1500 Atlantic salmon with minimum body weight of 2060 grams were transferred from two production cages to a common cage September 19. The salmon were fed a commercial Skretting growth feed.

The salmon were infected with sea lice copepods on October 5. The salmon were starved before and during the infection with salmon lice. During the infection procedure, a dense tarpaulin covering the outside of the cage was used to prevent spreading of salmon lice copepods to the wild. The salmon were infected with 50 copepods per salmon, a total of 7,500 copepods, using an exposure time of 3 hours. During this 3 hour period O_2 was added. After 3 hours, the water inside the cage was pumped through a filter to prevent free-swimming lice to be released to the wild.

Ballan wrasse

The wrasse used in the experiment was produced at Profunda (G 2010), and was transported to Nofima´s research station at Sunndalsøra in spring 2012. This was the first batch of wrasse produced at Profunda, and there was a high prevalence of some deformities, especially spinal fusions. Wrasse with no visible deformities were selected for the cage experiment with salmon at Ekkilsøy. The wrasse were transported to Ekkilsøy October 25 and kept in two separate cages until the start of the experiment with salmon on November 2. One cage held wrasse of 40-50 gram size and the other wrasse of 70-80 gram size. Both cages were equipped with plastic shelters (OK Marine).

Experiment with wrasse and salmon

Because ballan wrasse does not usually graze on younger stages of salmon lice, the experiment was started when the salmon lice reached sexual maturity.

On November 2, the wrasse were distributed to 8 of the 12 experimental cages (Figure 1); 4 cages with wrasse of 40-50 grams size, 4 cages with wrasse of 70-80 grams size, and four cages with no wrasse (control). Five wrasse were distributed to each cage, i.e. 5% of the number of salmon. Thereafter, the salmon previously infected with salmon lice on October 5 were gently netted from the common cage, anaesthetised (Finquil), and distributed to the 12 experimental cages (100 salmon per cage). The density of salmon is in the lower range for commercial farming, but in the upper range for small experimental cages.

Figure 1. Image of the 12 experimental cages (5x5x5 m) taken during the final sampling. Photo: Nofima

After distribution of the salmon, 100 of the remaining salmon in the common cage were anaesthetised and weighed. Lice were counted and classified into three life stages: chalimus, pre-adult and adult. These recordings were used as start-up values. Weights and number of lice per salmon were not recorded on fish transferred to the experimental cages because, based on previous experience, the handling usually causes losses of attached lice.

At the start of the experiment, welfare indicators were scored for all the wrasse plus the 100 extra salmon that were not used in the experimental cages (Figure 2). Scores were given for all fins - for fin erosion, fin split (number of spits between fin rays), and presence of bleeding in fin tissue. In addition, other exterior injuries such as eye damage (cataracts, bulging eyes, etc.), damage to the mouth, jaw, snout, and damage to or deformities of the opercula, and eventual skin lesions were evaluated. An external evaluation of skeletal deformities was made for both species.

On November 6 and 20, eleven and twenty salmon, respectively, were anaesthetised and the number of lice per individual was counted. The experiment was ended November 29-30. Weight, length and numbers of lice per fish were recorded for all salmon. Welfare scores were given for 25 salmon per cage. All wrasse were weighed, autopsied for stomach content and given welfare scores.

Figure 2. Welfare indicator evaluations of wrasse (left) and salmon (right). Photo: Nofima

Results and discussion

There was no difference between treatments in terms of the number of lice recorded on the salmon, i.e. no effect of adding wrasse to the salmon cages. At the first counting (November 6) the total number of lice per fish was 15.5±3.5 in the control group, 18.8±3.9 in the group with large wrasse, and 15.5±5.0 in the group with small wrasse.

The lice counts recorded November 20 and at termination of the experiment are shown in Tables 1 and 2.

Figure 3. Counting of sea lice on Atlantic salmon during the final sampling (November 29-30). Photo: Nofima

Table 1. Mean numbers of lice per Atlantic salmon in cages with small, large and no ballan wrasse, counted November 20 (mean ±SD).

	Control	Large wrasse 70-80 g	Small wrasse 40-50 g
Chalimus	0.1 ± 0.1	0.0 ± 0.1	0.0 ± 0.2
Pre-adult lice	6.8 ± 3.3	7.7 ± 3.8	7.7 ± 3.8
Adult lice	10.8 ± 5.5	12.6 ± 5.0	11.8 ± 4.6
Total number of lice	17.7	20.4	19.6

Table 2. Mean numbers of lice per Atlantic salmon in cages with small, large and no ballan wrasse, counted November 29-30 (mean ±SD).

	Control	Large wrasse 70-80 g	Small wrasse 40-50 g
Chalimus	0.6 ± 1.0	0.4 ± 0.8	0.3 ± 0.6
Pre-adult lice	6.1 ± 2.9	6.1 ± 3.1	6.1 ± 3.1
Adult lice	10.5 ± 5.1	11.6 ± 4.9	11.5 ± 4.9
Total number of lice	17.2	18.1	17.9

Since the numbers of salmon lice were far above the intervention level (governmental directions) in all treatments during the sampling November 20, it was decided to end the experiment quickly, and this was done 29-30 of November. The mortality of salmon was low during the experimental period; 5 dead salmon out of a total of 1,200 fish, i.e. 0.4% mortality. No ballan wrasse died during the experimental period. During the final sampling, all 60 wrasse used in the salmon cages were autopsied, but no salmon lice were observed in any of the intestines. In 3-4 wrasses some fish scales were found. Despite the fact that the wrasse looked healthy on the exterior, the intestines indicated that the wrasse had not been feeding for a while, i.e. the intestines were very thin (Figure 4).
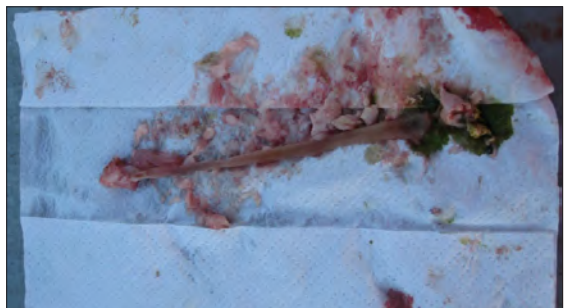
Figure 4. Autopsy of a typical ballan wrasse intestine at the end of the experiment. There was no feed in the intestine, and the gall bladder was full. Photo: Nofima

The growth of the salmon during the experimental period was calculated using the mean weight of the 100 extra fish not used in the experimental cages as initial weights. At end of the experimental period, all salmon in the 12 cages were weighed. The weights, specific growth rate and thermal growth coefficient are shown in Table 3. The growth of the salmon was relatively low, but the fish were in good health with no external damage. The relatively low growth can be explained by the large salmon being handled and moved from larger cages (7x7 m) to smaller cages (5x5 m). The salmon were held for less than one month in the small experimental cages. From experience, we know that large salmon needs time to adapt to smaller units before they start to grow well. However, fast growth of the salmon was not a critical factor for this experiment.

Table 3. Mean weights ±SD of Atlantic salmon at start and at the end of the experiment.

|	Control	Large wrasse 70-80 g	Small wrasse 40-50 g
Start weight of salmon (g)	2534 ± 283	2534 ± 283	2534 ± 283
Final weight of salmon (g)	2762 ± 369	2755 ± 409	2808 ± 391
Specific growth rate (SGR)	0.32	0.31	0.38
Thermal growth coefficient (TGC)	1.43	1.39	1.70

The temperature was 11.7°C when the salmon were infested with lice on October 5, and remained above 10°C until a week before trial started on 2 November.
During this week, a storm came in from the north, and the temperature dropped almost two degrees to 8.9°C. From the experiment started to the final sampling on November 29 the temperature gradually fell further to 7.2°C (Figure 5).

There were few external damages or injuries observed, both in salmon and wrasse, at the start and end of the experiment. Figure 6 shows an example of the observations in small and large wrasse at the end of the experiment while Figure 7 shows an example of the same type of injury (split fin) in salmon. The results show that split fins were observed among a relatively large proportion of the wrasse, but the number of split fins per fish was low (Figure 6, right). The same pattern was observed for fin erosion and bleeding in fin tissue. In total, the observed damages were not very serious, and only a few wrasse got notifications for jaw, mouth, snout, eyes, or skin damages.
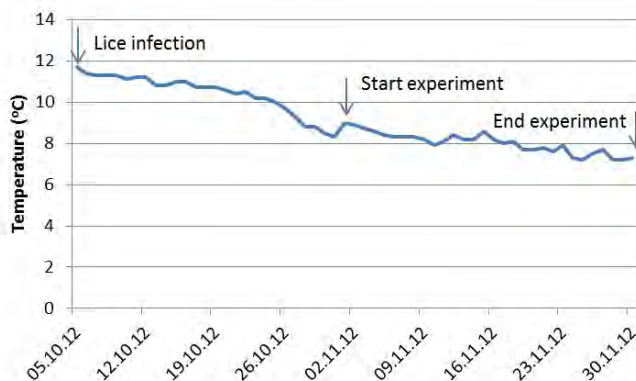
Figure 5. Sea water temperature during the period from October 5th when the salmon was infected with salmon lice until termination of the experiment November29th.
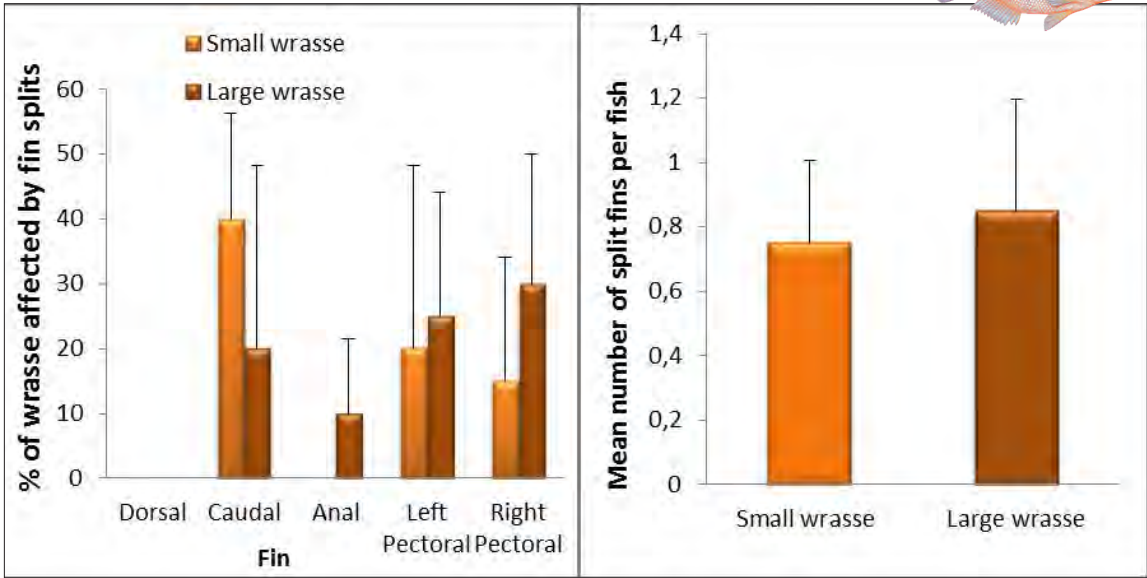
Figure 6. Example of welfare indicators of ballan wrasse showing proportion of fish with split fins (left) and average number of split fins per fish (right) at the end of the experiment.
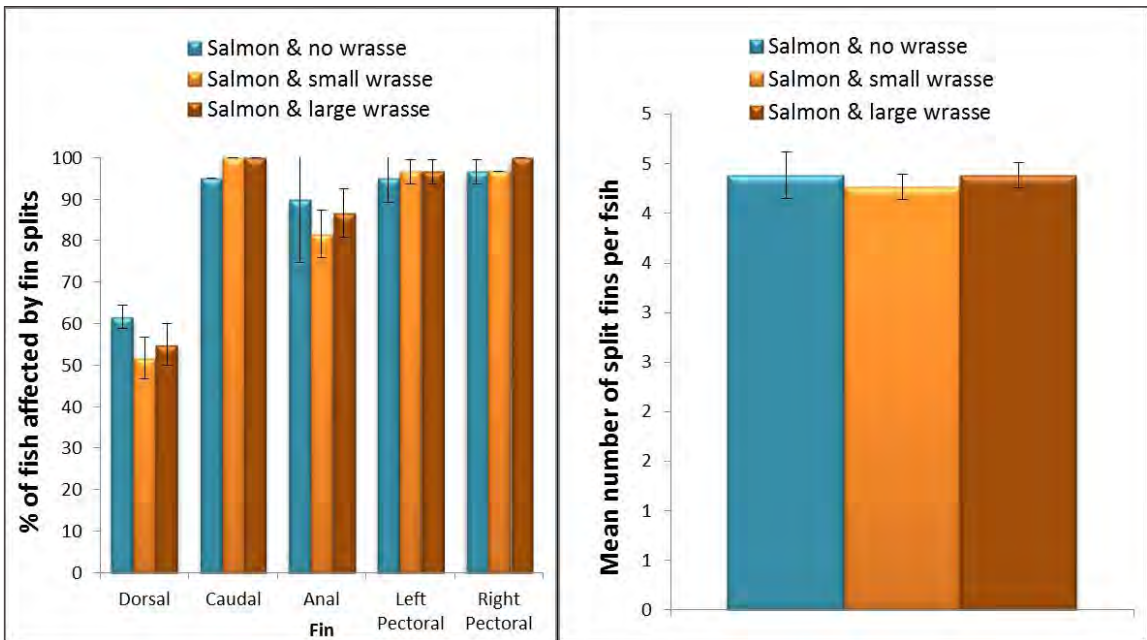
Figure 7. Example of welfare indicators of Atlantic salmon showing proportion of fish with split fins (left) and average number of split fins per fish (right) at the end of the experiment.

Fouling of the cage nets can influence foraging activity of wrasse, but there was no evidence of fouling on the nets during the trial period (Figure 8).

In the present experiment, no lice were observed in the wrasse intestines. These results contradict the findings of Skiftesvik et al. (2013) who, in a parallel experiment with smolts and farmed or wild wrasse, found up to 78 lice in single wrasse intestines. The majority of the inspected wrasse however had less than 10 lice in their intestine (see also "Gut content of ballan wrasse).

In the present experiment both the salmon and the wrasse were of apparently good health. The most likely explanation for why the wrasse did not graze on salmon lice is the low temperature (mean 7.7°C), and particularly the drop in temperature from 11.7 to 7.1°C.

Figure 8. Image taken at termination of the experiment showing no fouling of the cage net. Photo: Nofima

Observations made by salmon farmers using wrasse in cages show that wrasse become passive and have a low appetite at these temperatures. Skiftesvik et al. (2013) conducted their experiment with smolts and wrasse during August-September with a mean temperature of 12.2°C. At this temperature the ballan wrasse is known to be active. In the present study, some swimming activity was observed among the wrasse in the salmon cages but no grazing activity was observed. Prior to the experiment it was questioned whether the large salmon (>2 kg) would act aggressively towards the relatively small wrasse (50-80 g) but there were no signs of salmon attacking the wrasse. On the other hand Skiftesvik et al (2013) found some damage to the smolt in their study, indicating that the size relationship between salmon and wrasse can be of importance. Thus, there is still a need for more knowledge about what is the optimal size relationship between ballan wrasse and Atlantic salmon <2 kg.

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Recommendations

Based on the present experiment we cannot make a recommendation on the optimal size of ballan wrasse as a cleaner fish for large salmon (<2 kg).

The results also showed that there is a need for more knowledge about temperature ranges for optimal salmon lice grazing behaviour in ballan wrasse.

Delousing of Atlantic salmon (*Salmo salar*) by cultured vs. wild ballan wrasse (*Labrus bergylta*)

Anne Berit Skiftesvik, Reidun M. Bjelland,
Caroline M.F. Durif, Inger S. Johansen and Howard I. Browman

INTRODUCTION

The objectives of this study were to assess the prevalence of salmon lice on Atlantic salmon in sea cages, in the presence or absence of wrasse, thereby testing their efficiency as delousing agents, and the relative efficiency of wild-caught vs. cultured ballan wrasse.

EXPERIMENTAL SETUP

The wrasse were obtained from three sources: 1. Marine Harvest (MH) (ballan wrasse), 2. Institute of Marine Research (IMR) (ballan wrasse), 3. Wild-caught (ballan and corkwing wrasse) obtained from a local fisher. The cleaner fish (25 fish per sea cage) were set out on 30.08.2012 and the salmon (500 fish per sea cage, mean weight 429 ± 115 g) one week later. This is about the ratio of wrasse/salmon that is currently in use on commercial salmon farms in Norway. There were four replicates and five treatments: control (no wrasse), IMR ballan wrasse, MH ballan wrasse, wild ballan wrasse, and a mix of wild corkwing + IMR ballan wrasse (corkwing was used in this treatment because of an insufficient number of ballan wrasse). Treatments were randomly distributed over the floating experimental sea cage facility. Artificial shelters – dark plastic "kelp" - were placed in the nets as shelter for the wrasse. Control treatments had no wrasse. All other treatments had 25 wrasse each. Every week, ten salmon were randomly collected from each of the sea cages and anaesthetised (MS-222). Lice were counted and classified into three life history stages: chalimus, pre-adult and adult. The general status of these fish was visually assessed and classified into four categories 1- no damage; 2- skin damage most likely due to handling, 3- fin and gill bites by the wrasse, 4- both types of damage (skin damage and bites). After lice counting, the fish were released back into the sea cage from which they had been collected. At the end of the experiment (week 6) length and weight were measured on ten salmon per sea cage. To minimise the load of fouling organisms colonising the sea cage nets (and available as food for the wrasse), the nets were changed mid-way through the experiment.

RESULTS AND DISCUSSION

The number of chalimus-stage lice increased significantly throughout the experiment and reached a maximum after 6 weeks (Figure 1A). There was no significant difference in the number of chalimus across treatments. The number of pre-adult and adult lice increased every week in the control treatment (Figures 1B and 1C). Differences between control vs. cleaner fish treatments were significant after 1 week as pre-adult and adult lice remained low and stable in the sea cages with cleaner fish. There were no significant differences in the number of lice (whatever their stage) between the different cleaner fish treatments: HI, MH, wild or mix. Overall, louse prevalence (pre-adult and adult stages) decreased from 9 lice on average per fish in the controls to less than 1 in the sea cages with wrasse. In total, for all treatments and replicates, wrasse consumed approximately 4,000 lice in seven days. This represents a minimum consumption rate of 23 lice per wrasse per day.

Ballan wrasse from all treatments had significantly lower condition factor after 6 weeks. Maximum body mass reduction occurred in the MH wrasse (6%), while IMR and wild wrasse decreased by respectively 3.6 and 2.5% (Figure 2). Corkwing wrasse (only present in the mixed treatment) also decreased in condition factor; from 1.45 to 1.36. The weight of salmon increased from 413 g to 672 g on average at the end of the experiment. Final weight was not significantly different between treatments. The condition factor of salmon was not significantly different between treatments.
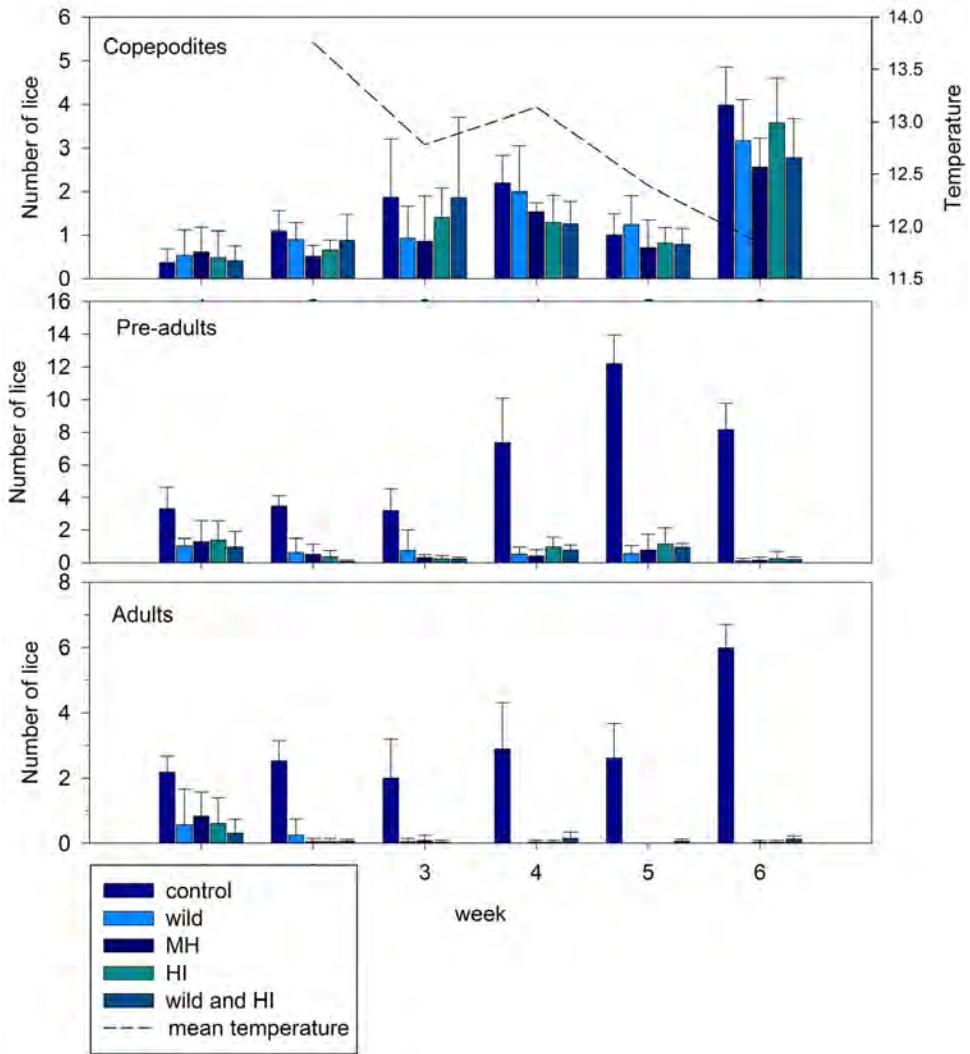
Figure 1. Number of sea lice (*Lepeophtheirus salmonis*) per salmon (*Salmo salar*) counted once a week for each treatment. Each bar represents the mean + sd. The bar at week 0 represents louse counts before the start of the experiment.

Ballan wrasse
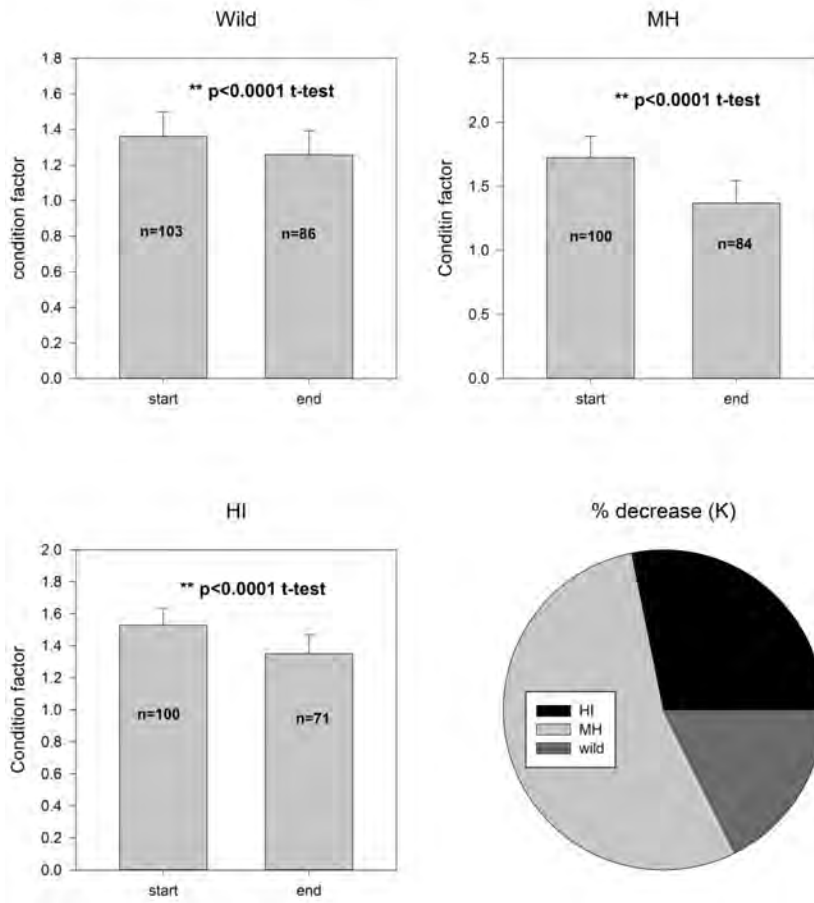
Figure 2. Condition factor (mean + sd) of ballan wrasse (*Labrus bergylta*) from IMR, MH and wild individuals at the beginning and at the end of the experiment. Panel D shows the decrease in percentage according to origin.

Damaged salmon represented 15% of the control fish, and from 23 to 43% of the treatment fish (Figure 3). Among these, skin damage was in equivalent proportions (9-13% of total) in control and treatments (Figure 3). Fin bites were present only in treatment salmon, representing 8 to 16% of the total. The number of individuals with fin bites significantly differed between treatments and were highest in the mixed treatment (wild + IMR).

Fish that had both types of damage were also highest in the mixed treatment (19%). No other type of bite-related damage was observed. Mortality in salmon (310 individuals died over the 6-week study period) was not significantly different between treatments. There were only 13 wrasse mortalities, however, when the wrasse were counted at the end of the experiment, a total of 59 individuals had disappeared (died or escaped) (IMR: 29; MH: 16; Wild: 14).
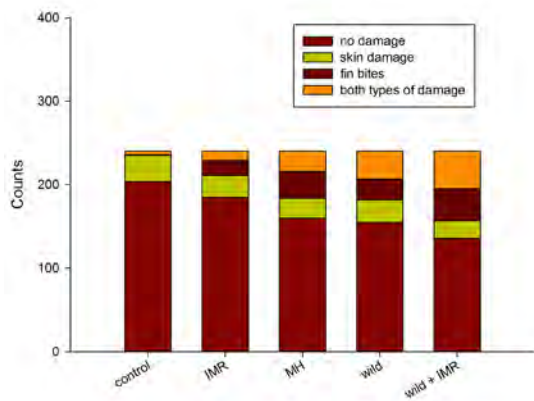
Figure 3. Damage to salmon (*Salmo salar*) over the experimental period (6 weeks).

Conclusions

Wrasse were extremely effective at delousing salmon. Although the wrasse were of different origin, there was no difference in delousing efficiency between the different groups. Intensively cultured wrasse were as effective as wild wrasse at removing lice, within just one week, despite having had no prior contact with salmon or salmon lice. At a ratio of 5% wrasse to salmon, the mean number of mobile lice life history stages on salmon was maintained at a level of less than one per fish. This is well below the allowable limit of 6 lice per salmon.

Cultured wrasse did not require prior experience with cleaner fish to be effective. Thus, our study demonstrates that intensively cultured ballan wrasse can be introduced into sea cages on salmon farms, naïve of either salmon or salmon lice, where they will delouse the salmon and keep salmon lice loads at very low levels.

All wrasse groups efficiently removed pre-adult and adult lice from the salmon, but not chalimus stage lice. The number of sea lice chalimus stages on the salmon increased throughout the experiment. However, the wrasse effectively reduced the older lice stages and, therefore, it would only be a matter of time until these chalimus stage lice developed into life stages that the wrasse remove.

Wrasse lost weight throughout the experiment. This indicates that 25 wrasse per 500 salmon may be too high a ratio and argues for a more systematic assessment of the wrasse:salmon ratio and monitoring of the wrasse welfare so that they can be fed if necessary. Intermittent dietary supplements might have improved their condition. Future research should assess procedures to supplement their food without impeding their delousing efficiency.

Damage to salmon was highest in the sea cage with corkwing wrasse indicating that this species may be more aggressive than ballan wrasse, that its food requirements are higher and/or that its dietary breadth is narrower. The inter-species dynamics that might result from introducing more than one species of wrasse to the sea cages simultaneously is a topic for future research.

The presence of wrasse did not affect the growth of salmon and salmon grew similarly in all treatment/replicate sea cages. Overall, they increased their body mass by 63% over 6 weeks. This is an acceptable growth rate compared to salmon growth in aquaculture (Kvenseth pers. comm.). Mortality remained relatively low -at least over the experimental period of 6 weeks - and equivalent regardless of treatment.

The present work is published: Skiftesvik, A.B., Bjelland,R.M., Durif, C.M.F.I., Johansen, I.S., & H.I. Browman. (2013). Delousing of Atlantic salmon (*Salmo salar*) by cultured vs. wild ballan wrasse (*Labrus bergylta*). Aquaculture 402-403 (2013) : 113-118

Recommendations
Farmed ballan wrasse is as effective as wild ballan wrasse to pick salmon lice off salmon in sea cages. However, when lice loads on salmon are low, the wrasse must be fed a supplemental diet to keep them in good shape throughout the season.

Anaesthesia of ballan wrasse

Caroline Durif, Reidun Bjelland and Anne Berit Skiftesvik

Introduction

The handling of ballan wrasse during sampling requires sedation. Dosage, induction time and recovery time may vary according to the size of fish and water temperature. In this experiment, juvenile fish were tested to determine the optimal dosage of MS-222.

Experimental setup

The fish used in the experiments were 237 dph. Fish were initially weighed, and then placed individually in 800 ml of water with anaesthetic at one of three different concentrations of MS-222; 50, 100, or 150 mg/L. Fifteen fish were tested at each concentration.

Induction time was defined as the time when the fish was no longer moving and had no physical reaction when handled. Once anaesthesia was induced, the fish was immediately placed in fresh seawater for recovery. Recovery time was defined as the time when the fish recovered its upright position.

The condition of the fish was monitored for 24 h after the trials.

Results and discussion

The fish weighed between 8 and 26 g (mean ± SD = 14.5 ± 3.7 g). There was no significant difference in mean body weight of fish between the different groups of fish that were tested.

Temperature varied slightly throughout the experiment from 12.3 to 14.8°C.

Induction time decreased significantly with increasing concentration of MS-222 from 2.5 minutes to less than one minute. Recovery time showed higher variation and increased with concentration, but the difference was not significant between treatments. Induction and recovery time did not vary according to the weight of fish or the temperature.

Fish anaesthetised at 150 mg/L exhibited a panic reaction when placed into the water. At this concentration, gill movements also often stopped. Although we did not test at higher concentrations, it seemed that 150 mg/L was a maximum level to be used without threatening the fish.
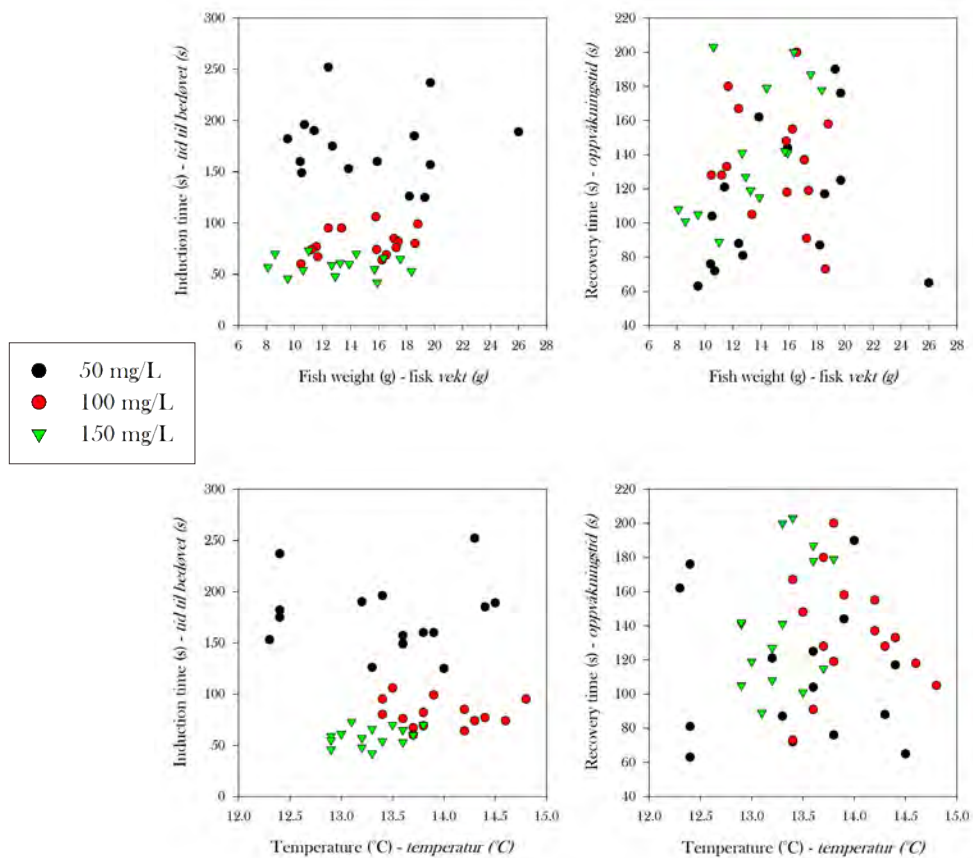
Table 1. Induction and recovery time of ballan wrasse using different concentrations of MS-222

	Concentration (mg/L)	Mean ± std (s)	Mean (m,s)
Induction time	50	176 ± 33	2'26"
	100	80 ± 13	1'20"
	150	59 ± 9	59"
Recovery time	50	111 ± 41	1'51"
	100	136 ± 33	2'16"
	150	142 ± 38	2'22"
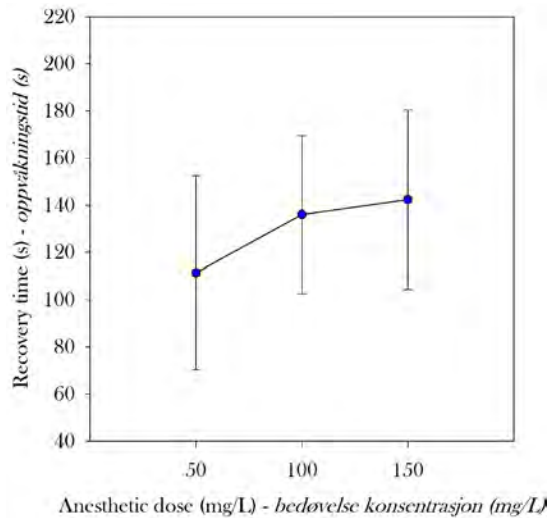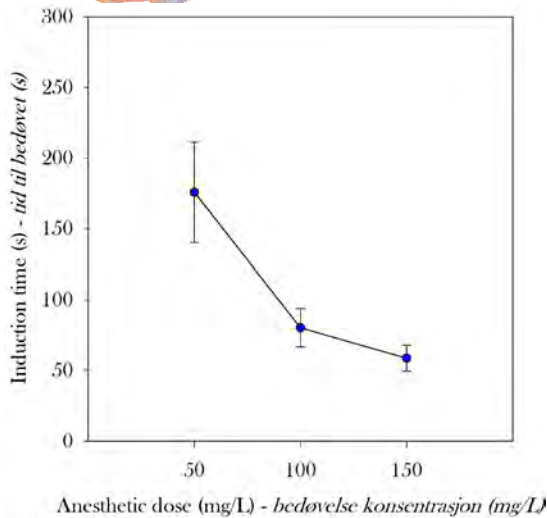
Figure 1. Induction time of ballan wrasse using different concentrations of MS-222

Figure 2. Recovery time of ballan wrasse using different concentrations of MS-222

- ● 50 mg/L
- ● 100 mg/L
- ▽ 150 mg/L

Figure 3. Induction and recovery time of ballan wrasse according to fish body weight and temperature at different concentrations of MS-222

Recommendations

Although we did not find any differences according to the weight of the fish, our recommendations are valid only for the size group used in this experiment: fish weighing between 8 and 26 g. This also applies to the range of temperatures used in the experiment (12-15°C).

Dosage and induction time should be chosen according to the type of sampling operation. When using MS-222, we recommend a dosage of 50 mg L^{-1} for weighing and measuring. Tagging, or any other invasive procedures require deeper anaesthesia and we recommend a dosage of 100 mg L^{-1}. Preliminary tests on a few individuals, however, are always necessary as fish reaction may vary according to their condition.

Flushing of intestine

Anne Berit Skiftesvik, Caroline Durif and Reidun Bjelland

INTRODUCTION

In experiments using ballan wrasse to control lice infestation on salmon, it has been a common method to kill a number of fish to look at the stomach contents so as to see how efficient the cleaner fish are at eating the salmon lice. In some experiments, and also in commercial farming, it would have been convenient to be able to look at this without killing the wrasse.

EXPERIMENTAL SETUP

In the preliminary tests, flushing of guts was conducted on fish killed with an overdose of MS222. A tube was inserted into the gut through the mouth of the fish, and a set amount of water was injected into the gut in order to flush out content. The fish was then dissected to see the result.

RESULTS AND DISCUSSION

It turned out that fish that had a full intestine (either with lice or other materials) did not respond well to the flushing procedure. Either the gut was not emptied or the intestinal walls ruptured.

We did not go any further with this experiment, as it conflicted with animal welfare regulations. No attempts were made on live fish.

RECOMMENDATION

Flushing of the intestine of ballan wrasse is not recommended as a method for studying gut content.

Gut content of ballan wrasse

Reidun Bjelland, Inger Semb Johansen, Caroline Durif and Anne Berit Skiftesvik

INTRODUCTION

The aim of these analyses was to examine whether all cleaner fish are efficient in delousing salmon, how many lice could be found in one fish, and what other organisms were found in the gut.

EXPERIMENTAL SETUP

Two net pens (5 X 5 X 5 m) were set up each with 500 salmon (mean weight = 700 g) and 87 ballan wrasse (mean weight = 35 g; produced by Marine Harvest and IMR). Shelters (plastic kelp) were provided in the net pens.

The ballan wrasse had never been in contact with salmon before this experiment and had been fed pellets. Lice were counted on the salmon at the beginning and at the end of the experiment. Wrasses were sacrificed on four occasions for gut content analysis in addition to the final sampling when all fish were analysed. This experiment lasted 14 days

RESULTS AND DISCUSSION

The wrasse had up to 78 lice in their gut, but most of them (68 %) of them had less than 10 lice (Figure 1).

There was no correlation between size of wrasse and presence of lice in the gut. Fish from MH were smaller in size than IMR fish, but this did not affect their diet during the experiment (Figures 2 and 3).
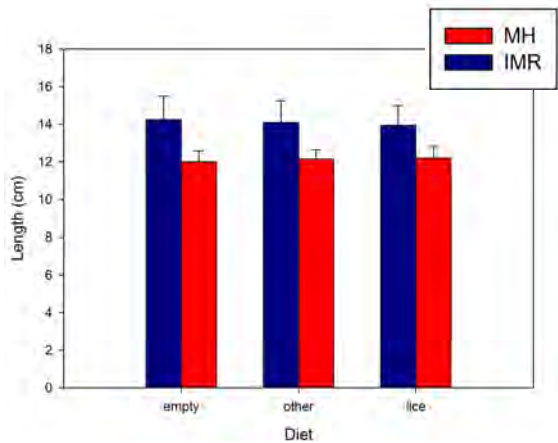

Figure 2: Average length (+ sd, n=3) of ballan wrasse raised at the Institute of Marine Research and Marine Harvest and gut content during a delousing experiment.
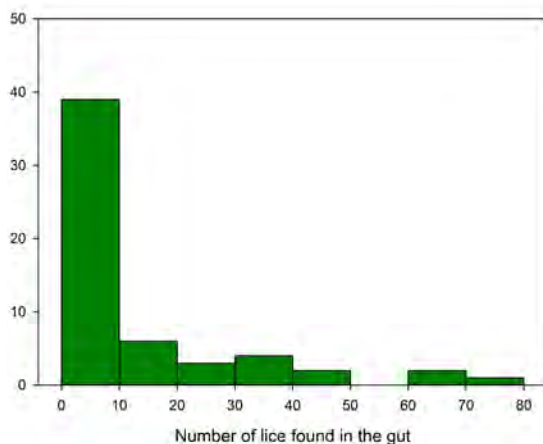

Figure 1. Number of lice found in the gut of ballan wrasse raised at the Institute of Marine Research and Marine Harvest and gut content during a delousing experiment (n=3).


Figure 3: Proportion of ballan wrasse having eaten lice, other organisms (ghost shrimp, copepods, gammarids, scales), or had an empty gut during a delousing experiment (n=3).

The salmon showed little reduction in lice counts (Figure 4) over the course of the experiment as the experiment only lasted two weeks and the fish were handled frequently which may have disturbed their cleaning behaviour.

Figure 4: Average number (± SD, n=3) of mobile sea lice counted on the salmon before and after exposure to ballan wrasse raised at the Institute of Marine Research and Marine Harvest.
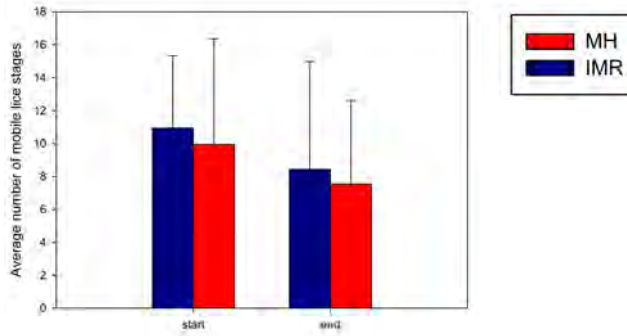
Observations

Farmed ballan wrasse is clearly able to eat salmon lice.

Chapter 7
Best practices for farming of ballan wrasse

Synnøve Helland, Stine Wiborg Dahle, Kristin Hamre, Anne Berit Skiftesvik, Ingrid Lein, Kateriana Kousoulaki, Gunvor Øie, Howard Browman, Jan Ove Evjemo, Elin Kjørsvik and Espen Grøtan

Broodstock and Spawning

Introduction of newly-caught brood stock

Until farmed brood fish are available, precautionary measures must be taken when introducing newly caught fish into wrasse farms and regulations from fish health authorities must be observed. Any fish brought in from the wild are potential carriers of pathogens (viruses and bacteria) and parasites. Thus, newly caught fish should be placed in quarantine in a bio-secure area for at least four weeks after catch to avoid contamination of existing brood fish, and injured fish should be treated adequately. In addition, fish brought in from another farm should be isolated during the first weeks.

Wrasse broodstock are caught either by fish traps or fish nets. For the catch of high numbers of fish, nets are most efficient, but this method causes more injuries to the fish than fish traps. Any fish with catch-injuries should be treated with antibiotics and an iodine solution to prevent bacterial infections. All newly caught fish should be treated with formalin shortly (first days) after arrival and 2-3 weeks later for elimination of ectoparasites.

Tanks, stocking density and gender ratio

Circular or square tanks of varying sizes and colours, and different light intensities are used for wrasse broodstock. A dimmed light seems to reduce stress. Artificial kelp (plastic) is important for the brood fish (Figure 1). Female:male ratios varying from 4:1 to 10:1 have been used successfully, but higher ratios of males can lead to aggressive behaviour in the males. Stocking densities varies from ~5 kg/m3 to 12 kg/m3. Number of brood fish per m3 vary from <10 to 25.

Feed and feeding

The ballan wrasse is picky about the palatability of feed, and shrimp seems to stimulate their appetite. In Norway a moist feed prepared from a commercial diet (Skretting Vitalis Calla), which is mixed with shrimps, fresh water and binder, is used. The feed is stored frozen until it is fed to the brood fish. The broodstock is most commonly fed every three days, but this is dependent on water temperature.

Figure 1. Ballan wrasse brood fish, Photo: Synnøve Helland, Nofima

Egg incubation and hatching

The ballan wrasse spontaneously spawns sticky eggs in the broodstock tanks. Mats of different materials are used for collection of eggs at the bottom of the tank. Some brood fish seem attracted to the mats, but a lot of eggs are lost down the tank centre drain by this method.

The egg mats are hung vertically in incubators, and the eggs are incubated at 8-12°C in filtered and UV-treated sea water (Figure 2A). Eggs are incubated in darkness except during rearing operations. Eggs are commonly disinfected daily in the incubators with Pyceze® according to the manufacturer's recommendations.

The water flow is reduced at start of hatching, and floating hatched larvae are transferred by overflow to another tank (Figure 2 B, C). This tank is wheeled to the first feeding tanks, and larvae are gently scooped and moved to a first feeding tank.

Live feeding period

Larval rearing

Temperature and light regimes

In Norway, ballan wrasse spawns from early to mid-summer characterised by continuous light and high and increasing water temperatures. Ballan wrasse larvae do not cope well with very low water temperatures, and can successfully start feeding at water temperatures between 12°C and 16°C. Larvae that start feeding at 12°C may grow faster if water temperature thereafter is elevated to 14-16°C.
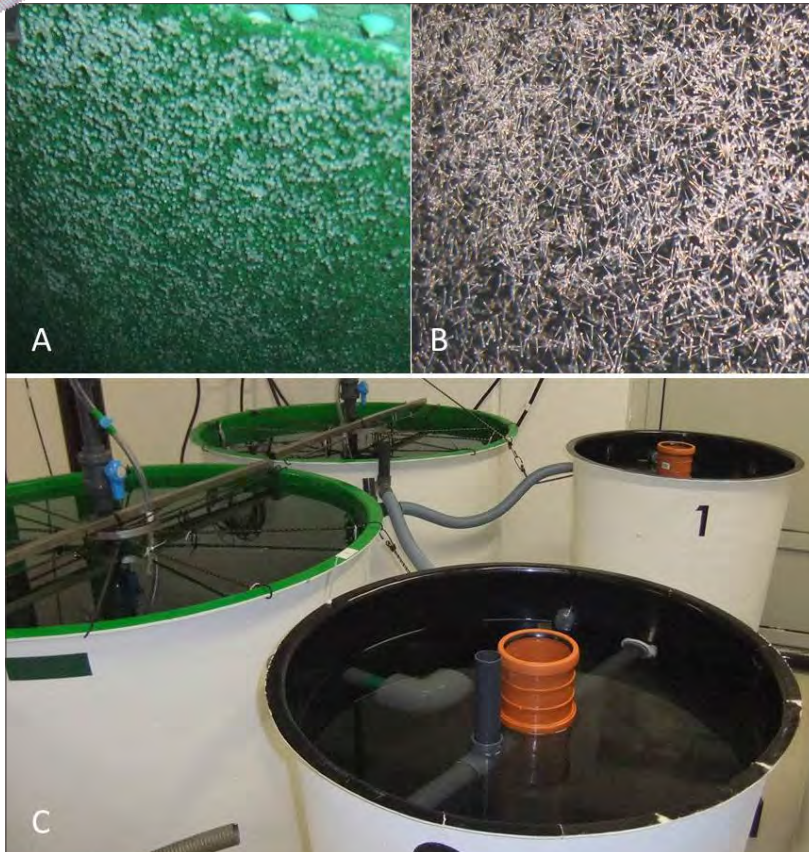
Figure 2. Rubber mats with eggs (A), newly hatched larvae (B), and incubation of spawned eggs and collection of newly hatched larvae (C). Photo: Synnøve Helland, Nofima

Ballan wrasse is a visual feeder during first feeding. Marine fish larvae have fast gut evacuation rates and thus long periods of darkness result in empty guts, starvation and reduced growth. Hence, as in most species and in the rearing of ballan wrasse, the common practice during the live feed and weaning periods involves continuous light and feeding.

Algae or clay for turbidity

Most hatcheries use algae or clay during the live feed stage to increase the turbidity of the water in the tanks, and prevent the larvae from aggregating near the tank walls. There seems to be no nutritional value of the algae except maybe some attractants. Most hatcheries choose to use clay to reduce the biological load and hence improve the tank hygiene.

Feeding regime of live feed

There are several live feed protocols that result in successful production of weaned larvae. Presently, the commercial ballan wrasse producers start feeding the larvae with enriched rotifers *Brachionus* sp., followed by *Artemia* sp. and then wean the juvenile to formulated feed.

There is no consensus regarding the optimal duration of feeding rotifers or *Artemia*, or the length of the co-feeding period with the two live feed organisms. The duration of each of these live feed phases, density of live feed and wrasse larvae, and time of weaning vary considerably. First feeding of ballan wrasse larvae starts at day 4 or 5 post hatching. Duration of the rotifer feeding vary from 25 to 58 days. The start of *Artemia* feeding varies from day 25 to 30 post hatch, and the period lasts for 20 to 70 days. Thus, the duration of co-feeding of rotifers and *Artemia* lasts from 5 to 33 days depending of strategy and protocol of the hatcheries.

Old prey organisms have a lower nutritional value than the newly enriched ones. Thus it is good hatchery practice to avoid accumulation of older live feed organisms in the tanks. This is also important for controlling the tank hygiene and water quality. In order to make sure that the larvae feed sufficiently, inspection of gut filling should be done regularly.

In experimental conditions the use of cultivated copepods as live feed gave better results than that with rotifers and *Artemia.* A short description of the three live feed organisms is given below. The nutritional composition of the live feed organisms is described in Appendix I.

ROTIFERS

ROTIFER SPECIES

In aquaculture, different species from the genus *Brachionus* are used in larvae culture (Figure 3). Genetic analysis has revolutionised the way *Brachionus* rotifers are viewed today and indicate that e.g. *Brachionus plicatilis* is not a single species, but is in fact, a complex of at least 15 putative species (Gomez et al., 2002), and this is still explored. In Norwegian hatcheries, the most used species are the *Brachionus* sp. species/biotypes 'Nevada', 'Austria' and 'Cayman'. The hatcheries often have a mixed culture of these different *Brachionus* species/biotypes (Dahle, S. W., pers. comm), which should be avoided since the different species exhibit different growth rate, lorica length, optimum for salinity and temperature, swimming activity, size preferences for food particles and biochemical composition.

Figure 3. The rotifer genus *Brachionus* sp. displaying the difference in sizes of rotifers used at the hatcheries. Photo: Tora Bardal, NTNU.

CULTIVATION OF ROTIFERS

Rotifers can be cultivated in a wide range of production systems. In hatcheries around the world there are round, square, cylindrical, conical or flat bottom tanks, with volume from 100 to 300,000 litres. The rotifers are often fed refrigerated and condensed freshwater *Chlorella*, and other feed types for rotifers are live algae, Baker's yeast, formulated diets and emulsified oils (Øie et al., 2011).

Different methods are used for rotifer cultivation include batch cultivation, continuous cultivation and methods described as combinations of these two cultivation methods. The choice of cultivation and enrichment diets will influence the nutritional value and the bacterial content in the culture. To decrease the bacterial level it is important to have good washing procedures, and washing water should have the same temperature and salinity as the culture. After the washing it is possible to cool down the rotifers to stabilize their nutritional value. For cultivation of rotifers see Chapter 4.

ARTEMIA

Artemia production is different from that of rotifers because *Artemia* are hatched from resting cysts that are commercially available. There is no need for biomass production and maintenance of cultures. In addition, the biochemical composition and nutritional value are more stable and reproducible. *Artemia*, where *Artemia franciscana* is the most commonly used species in aquaculture, are mostly used as freshly hatched nauplii or enriched nauplii (Figure 4).

Figure 4. *Artemia franciscana* nauplii Instar II. Photo: Synnøve Helland, Nofima.

Cultivation of Artemia

Artemia should be cultivated in tanks that ensure proper oxygenation and adequate mixing, and the process includes the following steps: decapsulation, hatching, enrichment/growth, harvesting, washing and feeding to fish larvae.

Copepods

Zooplankton constitutes a major part of the natural diet of marine fish larvae and can provide better growth, survival and development compared to enriched forms of rotifers or *Artemia*.

Copepods can be harvested from the fjords or from outdoor ponds, but are highly influenced by season and there is no control of nutritional value, species composition and parasitic contamination (van der Meeren & Naas, 1997; Helland et al 2003).

SINTEF have developed methods for intensive and controlled cultivation of the copepod *Acartia tonsa Dana* (Figure 5) (see Chapter 4). Intensive production can secure a stable supply of copepods regardless of season, in a controlled environment, with known size, species and nutritional value.

Figure 5. Nauplii (left) and copepodid stage of cultivated *Acartia tonsa Dana*. Photo: Andreas Hagemann, SINTEF.

The intensive production of *Acartia tonsa* eggs at SINTEF will, in the future, be commercially available for hatcheries. These eggs can be shipped to end-users worldwide and used as live feed in commercial hatcheries.

Weaning from live feed to formulated feed

There is variation between the protocols used both by the various industry partners and the experiments done in LeppeProd on when to wean the larvae from *Artemia* to formulated feed, and the duration of co-feeding. The earliest weaning started at day 40 with a co-feeding period of 10 days, i.e. a weaned wrasse at day 50. The latest weaning started on day 76 and co-feeding lasted for 4 days, i.e. a weaned wrasse at day 80. The shortest and longest co-feeding of *Artemia* and formulated feed were 1 and 10 days, respectively. Some of these differences can be explained by temperature, the rest on differences in protocols and strategies.

Ballan wrasse larvae have a significantly higher initial feeding response when the formulated weaning diet contains shrimp meal and no fishmeal irrespectively of the nutritional quality of the feed (see Chapter 5). It seems that in the first days of weaning, shrimp meal cannot be substituted by krill meal without significant loss of feeding response and consequently loss of fish. In absence of fish meal or whole fishmeal hydrolysate, ballan wrasse larvae may wean to formulated diets with high survival rates and good growth but they will develop severe head deformities. Start feeding with weaning diets without fishmeal, shortly afterwards changed to a fishmeal, or fishmeal stick-water rich diet, containing either krill meal or shrimp meal can safeguard good larval quality, growth and survival rates.

JUVENILE AND ON-GROWING

TEMPERATURE

Weaning of ballan wrasse (Figure 6) from live to formulated feed was most effective with respect to growth and survival at 16°C in an experiment where temperatures ranged from 12 to 18°C (Opstad & Skiftesvik, unpublished). They will continue to grow well at 16°C throughout the production period, but they will also do quite well at 14°C.

VISION

A survey of the photoreceptor cell population in the retina defines the fish's spectral sensitivity. The majority of cones in wrasse fall into three λ_{max} ranges – violet, blue and green. There were essentially no red-sensitive cones. The violet cone is only found in larval/juvenile wrasse, and not in the adults.

Warm white LEDs provide suitable stimulation of both of the main cone classes in the ballan wrasse and is an excellent replacement for current lamps. For larvae and juveniles the 'fit' is not as good given the output of the LEDs in the violet region where the third visual pigment is located. However, there is enough overlap between the LED emission spectrum and the violet pigment absorbance to allow for adequate stimulation of this cone.

Regarding tank colour, black is be the best choice since it provides maximum possible contrast between 'target' and background when the light source is above the tank (the 'darkfield' situation).
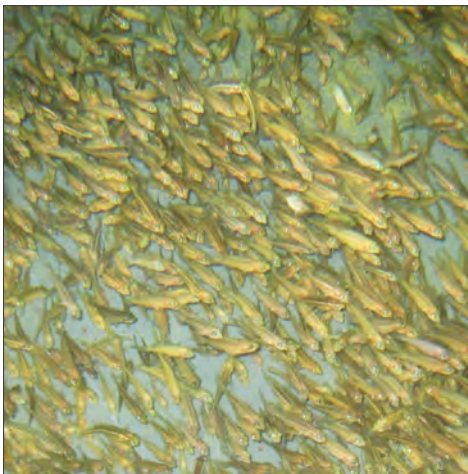
Figure 6. Image of juvenile ballan wrasse. Photo: Synnøve Helland, Nofima.

LIGHT REGIME

Continuous photoperiod regimes can be less efficient than constant extended photoperiod regimes (i.e. beyond 12 hours of light per day). Too much light can be stressful or even lethal for the fish (Boeuf & Le Bail, 1999). Moreover, long-term extended fixed photoperiod (LD20:4) may act as an irritant, inducing stress, suppressing growth and reducing feed utilisation in turbot (Stefánsson et al., 2002). The latter example shows that one should evaluate the need for a gradual shift towards days with dark periods as the fish grow.

Larger fish have a slower gut passage rate and hence a dark period should not necessarily induce starvation (visual feeders). The ballan wrasse showed no effect of different light regime on growth, and it has been observed that the gut passage time is around 12-14 hours. However, we have seen that an abrupt change in light regime had negative effects on growth and a gradual transfer should be used until further testing.

FEEDING REGIME AND FEED NUTRIENT COMPOSITION

It is difficult to determine the critical point at which fish are satiated, so fish are frequently overfed. When fish are fed insufficiently or excessively, their growth or feed efficiency may decrease, resulting in increased production cost. Overfeeding will also lead to deterioration of water quality. Information on gastric evacuation rate of fish is useful for assessing appetite return and allows one to estimate proper feeding frequency. In LeppeProd the passage of feed in ballan wrasse was about 12 to 14 hours (see Chapter 5).

The gastric content of fish is affected by the properties of the diet such as its composition of ingredients or its moisture content. Based on the feeding frequency experiment in LeppeProd, the ballan wrasse seems to be able to adapt to different feeding regimes. The survival was slightly higher when the fish were offered feed continuously, but growth and intestinal health was unaffected by feeding regime. Based on this, a continuous feeding of dry alternatively moist feed seems to function well.

Ballan wrasse performance in the on-growing phase is affected by protein, raw material quality as well as the feeds' technical properties. Dietary krill meal, krill hydrolysate and shrimp meal efficiently stimulate feeding response in ballan wrasse juveniles during the on-growing phase. The on-growing results of ballan wrasse given the available commercial diets is not yet satisfactory, and there is need for development of nutritionally complete diets of appropriate technical quality that suit the stomachless, short and basic pH digestive system.

Use of ballan wrasse as cleaner fish in salmon cages

As lice pickers, farmed ballan wrasse will do as good a job as wild ballan wrasse (Skiftesvik et al. 2013), but like wild wrasse, they have some specific requirements when living in salmon cages. They have to have a place to hide and rest - artificial kelp (made of plastic) works fine in that regard. Ballan wrasse should be added to the salmon cages after salmon are fed. Ballan wrasse should not be introduced into the sea cages during the winter-spring since wrasses are not effective lice pickers at low temperatures.

Wrasse will only be effective cleaner fish if the availability of alternative feed is low. Thus, the net cages must be kept clean. If they have alternatives to salmon lice to eat, they will eat that simply because it is easier for them. On the other hand, if the nets are clean, and there are not a lot of lice on the salmon, the ballan wrasse has to be fed. If not, they will rapidly starve and possibly be susceptible to disease. The recommendation is to feed them about twice a week when lice loads on the salmon are low.

Figure 7. Photographic illustration of ballan wrasse. Photo: Synnøve Helland, Nofima.

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Nutrient composition of live feed after culture and enrichment

Kristin Hamre

There is large variation in the composition of nutrients in commercial diets for culture and enrichment of rotifers and in enrichment diets for *Artemia*. Recently we have worked extensively with culture and enrichment diets for rotifers, which will be covered here. Some nutrients, such as fatty acids, iodine, selenium and taurine may occasionally be present at levels below the larval requirements in rotifers ready to be fed to fish larvae, leading to larval mortalities and unpredictable rearing results. There are at least two good approaches to this problem: Either one can identify which nutrients that are outside the safe window of supplementation and remove or add ingredients to adjust the rotifer diets, or one can feed a varied diet. Some information about composition of rotifer diets is present in Mæhre et al. 2012 and more will be published soon.

The requirement for fatty acids in most species of marine fish larvae has not been determined, but DHA in line with the lower levels found in copepods (20% of total fatty acids; TFA) can be considered safe. When rotifers were enriched with Multigain in the present study, the level of DHA increased with 10% of TFA. When unenriched rotifers had been fed yeast-based diets not fortified with DHA, the Multigain enriched rotifers had less than 13% DHA of TFA. This may be similar for other nutrients and it is therefore important to consider the nutrient composition of the culture diet as well as the enrichment diet. A level of more than 20% DHA in rotifers can be obtained through the culture period by feeding synthetic oil with 50% DHA (See Table 2). In this case it is not necessary to enrich the rotifers with additional long chain n-3 PUFA. A similar argument may be true for other nutrients. Therefore, if the rotifers are cultured on a nutritious diet, the enrichment step may be unnecessary.

The best way to maximize the amount of rotifer protein and phospholipids is to produce lean rotifers. Another method is to short term enrich the rotifers with a protein source with higher protein concentration then the rotifers (Helland et al. 2010). It is difficult to enrich rotifers with these nutrients because they are processed by the feed organism to body protein and membrane lipids where the levels are genetically determined. A lean rotifer will contain relatively more protein in the dry matter and more membrane lipids (PL) relative to storage lipids (TAG) and total lipids than "fat" rotifers.

The taurine level is very low in rotifers compared to both copepods and *Artemia*. Starvation of *Artemia* is shown to result in an increasing level of taurine (Helland et al. 2000). The commercial producers of rotifer diets do not seem to add taurine to their products and we are still missing validated methods for taurine enrichment. Water soluble components like taurine can be included in liposomes that can be fed to rotifers and results in rotifers with copepod levels of taurine (Helland et al. 2010). However these methods are being developed at the moment and taurine enrichment seems to promote growth in fish larvae (Hawkyard et al., 2014).

The commercial producers of rotifer diets have good control of most of vitamins, but vitamin levels may sometimes be very high. This is especially the case for vitamin A which may give skeletal deformities in the larvae at dietary levels above 10 mg/kg DW. There is little knowledge about vitamin D and K, and especially vitamin K seems to be present at low levels in some cases. Enrichment of rotifers with vitamin D and K and possible effects on fish larvae needs further study.

The levels of micro-minerals are very high in copepods, sometimes more than 300 times above the requirement in fish (NRC (2011). We have shown that rotifers enriched with iodine to copepod levels become mildly toxic for the fish larvae. The optimum iodine level for cod larvae seems to be only 3-4 times above the requirement for fish of 0.6-1.1 mg/kg DW (NRC 2011). A similar relation between copepod levels and larval requirements may be true for the some of the other micro-minerals also. Rotifers occasionally contain iodine below the requirement in fish and methods for iodine enrichment are not sufficiently developed. However some information can be found in Shrivastava et al. (2012). Selenium levels are low in many of the rotifer batches that we have analyzed. The concentrations may be down to 1/3 of the requirements in fish according to NRC (2011). A procedure for enrichment of rotifers with selenium can be found in Penglase et al. (2011). The other micro-minerals as well as the macro-minerals need further study.

Table 1. Basic levels of macronutrients, vitamins and minerals in unenriched rotifers, *Artemia* nauplii (EG- type, Great Salt Lake UT, USA, INVE Aquaculture) ongrown *Artemia* and zooplankton, mainly copepods, harvested from a fertilised seawater pond in western Norway (Svartatjønn). The ranges of requirements in juvenile and adult fish given by NRC (2011) are listed for comparison. This table is modified from Hamre et al. (2013).

			ONGROWN		
	Rotifers[a]	Artemia[b]	Artemia[b]	Copepods[c]	NRC (2011)
Macronutrients (g kg⁻¹ DM)					
Total amino acids (TAA)	396±12	471-503	596±59	634±89	-
Nitrogen	89±2	85-102	101±10	119±5	-
Protein/Nitrogen factor	4,46	4.95-5.57	5.79±0.85	5.30±0.44	-
Soluble AA (% of TAA)	44-61(*)	54±4*	Na	na	-
FAA (% of TAA)	5-7	9-10	Na	12-13	-
Taurine (% of protein)	0.08±0.04	2.1±0.1	-	1.5±0.2	-
Lipid (TL)	95-110	102	178±34	156±31	-
PL(% TL)	34	31	33±2	50±12	-
NL(% TL)	66	69	67±2	50±12	-
Total fatty acids	90±21	119	84±8	na	-
Glycogen	na	74-96	21±1	5±2	-
Ash	78±20	90	197±12	73-170	-
Vitamins (mg kg⁻¹ DM)					
Vitamin C	117-190	798	400-1000	500	50
Riboflavin	22-44	37	27-60	14-27	4-7
Thiamine (B1)	2.0-57	4,2	3-12	13-23	1
Folic acid	4.0-57	14	6-11	3-5	1
Pyridoxine (B6)	20-25	28	2-33	2-6	3-6
Biotin	1.6-1.8	4,5	2-5	0.6-0.9	0.15-1
Cobalamin (B12)	23-43	0	2-5	1-2	0,02
Niacin	191-249	159	160-250	100-150	10-28
Vitamin E	85-294	70	64-500	110	50
Carotenoids	24	630-750	650-750	630-750	
Vitamin A	0	0	0	0	0,75
Minerals (g kg⁻¹ DM)					
Phosphorus	9.4±0.7	12-19	Na	12.4-15.0	3-8
Calcium	1.9±0.2	1.9-2.0	Na	1.1-2.4	ND[c]
Magnesium (mg kg⁻¹ DM)	4.8±0.5	2.0-5.0	Na	2.4-3.1	0.4-0.6
Iodine	3.2-7.9	0.5-4.6	2.2±0.4	50-350	0.6-1.1
Manganese	3.9-5.1	4-30	Na	8-25	2-12
Copper	2.7-3.1	7-40	Ns	12-38	3-5
Zinc	62-64	120-310	Na	340-570	15-37
Selenium	0.08-0.09	2,2	Na	3-5	0.15-0.25
Iron	84-114	63-130	Na	85-371	30-150

[a]The rotifers were grown on yeast and cod liver oil, yeast and Algamac™ (Aquafauna Bio-marine, Inc., California, USA) or yeast and Chlorella (Chlorella Industry Co. Ltd. Tokyo Japan) for 4 days. [b]The Artemia were either newly hatched or grown on micronised fish meal for 4 days after hatching. [c]Not determined.

Table 2. Fatty acid profiles of unenriched rotifers grown on yeast and cod liver oil (CLO) or yeast and EPAX 2010 (a synthetic oil from Pronova, Norway with 50% DHA and 10% EPA of total fatty acids), unenriched *Artemia* (EG- type, Great Salt Lake UT, USA, INVE Aquaculture) and copepods harvested from a fertilised seawater pond in western Norway (Svartatjønn). Table from Hamre et al 2013.

Fatty acids (% TFA)	Rotifers/CLO	Rotifers/EPAX 5010	*Artemia* nauplii Unenriched	Copepods
14:00	2.7±0.3	1.1±0.2	0,7	5.9±1.8
16:00	9.7±0.5	6.9±0.2	10,8	13.2±1.5
16:1n-9	1.1±0.2	1.1±0.1	1,2	0.5±0.3
16:1n-7	8.9±1.3	9.6±1.8	2,6	5.2±3.1
18:00	3.2±0.4	2.4±0.6	4,6	1.9±0.6
16:3n-3	1.2±0.0	0.0±0.0	0	0.7±0.9
18:1n-11	2.4±0.3	0.5±0.3	0	0.0±0.0
18:1n-9	22.2±0.1	13.2±1.2	17,3	1.5±0.4
18:1n-7	3.4±0.2	1.9±0.2	7,2	2.5±1.2
18:2n-6	5.9±0.3	5.9±0.7	6,3	2.7±2.5
18:3n-3	0.9±0.0	1.7±0.5	30,3	2.2±0.7
20:1n-11	1.4±0.2	0.2±0.3	0	0.0±0.0
20:1n-9	7.0±0.9	2.5±1.1	0,5	0.2±0.1
18:4n-3	0	0	0	4.0±1.7
20:4n-6	0.6±0.0	1.5±0.4	1,6	0.7±0.2
22:1n-11	4.0±0.8	1.1±0.7	0	0.1±0.1
22:1n-9	1.5±0.2	0.9±0.4	0	0.0±0.0
20:4n-3	1.3±0.1	1.0±0.0	0,8	0.5±0.1
20:5n-3	5.1±0.3	9.2±1.7	2,2	18.6±3.2
24:1n-9	0.5±0.0	0.4±0.2	0	0.3±0.1
22:5n-3	1.5±0.1	5.6±0.6	0	0.9±0.2
22:6n-3	8.3±0.7	21.4±1.7	0	28.5±4.8
Saturated	16.8±0.8	11.1±0.5	16,9	22.5±1.0
Monoenes	52.6±0.9	31.4±2.4	29,2	10.4±4.0
n-3	19.0±0.7	39.3±3.3	39,3	55.9±4.8
n-6	6.8±0.2	7.9±0.4	7,9	4.3±3.3
Polyenes	25.8±0.8	47.2±3.0	47,2	60.9±5.1
n-3/n-6	2.8±0.0	5.0±0.7	5	19.7±11.5
DHA/EPA	1.6±0.2	2.4±0.5	0	1.6±0.4
EPA/ARA	9.1±0.1	6.9±2.7	1,4	29±10

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Identified knowledge gaps

BROODSTOCK AND EGG:

- Further development of procedures for safe prevention or removal of gelatinous layer on the egg surface

- Further development of procedures for short and long term storage of sperm to secure availability of good quality sperm

- Further development of procedures for synchronisation and completion of final maturation

- Further development of egg disinfection methodology

LARVAL NUTRITION AND REARING CONDITIONS:

- Further development of culture and enrichment protocols for rotifers, and feeding strategies for larvae, to supply all nutrients at adequate levels

- Nutritional requirements of taurine

- Effects of ARA on stress and clumping behaviour

- Test of cultivated copepods as start feed in commercial hatcheries

- Further improvement of water quality during start feeding

GROW-OUT AND BROODSTOCK NUTRITION AND REARING CONDITIONS:

- Effects of easily digestible protein sources on feed intake growth and intestinal health

- Further studies into the requirements of ARA, iodine, vitamin A, D and K, zinc, bone minerals and taurine

- Designing optimal shelters for ballan wrasse juveniles

- Further development of anti-fouling materials

- Tank design and water treatment with regard to fish health, growth and survival

USE OF BALLAN WRASSE IN SALMON CAGES:

- Delousing efficiency of wrasse in cages with large salmon

- Optimal size relationship between salmon and wrasse for efficient delousing

- Impact of temperature (high, low and temperature changes) on delousing efficiency of wrasse

- Cost benefits of delousing methods

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Production of ballan wrasse *(Labrus bergylta)*

Science and Practice

The salmon louse *(Lepeoptherius salmonis)* is a well-known salmonid parasite in nature, also commonly known as 'sea lice'. It lives of the mucus, skin and blood of the salmon and causes damage to the fish when infestation rates are high. Increasing resistance of sea lice towards chemical treatments can affect the viability of the salmon industry as there are few alternative means to limit the numbers of lice in salmon cages today. Control of potential impacts in Norwegian coastal areas is also important for the public image of the salmon industry.

Over the last years there has been increasing interest in using cleaner fish as a primary mean to fight lice infestations. The cleaner fish eats lice of the salmon when stocked in salmon cages. The 'LeppeProd' project represents a major effort to develop the production of farmed wrasse for this purpose.

In the 'LeppeProd' consortium scientists and partners from the industry joined forces to develop new knowledge on reproduction, juvenile production, on-growing and use of ballan wrasse *(Labrus bergylta)* in salmon cages. The 3-year project was supported by the Norwegian Seafood Research Fund (FHF).

'LeppeProd' has prioritised the most important challenges identified by the industry, a strategic approach to ensure that the research results and recommendations were rapidly implemented in commercial production through combining the expertise of hatchery personnel and scientists.

This manual "Production of ballan wrasse (*Labrus bergylta*) – Science and practice" has been prepared to demonstrate both the results and the recommendations of the research activities of 'LeppeProd', and targets the operators and managers of the hatcheries, students and researchers. It is a compilation of the knowledge gained during the 'LeppeProd' project and aims to be a practical tool for efficient and reliable protocols, professional advice and recommendations for the farming of ballan wrasse.

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