NORA

Final report

Lumpfish: Eco-friendly biological delousing of farmed Atlantic salmon (NOLICE)

Rognkjeks: Miljøvennlig biologisk avlusning av Atlantisk laks

PROJECT CO-ORDINATOR

ALBERT K. IMSLAND

Applicants:

1. Akvaplan-niva AS (Norway)
2. Fiskaaling (Faroe Islands)
3. Nordlaks AS (Norway)
4. The Faroese Fish Farmer Association (Faroe Islands)
5. Fjarðalax (Iceland)
6. Norsk Institutt for Vannforskning (Norway)
7. Holar Agricultural University (Iceland)

Applied from NORA:
A three year project, applied for a total of 500 thousand DKK for each year, total support from NORA 1.5 m DKK.

Progress in the project period 2013-2019

Overall summary

The project is on track according to the original work-plan and below is a short description of the progress during the first two years.

WP1 Domestication of lumpfish and effective use for delousing salmon

During the first two years of the project significant progress has been made in all three tasks of the first work package. For Task 1.1 (Domestication of lumpfish: broodstock management, larval rearing and juvenile production), work has been made in all three participating countries (Norway, Iceland and the Faroes). Initial trials were started in 2011-12 at Akvaplan-niva (Partner 1) research station, Kraknes, Tromsø, leading up to further developmental work in 2013-14. First draft of protocol for domestication of lumpfish has been made and is included in this report. Staff members from Fiskaaling (Partner 2) and Hólar (Partner 7) have travelled to Partner 1 to gain insight into lumpfish rearing ensuring a direct transfer of biological and technical know-how. Work on Task 1.2 (Thermal optima and thermal tolerance of juvenile lumpfish) and Task 1.3 (Sperm quality parameters, short and long term storage of milt) was started during the second year and is finished. Progress report is included in this report.

WP2 Effective use of lumpfish for delousing salmon

The first part of Task 2.1 (Feeding behaviour and population dynamics of lumpfish) i.e. studies to elucidate behavioural interactions between Atlantic salmon and lumpfish were performed during winter 2013-14. Next step was to investigate different sizes of lumpfish in relation to the size of the salmon and this was investigated in under semi commercial conditions during 2015 and results reported in this report.

Initial work in Task 2.2 (Development of lumpfish breeding programme and search for QTLs) was performed during spring and summer of 2015. The work involved the production of 9 different lumpfish families at the research station of Partner 1 (APN). Sea lice grazing efficiency of these families was investigated during spring and summer of 2015 and is reported here.

WP3 Commercial scale protocols for biological delousing

Planning of activity in WP3 was started in autumn of 2014 and first trials started in 2015. Final findings are reported here.

In the following project description we have included status reporting for each task in the project printed out with different letter type and using red font to indicate the progress during the project period.

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1. Objectives of the proposal

1.1 Main objectives

- **Improved rearing environment, health status and animal welfare in Atlantic salmon farming.**
- ***NOLICE* will develop economically and environmentally sound strategies for biological delousing for the NORA region.**
- **With the success of *NOLICE* the current use of pesticides for delousing will be drastically reduced.**
- **Improvement of competitive position for the farmers of Atlantic salmon in the Nordic Atlantic region using biological delousing.**
- **Lower sea lice infections that will lead to improved fish welfare and quality of the produced products.**
- **More cost effective production and better use of the resources involved.**

General concepts and needs of the salmon farming industry
Sea lice infestation of Atlantic salmon by *Lepeophtheirus salmonis*, is the largest threat facing Atlantic salmon aquaculture (Denholm et al. 2002; Johnson et al, 2004). The control of this parasite, which grazes on the skin and mucosal tissue of the fish (Kabata 1974) leaves the fish open to secondary infections, imposes an osmotic stress that ultimately causes death in the fish. Infestation and the wounds it causes downgrades the product at market. The problem exceeds that of aquaculture with wild fish stocks (predominantly Atlantic salmon) acting as a natural reservoir of the parasites and the effects of parasitism has been linked to wild population declines. There are also other ectoparasites that may pose a threat to Atlantic salmon (Mustafa and MacKinnon 1999) e.g. *Caligus curtus* and *Caligus elongatus*. *C. elongatus* has been found on over 50 marine species, many of them with aquaculture potential (Mustafa and MacKinnon 1999).

The aquaculture industry has been struggling with the issue of sea lice control for many years and has relied heavily upon the use of chemotherapeutic treatments either as bath treatments (such as hydrogen peroxide and organophosphates) or, more recently, synthetic pyrethroids (Cypermethrin and Deltamethrin) or using an in-feed treatment with emamectin benzoate (SliceTM) (Denholm et al. 2002). However, recent evidence is suggesting that accumulative resistance to all of these compounds has occurred in the sea lice population (Lees et al. 2008; Anon. 2010).

The fundamental problem is that current methods of sea lice control are proving ineffective and unsustainable. Development of effective and biologically sustainable methods is urgently needed. The innovative approach of biological control addresses the basic need of the producers of Atlantic salmon to remain financially competitive with regard to Atlantic salmon aquaculture within the EU and the world markets. Moreover, since this innovative approach will reduce or eliminate the reliance on chemical sea lice control, the producers have a distinct competitive edge with regard to ethical and organic labelling of their products.

1.2 Innovation aspects

Lumpfish – a new candidate for biological delousing under low temperatures

The cleaner fish in use today (e.g. Ballan wrasse) are temperature sensitive, making them ineffective for biological delousing under low (< 6°C, Sayer and Reader 1996; Kvenseth 2010) temperatures frequently observed during winter time in the Nordic Atlantic region. As Atlantic salmon is presently being farmed under such low water temperature conditions in large parts of Nordic Atlantic region, there is a need for developing an alternative method under those climatic conditions. The common lumpfish (*Cyclopterus lumpus* L.) has been suggested as an alternative (Willumsen and Johansen 2001). As a cold-water alternative the common lumpfish (*Cyclopterus lumpus*) has been suggested and trials have started in Norway and Faroe Islands with this species. Initial results are very promising with 93-97% less sea lice infection (adult female lice) in sea pens with lumpfish.

Lumpfish are found in the eastern Atlantic from the Portuguese coast north to Iceland and Norway and tolerates low temperatures very well (Kudryavtseva and Karamushko 2002). It is common from January-September along the northern European coast (Stein 1986). The lumpfish migrates to the coast in the spring to spawn. The male lumpfish guards the eggs until hatching. The larvae are well developed at hatching and will stay near the bottom for the next two years. Some initial rearing of larval and juvenile lumpfish have been made (Benfey and Methven 1986; Klokseth and Øiestad 1998). Klokseth and Øiestad (1998) performed pilot scale spawning and larval rearing trials with lumpfish. Although they managed to produce over 20 thousand juveniles they later died in a Vibrio outbreak. Until now, no commercial production of lumpfish has been performed. In *NOLICE* it is intended to develop protocols for successful juvenile production of lumpfish, so that early behavioural weaning to live in sea pens can be performed.

As for any new aquaculture species it is important to map the optimum rearing range. In *NOLICE* it is intended to investigate in details the temperature range the species can be reared under and used for effective biological delousing.

Lumpfish is an opportunist who has a large capacity for feed intake and initial observations made by farmers (http://www.youtube.com/watch?v=xhgxUzPRhlc) and previous attempts (Willumsen and Johansen 2001) clearly indicate that lumpfish will eat sea lice and may be used for biological delousing under low temperatures. The lumpfish is therefore a prominent candidate for delousing in the Nordic Atlantic region.

1.3 Benefits for the producers of Atlantic salmon

Infestations by the salmon louse (*Lepeophtheirus salmonis* Krøyer) an ectoparasitic copepod have a large impact on fish farmers economy in form of costly treatment procedures, reduced growth, feed waste and downgrading of the final product. It is expected that the cost of sea lice to Norwegian fish farmers alone is more than 150 m€/year and in the Faroes more than 10 m€/year. There are also other sea lice species that may pose a threat to Atlantic salmon (Mustafa and MacKinnon 1999) e.g. the cod lice (*Caligus curtus*) and the *Caligus elongatus*. *C. elongatus* can be found on over 50 marine species many of them with aquaculture potential (Mustafa and MacKinnon 1999; Oines et al. 2006) including the Atlantic salmon. Thus, the effect of parasitic sea lice on farm stocks has emerged as one of the major health issues for both established and emerging aquaculture species. There is also evidence of serious negative environmental impacts (Costello 2009) including the possibility of transfer of sea lice infestations from farmed to wild fish as well as treatment chemicals being released to the environment (Heuch et al. 2005). Over time, greater dependence on sea lice medicines has resulted in lice developing resistance against these agents, thereby further exacerbating the problem both in farms and in the wild. Sea lice medicines available to the salmon farming industry is limited with continuous and frequent use of cypermethrin and emamectin benzoate,

potentially leading to the development of resistance. Reduced efficacy and the proportion of ineffective treatments have risen from zero in 2002 to 50% of reported cases in 2006 (Lees et al. 2009; Anon. 2010). It is therefore only a matter of time until resistant sea lice will pose a serious threat to the whole salmon farming industry. Consequently it is urgent to develop new ways of dealing with this imminent problem.
At the same time, the organic farmed Atlantic salmon market has been identified as an important niche for the salmon aquaculture industry and the use of medicated/treated feed for the control of sea lice may not be accepted in the future if organic certification is to be achieved. A cost-effective and environmentally sustainable solution to prevent the development of resistance by sea lice to medicines is an urgent priority for the aquaculture industry.

Exploitation of the results of the proposed project will greatly contribute to development of environmentally friendly and more sustainable culture of Atlantic salmon in the NORA region. The biological part of the project will result in a number of distinct protocols for effective biological delousing with lumpfish suited for farming in the different countries and under variable farming conditions. Thereby the project provides evaluation of different sustainable delousing methods suited for salmon. This is very important for the implementation and exploitation of the project results. The project is designed such that each producer works with its own production system, ensuring easy implementation as experience is gained during the project. However, all project results are of course directly available to all partners in the project, so one producer can implement the results from other if desirable. The results will be made accessible and open to all interested in the NORA region.

1.4 Benefits of a joint venture

In the NOLICE project the aim is to develop a tool that can be used in the whole NORA region to tackle a major obstacle faced by the Atlantic salmon farming industry, a problem face by the Atlantic salmon farming industry across the NORA region.
An joint venture with collaborating participants across the NORA region will accelerate the progress in developing lumpfish as an effective delousing tool for producers of Atlantic salmon. The commercial production of a reliable, sustainable and biologically safe supply of lumpfish for use as cleaner fish for sea lice control is the only remaining realistic option if sea lice are to be controlled in Atlantic salmon aquaculture in an environmentally and sustainable manner.
The joint effort of scientists and producers in the NORA region will ensure quick spread of know-how and experience related to development of the new delousing methods. An effort to develop lumpfish as a delousing agent is already under way in Norway, Faroe Island and Iceland. Integrated cooperation across the region will strengthen the current development of farming of Atlantic salmon especially in the colder part of the region. Farming of Atlantic salmon is frequently located in remote areas where depopulation and unemployment is a problem. The cooperation presented in the current project is likely to enforce the further development of fish farming in such remote areas and ensure they're future economic and environmental sustainability.

The main idea of the project is to outsource research and development activities to experienced and specialised industrial partners in order to develop adequate technology to produce healthy Atlantic salmon in ecologically-friendly way. Both species involved (Atlantic salmon and lumpfish) have great potential for sustainable growth in production, but sea lice is currently one of the main obstacles for further growth of the industry. To fully exploit this potential, it is very important to establish a critical mass with respect to human and financial resources across the Nordic Atlantic region. Projects that lack such critical mass cannot assess the development of novel methods for biological delousing sufficiently as individual producer

cannot be expected to be able to cover all aspects associated with such a development. For the industrial partners with limited resources the value of putting together a consortium with various expertise will be significant. Together they have a multidisciplinary field of expertise that only large companies may afford to hold within one company. It could be envisaged that the partners will maintain and reinforce their co-operation after the end of the project. The partners would not be able to establish an equally competent consortium within their own countries.
The consortium consist of companies that realise the need for, and wish to improve technology from the present level to a level that ensures further sustainable growth in production of salmon bearing in mind the consumers need for environmentally friendly and ecological driven production. The commercial farms included will implement the new technology for sea lice control in full-scale modules and test the feasibility of the new approaches.

The aquaculture industry is of a major importance for the remote coastal regions of Northern Atlantic region where aquaculture businesses and associated activities make up a considerable part of the local economy and where alternative employment opportunities are limited. In terms of market challenges the sector has been and will be increasingly exposed to international competition in the finfish sector. Thus by sharing the knowledge transferred, the salmon farming sector would be in a better position to tackle this competition.

The partners are from three of the biggest salmon producing nations in Europe – Norway, Faroe Islands and Iceland, where the latter two are focusing heavily on production of organic labelled farmed fish. The rapid expansion of finfish farming has focussed attention on problems caused by a variety of pest organisms such as sea lice which sometimes occur at high densities in coastal waters. This project will provide the Nordic marine finfish aquaculture industry with a practical solution to control lice in an environmentally friendly way by using a lumpfish to control lice in sea cages.

Successful biological delousing as foreseen in the project will represent a significant step in the development and up-scaling of Nordic aquaculture production. It will facilitate increased production throughout the production area for salmon, which in turn, will open for new employment development in those countries. Activities related to farming of lumpfish will also create new jobs and investment opportunities.

In conclusion: by its nature and structure the proposed project will strengthen transnational relationships within the aquaculture production and research in the NORA region.

2. Expected results and exploitation

2.1 Expected results and milestones

The main project results of the present project are:

- Improved rearing environment, health and animal welfare in Atlantic salmon farming.
- Economically and environmentally sound strategies for biological delousing.
- Reduction in use of pesticides in aquaculture.
- Improved quality of the final product.
- New methods for biological delousing of farmed salmon.
- More cost effective production and better use of the resources involved.
- Feasibility evaluation of farming of lumpfish in the NORA region

This project is aimed at further strengthening the competitiveness of producers of Nordic salmon farmers by developing successful protocols for large scale lumpfish culture and efficient use for sea lice control thereby maximizing the productivity of the farms per unit input. To this end, the project will investigate, and define, several topics in relation to successful launching of biological delousing for salmon culture. Emphasis is on testing the method under wide range of environmental conditions experienced by farmers in the whole production area of salmon in NORA region, particularly with respect to water temperature.

2.2 Importance for the NORA region

A successful production strategy will be transferable to other farmers of Atlantic salmon in the whole NORA region, *increasing the NORA-regions competitiveness in fish farming*. This project will contribute to the development of aquaculture in North Atlantic region which faces ever increasing competition from South America and Southeast Asia. Results from the proposed project, and the new biological delousing methods are *available to the whole NORA region aquaculture sector (end-user).* The successful launching of new sustainable and environmentally sound rearing concepts will make it far more attractive to start new businesses and ensure better use of the resources available, because of lower investments per production capacity and lower cost per unit produced. In addition the project will create a basis for a new industry related to production of farmed lumpfish.
Overall, the project will have a clear economic impact for the industrial participants in terms of economic growth (higher production efficiency) thereby stimulating increased employment in the aquaculture sector.

2.3 Dissemination of results and know how

Dissemination activity is given a strong focus in the NOLICE project. WP 4 in the project is deals specifically with the exploitation and dissemination of results. Thus, the absorption of the technology by the partners will be ensured through confidence-promoting steps:

- Install in close communication with the key staff the optimized rearing system on their site, based on the actual state-of-the-art for the system;
- Through discussion with key staff at each industrial partner, make site-specific modifications in regard to requirements they might launch;
- Implementation of the optimized rearing system at each industrial partner in close collaboration with their technicians;
- Training the key staff in the basis of experimental design and basic statistical principles for analysis and validation of results, and the use of the quality assurance

system; consequently, they will feel confident in replicating the experiments by themselves and performing new ones, if required.

- Train the key staff at the industrial partners in the use of the prototype for biological delousing of Atlantic salmon.

For the industrial partner, the work undertaken in this project does not finish with the successful demonstration of the technology applied on a variety of fish species.
For the Atlantic salmon farming industry, this is the first phase of a process that subsequently will lead to:

- Effective implementation of the eco-friendly biological delousing method at their farm. This phase will possibly last through subsequent year(s), during which the progressive adaptation of facilities and methodologies will be done as well as some further studies. Training of the staff in the new way of working will also be needed.
- Commercialisation of the results of *NOLICE*. It is expected to emerge interest in the protocol for the optimized rearing system for the whole production. Equally, the adaptation of some facilities for intensive fish culture can be commercialised.

In order to ensure that the Atlantic salmon farming industry is able to assimilate and exploit the results of the project, a number of steps are foreseen. The consortium clearly sees the importance of active research participation by the salmon producers in cooperation with the research companies and Universities, using their own hatchery, on-growing system and facilities for biological delousing. All of the producers have adequate scientific expertise for such participation. During the project, industrial partners and researchers will work together, be in close touch, and communicate extensively. Outside the set meetings, communication between the partners will be intensive and will involve practical experience exchange at the various sites of the project across the NORA region. In order to promote and facilitate the spread of knowledge, the project has been designed in such a way that industrial partners and researchers work together on the same tasks. In addition, involved partners have bilaterally co-operated in the past. As result the barriers between participants are low and informal contacts already exist. In addition the idea of turning the project results into protocols for production, originates from the industrial partners themselves ensuring fast and effective implementation of the project results.
The protocols developed will be established according to the needs, and wishes of the Atlantic salmon farming industry ensuring accessibility to the results. The protocols will be practical manuals rather than scientific papers. In addition, as a result of the short communication lines and close co-operation between industrial partners and researchers during the whole project, transfer of information is natural and direct, as the industry partners are basically involved in establishing the protocols. The industry partners are well established salmon producers representing a significant part of the NORA region.

In the first half of the project, dissemination of information about the project will remain limited to the consortium partners. During the second half of the project, articles may, at the discretion of the industrial partners, be published in scientific journals as well as in more industrially oriented magazines. These publications will include all results in all areas of the projects. The project coordinator will coordinate the publication effort of the consortium.

3. Work and milestone description

3.1 Work package descriptions

Work package number			1		**Start date or starting event:**		1						
Work package title			***Domestication of lumpfish and effective use for delousing salmon***										
Participant number	1	**2**	3	4	5	6	7						
Person-months per participant:	3.5	**3**	1	1	1	4	3						

Objectives:

- To develop full-scale production protocol for successful domestication and production of juvenile lumpfish.
- To identify and quantify factors that affect food choice of lumpfish in tanks and net pens with Atlantic salmon.
- Secure access to sperm of good quality, milt can be collected frozen and used at the most convenient time

Description of work

This WP will concentrate on the complete rearing cycle of lumpfish. The aim is to develop production protocols for successful domestication of lumpfish under aquaculture conditions. This Work Package consists of three main tasks that together cover early juvenile production of lumpfish and protocols for effective use in delousing salmon :

- *Task 1.1. Domestication of lumpfish: broodstock management, larval rearing and juvenile production*
- *Task 1.2. Thermal optima and thermal tolerance of juvenile lumpfish*
- *Task 1.3 Sperm quality parameters, short and long term (cryopreservation) storage of milt.*

Task 1.1. Domestication of lumpfish: broodstock management, larval rearing and juvenile production

This task will deal with establishment of juvenile lumpfish production including establishment of broodstock, collection of eggs, incubation, first feeding and weaning. Some initial trials have been done (Benfey and Methven 1986; Klokseth and Øiestad 1998), but those earlier findings need to be scaled up to industrial use. Wild caught adult lumpfish will make the basis of the initial broodstock. Eggs will be artificially fertilized (stripping) and natural spawning will also be tested. Fertilized eggs will be placed in egg incubators (different types will be tested). Different incubation temperatures and first feeding methods (Artemia, live zooplankton, dry feed) will be tested.

Task 1.2. Thermal optima and thermal tolerance of juvenile lumpfish

Controlled temperature studies (4-14°C) will be performed to elucidate the effect of temperature on growth potential, survival and feeding efficiency of juvenile lumpfish. The identification of the primal temperature effect will have large practical value to predict the optimal feeding efficiency and survival for lumpfish in sea pens during an annual production cycle.

Task 1.3. Sperm quality parameters, short and long term (cryopreservation) storage of milt.

To date, spawning of lumpfish in captivity has proved problematic, most likely due to the unique reproductive traits of the lumpfish, including complex spawning behaviour and parental care. For these reasons, spawning under commercial conditions should reply on artificial insemination (AI). However, a major disadvantage of the lumpfish is the small quantity of milt produced by mature males. For this reason AI procedures should ideally involve sperm cryopreservation. As fertilization success is usually positively correlated to sperm quality traits (sperm density and sperm motility), sperm quality of cryopreserved sperm will be assessed by Computer Assisted Sperm Analysis (CASA) and by high resolution video images.

Role of the partners in WP1

Partner 2 (WP leader, FISKA) and Partner 1 (APN) will be the participants with the main responsibility for WP1. These partners will cooperate with the industrial partners 3-5 in the tasks in WP1. Once established partner 1 (APN), partner 2 (FISKA) and partner 7 (HÓLAR) will use the production protocol for lumpfish production to produce juveniles for field tests at all industrial partners. All experiments in Task 1.1 will be carried out by Partners 1, 2 and 7 in cooperation with industrial partners 3-5. Task 1.2 will be carried out by Partner 1 in cooperation with partner 3. Task 1.3 will be carried out by Partner 6 (NIVA) in cooperation with partner 1, 2 and 7.

Milestones

- M1.1. Protocols for broodfish management and spawning of lumpfish (Month 12).
- M1.2. Optimal rearing protocol of juvenile lumpfish (Month 24).
- M1.3. Protocols for cryopreserving lumpfish sperm and optimal artificial insemination protocol of lumpfish (Month 30).

WP1 Progress 2013-18

Task 1.1. Domestication of lumpfish: broodstock management, larval rearing and juvenile production

Work in this task has been made in all three participating countries (Norway, Iceland and the Faroes). Initial trials were started in 2011-12 at Akvaplan-niva (Partner 1) research station, Kraknes, Tromsø, leading up to development of protocol for domestication of lumpfish. Staff members from Fiskaaling (Partner 2) and Hólar (Partner 7) have travelled to Partner 1 to gain insight into lumpfish rearing ensuring a direct transfer of biological and technical know-how. Below we will describe the work at all three partners.

1. Norway (Partner 1, Akvaplan-niva, Kraknes, Tromsø)

General rearing environment

Lumpfish for breeding purposes was collected from wild brood fish from local populations caught off the coast of Tromsø in the period from week 14 to 20. The following protocol was developed using stripped eggs and milt and/or spawning in tanks from these wild specimen. The broodstock consisted of 41 females and 6 males.

The lumpfish were commercially bred at the research facility Troms Marin Yngel, Kraknes, Norway (69° 45" 53" N, 19° 02" 46" E).

Brood stock management

Establishment and Maintenance

When collecting wild broodstock emphasis should be on minimising the stress of the fish. Only individuals free from or having minimal external wounds or scratches should be sampled. Maintain good water quality during transfer to rearing facility.

The broodstock was reared in seawater and kept at natural photoperiod.

Collection of eggs
Spawning/stripping
The lumpfish will spawn in tanks but there are two main disadvantages related to this method. Firstly, eggs tend to clump together resulting in higher mortality during incubation period. Furthermore, spawning in tanks has also resulted in low fertilization rate compared to stripping, as lumpfish roes are fertilized externally. When stripping the eggs the behaviour of the female fish has to be monitored closely to catch them during ovulation. Eggs and milt are collected by stripping (Fig. 1.) and are then dry fertilized. The stripping and artificial insemination of the eggs has resulted in good overall fertilization rates (>90%) and seems to be much more viable method compared to spawning in tanks.

Fig. 1. *Stripping of eggs from lumpfish*

Egg incubation
Eggs should be counted and measured (L) to attain good estimate of the total number of eggs. This will result in a more accurate evaluation of survival through later life stages.
The eggs are incubated in hatching trays and care should be taken to spread of the fertilized eggs in a thin layer. This is to ensure good water flow to maintain good oxygenation of all the eggs as well as easier removal of decaying eggs during the incubation period.

Fertilized eggs are treated with Buffodine (2.5ml l^{-1} water for 10 min) prior to incubation in hatching trays submerged in raceways. This antibacterial treatment is applied twice during the incubation period, and decaying eggs must be removed regularly to reduce fungus growth. During removal of dead eggs care should be taken to cause as little movement distress to the neighboring eggs.
An incubation temperature of 11°C has been proven to ensure good survival and growth, and uniform hatching within the egg-batch. Light during the incubation period should be dimmed (not darkness).

The larvae hatch after about 250 day degrees.

Larvae rearing
Rearing conditions
Larvae are carefully transferred to start feeding units subsequently upon hatching. During start feeding and subsequent juvenile stages, water temperatures should be 11-12°C. The photoperiod should be continuous dimmed light (LD 24:0).
Two types of rearing units have been tested. Shallow raceways have proven to work well (Fig. 2). Upwelling incubators (250 – 600 L) have also been used with good results and were evaluated to result in better distribution of the feed and facilitate easier cleaning of the tanks.

Feeding
Two different start feeding regimes have been tested; live feed (Artemia) or dry feed.
For start feeding using live feed, Artemia nauplii enriched with Multi Gain (BioMar, Norway) was used. After 21 days, juveniles are weaned onto AgloNorse formulated feed (300-500 µm) and 14 days later 500-800 µm dry feed pellets (Gemma Wean Diamond, Skretting, Norway) are introduced to the diet.

Start feeding lumpfish larvae directly onto dry feed gives good results in terms of growth rate and survival. The initial phase is based on formulated feed with small particle size (200-300 µm). About ten days later larger particle feed was gradually introduced (300-500 µm). Fourteen days later the fish was introduced to feed with 0.5 mm particle size (one week transition time). Fish were then fed particle size 0.5 mm for 1 month.

Fig. 2. *Start feeding of lumpfish in shallow raceways*

Daily maintenance and cleaning of tanks.
Daily routines included measuring oxygen levels in the effluent water. Removal of dead larvae and cleaning of tanks was conducted as required to minimize stress.

Juveniles
Tanks and environment
Throughout the first juvenile stages the same rearing units are used. Fish are transferred to larger rearing units in accordance with increasing biomass in the tanks. Currently there have not been established recommended rearing densities, and care should be taken to ensure good water quality in the tanks.

When juveniles reached the size of 1 gram, some fin nipping and aggression between individuals may be observed. Damages from this behaviour may result in mortalities. Size grading must therefore be applied to reduce the size variation within each tank. It is assumed that sufficient access to food and reduced fish densities in tanks may reduce this negative behaviour.

A rearing temperature of 11-12°C has given good results and survival during the juvenile phase. Increased temperature during the initial phase of the juvenile period, prior to a stepwise reduction in temperature could be applied to achieve better growth rates through the juvenile phases (see optimum temperature trials).

2. The Faroese Islands (Partner 2, Fiskaaling)

Removal of lumpfish egg stickiness and incubation of treated lumpfish eggs
Background: A few minutes after lumpfish eggs are exposed to seawater, the eggs stick together. We believe that this is a part of the reproductive strategy of this species, as this both facilitates a way for the lumpfish male to position the egg mass on a favourable spot as well as guarding the whole egg mass in a small area.
On the other hand, this feature imposes challenges in the production of lumpfish juveniles. Basically, a secure method of disinfection of lumpfish eggs must be found which effectively insures that eventual pathogenous viruses or bacteria are not transferred from parent fish to the next generation. This is extra relevant as the current lumpfish production still relies on broodfish caught from the wild. Another challenge lies in the incubation period of lumpfish eggs where mortality in egg masses may occur. This may be due to uneven individual egg access to fresh seawater which may cause high mortality and in deed create an uneven development in the incubated egg batch. This may in turn prolong the hatching period leading to an unwanted spread in larval size during start feeding.

Activities: To in attempt to solve these challenges Fiskaaling has done a few experiments in 2014 with the first aim to find a method to remove lumpfish egg stickiness (a) and after this objective reached – to find suitable containers for incubating the treated eggs (b).

a) Removal of egg stickiness.
Previous work has shown that it is possible to remove egg stickiness in Ballan wrasse, another marine fish species (Ingrid Lein et al. 2013). We tried a similar approach for lumpfish eggs including five different treatments (incl. control). Each treatment contained about 2.300 eggs, fertilised with about 30.000 spermcells per egg. The tests were done at the Fiskaaling Marine Research Station at Nesvík, Faroe Islands.
The best result to remove egg stickiness was obtained by using 10% alcalase mixed with Ringer's solution (10 ml alcalase in 100 ml Ringer's). This amount was sufficient for 5 dl eggs. Fertilisation % (observed after 180 day degrees) was more than 75.

b) Incubation of the treated eggs.
Lumpfish eggs do not float freely in the water column as cod or halibut eggs. This is also the case for lumpfish eggs when the egg sticking material is removed and the eggs sink to the bottom when transferred to egg-incubators. Therefore similar incubation technology used for incubation of salmon eggs is useful for treated lumpfish eggs. After fertilisation, salmon eggs are incubated in up-flowing incubators until the eye-stage is reached and then transferred to trays in long race-way tanks. Normal flow rate in these tanks is about 15L/min. By using similar incubators with up-flowing seawater and bottom, we have obtained very good results for treated lumpfish eggs. An important finding is that one should not rely on volume waterchange/hour or minute in these incubators, but rather ensure that the flow on each square centimetre of incubator bottom is $\geq$ 0.04L/min. An interesting observation is, that compared to conventional incubation with "sticky-eggs", treated eggs develop at a much faster rate.

Current conclusions for the work in a) and b) is that we have successfully removed lumpfish egg stickiness and found suitable incubators for the treated eggs. We did not test whether removal of egg stickiness ensures effective disinfection of the eggs or if this method has negative effect on the development of lumpfish larvae. However, we have not observed any negative effects at this date. Although these preliminary findings are of immediate importance for the current production of lumpfish eggs/larvae, more thorough research on the mentioned topics is needed.

3. Iceland (Partner 7, Hólaskóli)

1.1 Spawners

Number of spawners:

Approximately 40

Facilities and breeding conditions:

8 corner-cut tanks of 1m^3

Flow through system – sea water (4-5°C – 26-29‰)

5 fish per tank

No feeding – the brood fish did not eat any food at any time

1.2 Eggs

Fertilization technique:

Naturally spawned eggs and stripped artificially fertilized eggs.

Number of eggs:

12 incubator trays were filled with eggs (about 2 cm layer) – 4 trays filled with stripped eggs and 8 trays contained bits of naturally spawned eggs.

N.B.: It was not possible to estimate the total number of eggs.

Facilities and breeding conditions:

Incubation trays in raceways (thin layer of stripped eggs or broken clusters of naturally spawned eggs)

Aeration column + low head

Flow through system – sea water (4-7°C – 19-26‰). Note salinity limited by maximum salinity of the rearing water.

Seawater treatment:

Degassing and oxygenation of the water

Daily maintenance:

Removal of dead and decaying eggs

Removal of air bubbles stuck under the incubation tray by gentle shaking/tilting.

Daily temperature measurement.

1.3 Larvae

Number of larvae:

56 000 (+/- 5 000) larvae

Handling:

The larvae were moved from the incubation trays to the rearing tanks using a large pipette and a small scoop depending of the number of hatched larvae.

Facilities and rearing conditions:

8 silos of 1 m^3: Water flowing from bottom to the top

Aeration column + low head

Flow through system – sea water (6-9°C – 19-26‰)

Water treatment:

Degassing and oxygenation of the water

Food:

Fine pelleted food from AlgoNorse.

D2 to D26	D21 to D110	Not used
200µm	300µm	600µm

Daily maintenance:

Temperature measurement.

Cleaning of the exposed sides of tanks + outlet mesh

Feeding: 3 times per day during the week, twice on Saturdays, once on Sundays

Cleaning protocol

Cleaning of tank bottom with a “siphon cleaner”, once a week

Cleaning of submerged sides of tank with a “siphon cleaner”, once every other week

Disinfection (D-San) of all materials after use

Production results

Due to water quality problems the majority of the produced larvae were lost during the first 4 months of the rearing period. Mortality was steady during those 4 months. Few signs of malformation (crooked tails) were detected but these larvae were quickly eliminated. Afterwards all larvae seemed to have a good morphology according to literature. The growth rate was probably very low as the larvae did not reach more than half a cm, which is probably due to a lack of food.

Throughout the trial, they seemed to show "normal" behavior as they were resting on the walls or clinging at the surface most of the time, and then showed interest in the food by swimming actively towards the particles and many snapping were observed. Few agonistic actions were seen, nothing excessive though.

Analysis and propositions

2.1 Spawners

Number of spawners:

During spring 2014, 40 wild lumpfish were been brought to Verið. Some individuals looked quite moribund and/or heavily stressed at their arrival – suggesting poor transfer conditions in the fishing boats. Thereafter, none of the fish showed interest in the pelleted food, even after many weeks of starvation.

An initial low number of larvae results in a small margin of error and therefore less flexibility of the protocol of production.

Proposal for improvement for next spawning season: Given the results from this year, it seems essential to make sure the suppliers of brood fish are equipped with water tanks properly oxygenated. A strict protocol from the capture at sea to the acclimation in Verið facilities should be created to provide guidance for the fishermen.

2.2 Eggs

Fertilization technique:

Two different ways of egg collection/fertilization produced very distinct hatching results. We observed that stripping and artificial fertilization was by far the most efficient technique compared to the natural spawning, for two plausible reasons. Firstly, the fertilization rate was lower for the natural spawning fish. Furthermore, eggs from natural spawning and fertilization stick together by clusters reducing survival during incubation. During incubation, it looked like the inside of the clusters was not well supplied in oxygen resulting in the death of the embryos upon which developed some fungus.

Proposals: For next breeding season focus only the stripping method. Moreover, a clear protocol of stripping has to be established and followed. A count of the number of eggs per volume is necessary to estimate the total number of eggs and therefore calculate the hatching and survival rates.

Securing good water flow and oxygenation of the eggs is an important issue. Spreading of the eggs in the incubation trays is important and as well as sufficient water flow.

Seawater treatment:

Water quality needs to be improved for the next season. The absence of water filtering allowed sedimentation of mineral and organic materials on the eggs and at the bottom of the raceway. Two impacts can be highlighted: a risk of hypoxia for eggs and a favorable environment for pathogenic development.

Proposals: Filter the seawater: by sedimentation (extra tank before rearing tanks, particle trap) or by mechanical action (sand filter, bag filters, protein skimmer for a finer filtration).

2.3 Larvae

Handling:

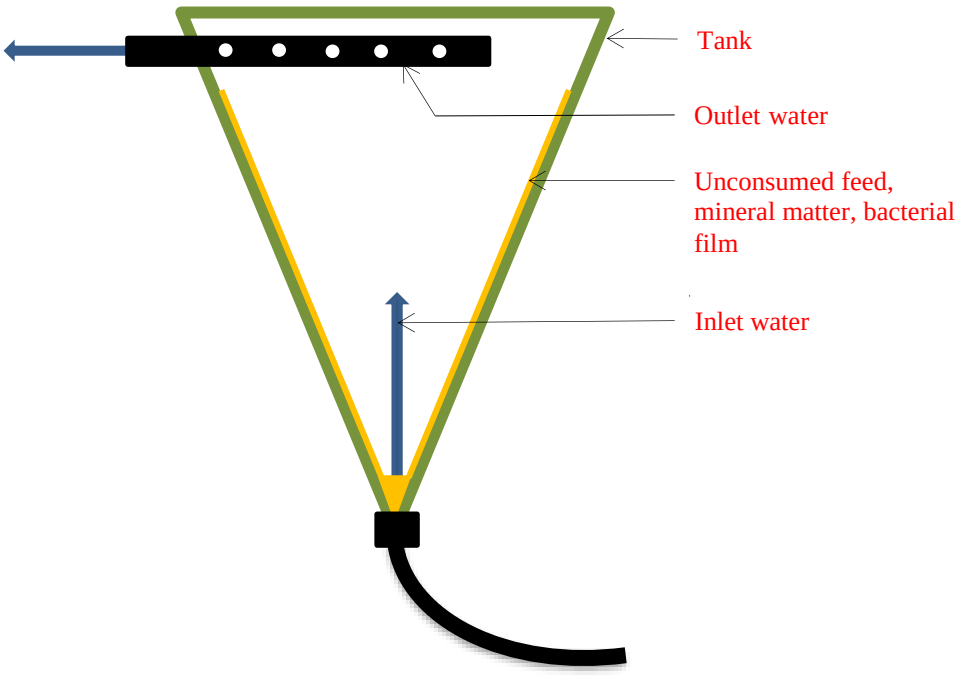
Even if lumpfish larvae are considered as strong larvae, we can wonder if the techniques used to harvest them and clean the silos haven't had an impact on their health.

Proposals: Move the incubation trays directly into the out-growing tanks at the beginning of the hatching period.

Rearing tanks and water treatment:

Many observations have been made about the use of silos for larvae rearing. First of all, it appeared that the water circulation into the tank is inefficient: it didn't allow a good disposal of the organic and mineral matters. It resulted in a spread and accumulation of matter all over the tank walls. No filtration of the seawater amplified this phenomenon. The consequences were that we often had to clean every part of the tank, disturbing lumpfish resting on the walls. Part of the lumpfish was sucked by the siphon-cleaner during cleaning. They were collected on the mesh of the bucket before being released to the silo. The induced stress and extra handling may have had an impact on the mortality rate.

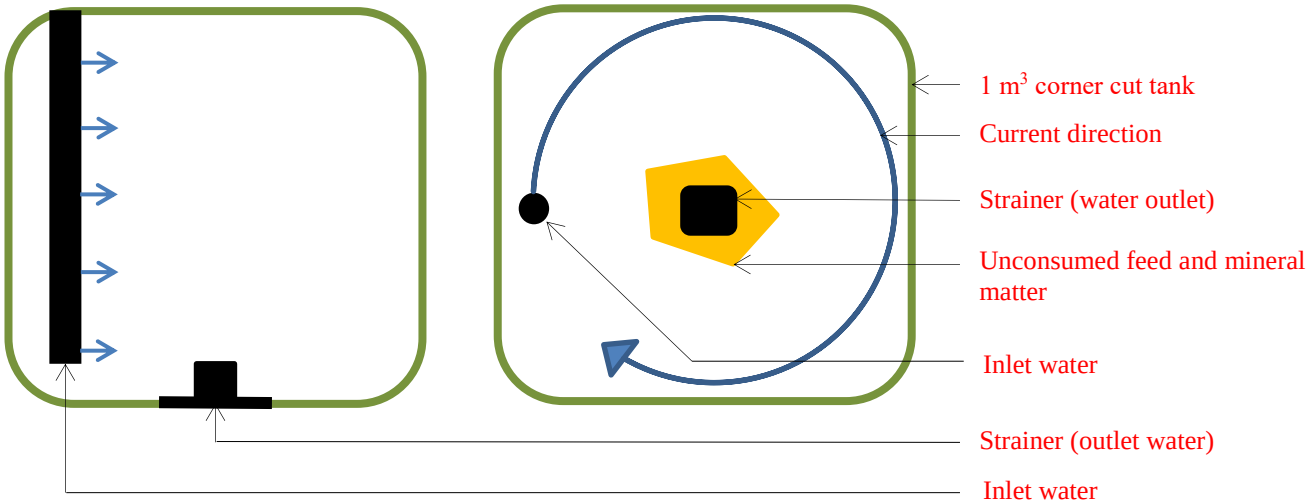
Sectional view of a silo tank used in 2014



Proposals: Use the following recirculated system for the lumpfish larvae (figure below). The main advantage is the circular current, allowing a good concentration and evacuation of the organic and mineral matters. The cleaning can be done more often and effectively, and lumpfish are less disturbed. The full effectiveness of this system would be reached by adding a filtration process to the seawater. We are currently trying to access a borehole providing better quality seawater. In case of success, we could just set up a large and high particle trap. If we still have to use our current seawater supply, we may have to set up a protein skimmer after the particle trap.

Similarly to another lumpfish larvae breeding program (Hafró), it would be interesting to put some small flexible pipes into the tanks to supply more surface for the larvae to rest – "recreating" macro algae where they are used to live on in the wild.

Sectional view and top view of a suggested tank for next season



Feeding:

Feeding could have had an important impact on mortality. Feeding only three times per day during the week and between 1 and 2 times during the weekend appear to be very insufficient for the larvae. Attempts of increasing the feeding times and quantities were made but it induced very intensive cleaning of the silos as the uneaten food was not flushed out properly – creating massive bacteria colonies (plus H_2S production). We stored the feed in a fridge to attempt to preserve it.

Proposals: Use an automated feeder over 24-hr periods. Apply pulse feeding to ensure better feeding of the whole group. Lumpfish prefer moving pray. Therefore ensure good water circulation to keep water floating for longer time and also deeper start feeding tanks may improve appetite. Take the food every day from the fridge. Regular control of the growth rate should be included into the rearing protocol.

Light:

No photoperiod or light intensity was defined for the rearing conditions. The eggs and larvae were exposed to a constant source of light from an experiment going on nearby and to additional light during working hours from time to time.

Proposal: As diurnal species, the fish should probably be exposed to similar light conditions as those of the Icelandic shores during Spring/Summer time. Unless a rearing protocol regarding light has already been approved – in which case it should be applied at Verið.

Task 1.2. Thermal optima and thermal tolerance of juvenile lumpfish (Akvaplan-niva)

Materials and methods

Fish stock and rearing conditions

The lumpfish used in this study were commercially bred at the research facility Troms Marin Yngel, Kraknes, Norway (69° 45" 53" N, 19° 02" 46" E). Eggs and milt were stripped from wild brood fish from local populations caught off the coast of Tromsø. Broodstocks consisted of 20 females and one male for experiments (Ex.) 1, and 20 females and 4 males for Ex. 2. Experiments 1 and 2 were carried out from 11 April – 16 July 2013 and 19 March – 26 June 2014, respectively.

Fertilized eggs were treated with Buffodine (2.5ml l^{-1} water for 10 min) prior to incubation in hatching trays submerged in raceways. This antibacterial treatment was applied twice during the incubation period, and decaying eggs were removed daily. Larvae were transferred to shallow raceways subsequently upon hatching and start-fed with Artemia nauplii enriched with Multi Gain (BioMar, Norway). After 21 days, weaning onto AgloNorse formulated feed (300-500 µm) was initiated, and 14 days later 500-800 µm dry feed pellets (Gemma Wean Diamond, Skretting, Norway) were introduced to the diet. At experimental start-up the lumpfish on average weighed 6.2 (Ex. 2) and 26.5 g (Ex. 1) and pellet size was increased accordingly to size. During the experiments fish were fed a commercial formulated feed (Amber Neptun ST, Skretting, Norway) to satiation.

In order to obtain the appropriate temperatures for the different treatment groups, natural, untreated (ambient) temperature seawater, cooled seawater (Ex. 1, 4.2°C ± 0.4 and Ex. 2, 3.7°C ± 0.4) and heated seawater (Ex. 1, 12.9°C ± 0.7 and Ex. 2, 12.7°C ± 0.8) were mixed in four header tanks. Seawater was pumped from 60 meters below sea level and was UV treated and particle filtered before entering the header tanks. Water for the ambient temperature tanks was supplied directly from the main header tank of the research facility without further temperature manipulation. Each header tank supplied two replicate experimental units, and an additional heater was inserted in holding tanks for groups reared at 16°C in order to increase the water temperature. Temperature was adjusted according to daily temperature measurements in the experimental units, and water flow was set to 20 l min^{-1} in each experimental unit in all groups. Daily routines included re-filling feeders, monitoring fish welfare, measuring temperature- and oxygen levels and flushing tanks.

All fish were reared at ambient temperature until initial temperature adjustment on 16 April 2013 (Ex. 1) and 22 March 2014 (Ex. 2). Juveniles in both experiments were reared at continuous light (LD 24:0) at approximately 90 lux throughout the experimental period.

Experimental design

On 11 April 2013 and 26 March 2014 lumpfish were anaesthetized with FINQUEL (Scanvacc, Norway, 0.1 g l^{-1}) before intra-peritoneal tagging with Trovan ® Passive Integrated Transponder (PIT) tags. Fish were subsequently weighed to the closest 0.2 g and length measured to the closest 1 mm before being randomly distributed in 10 experimental units. Mean initial weight (± S.E.) of tagged fish was 26.5 g (0.6) in Ex. 1 and 6.2 g (0.1) in Ex. 2. Each replicate consisted of 32-37 (Ex. 1) and 26-30 (Ex. 2) tagged lumpfish per unit. A sub-group of approximately 30 and 60 untagged fish per unit (Ex. 1 and Ex. 2, respectively) was added to provide sufficient biomasses in each tank. Fish were allowed to recover after tagging and adapt to new environmental conditions for four days prior to initial temperature adjustment on the 16 April in Ex. 1 and 23 March in Ex. 2. Two replicates of each of the five temperatures 4, 7, 10, 13ºC and ambient temperature were set up in Ex. 1, while Ex. 2 consisted of two replicates of the five temperatures 7, 10, 13, 16ºC and ambient temperature.

The experimental tanks were ten quadratic (70x70x60 cm) gray fiber-glass units (Bia Miljø, Norway), each with a rearing volume of 245 l. Automatic feeders (Billund Aquakulturservice, Denmark) were installed for each tank, and a computer program (SB 1500, Torp Aquateknik, Denmark) estimated daily feeding rates. Oxygen saturation and temperature were measured daily with a hand-held Oxyguard Handy Alpha (Sterner Aquatech, Norway) in the effluent water in each unit. Pure oxygen was added to the holding tanks of the highest temperature treatment groups in order to maintain oxygen saturation above 80%. Average oxygen saturations (% , (± S.D.)) for Ex. 2 were recorded to 103 (3.6), 89 (2.4), 98 (3.4), 91 (4.3) and 89 (4.7) for temperature treatments ambient, 7, 10, 13 and 16 °C, respectively. For Ex. 1 oxygen saturations were recorded to 84 (4.5), 88 (4.8), 89 (6.5), 89 (4.9), 93 (5.8) and 92 (6.4) for temperature treatments ambient, 4, 7, 10, 13 and 16 °C, respectively.

Sampling procedures
Sampling procedures were identical in Ex. 1 and 2. All fish were starved 24 hours prior to sampling. For tagged fish the individual PIT-tag number was recorded prior to weight-measurements to the nearest 0.2 g and length measured to the nearest 0.1 cm. All untagged fish were individually weighed to the nearest 0.2 g to estimate biomass and adjust daily feeding, but were not incorporated in the experiment results. After measurements fish were allowed to recover before being returned to their respective tanks. Five samplings were performed at approximately 3 week intervals, including experiment termination and initiation.

Data analysis and statistical methods
Specific growth rate (SGR% day^{-1}) was calculated according to the formula of Houde and Schekter (1981):

$$SGR = (e^{g} - 1) \times 100$$

where g is the instantaneous growth coefficient defined as $g = (\ln W_2 - \ln W_1) / (t_2 - t_1)^{-1}$ where W_1 and W_2 are the mean wet weights for individually tagged fish in grams at days t_1 and t_2, respectively.
Geometric mean weight (GM) was calculated as

$$GM = \sqrt{(W_1 \times W_2)}.$$

Specific growth rate was regressed against geometric mean weight in the time interval. To avoid pseudoreplication, data collected from tagged fish from both replicates of each temperature were combined. The temperature effect (Q_{10}) on growth rate was calculated according to Schmidt-Nielsen (1990) as

$$Q_{10} = (SGR_2 / SGR_1)^{10(T2 - T1)})$$

where SGR_1 and SGR_2 are specific growth rates for two treatment groups where T1 and T2 are temperatures for the two groups, respectively.

All statistical analyses were performed with STATISTICA™ 10.0 (StatSoft, 2010). A Kolmogorov-Smirnov test (Zar, 1984) was performed to assess normality of distribution. The homogeneity of variances was tested using Levene's F-test, while the effect of temperature on weight and growth rate was tested using a two-way nested Analysis of Variance (ANOVA) (Zar, 1984), where the replicates were nested in temperature treatment groups. In order to observe differences among treatment groups a Student-Newman-Keuls (SNK) multiple comparison test was conducted for significant ANOVAs (Zar, 1984).

Size ranking (initial size rank versus final size rank) was tested using Spearman's rank correlation (Zar, 1984). Parabolic regression (Brett and Groves, 1979) was used to analyze the relationship between size specific growth rate and temperature. The regression was made using the average growth rates of tagged fish in six size groups; <6.5, 6.5 – 11.0, 11.0 - 20.0, 20.0 - 40.0, 100.0 - 110.0 and 120 - 200.0 g. An equal number of fish from the same size class at each temperature was selected in terms of individual wet weight in g. The optimum temperature for growth (T_{optSGR}) was calculated as the zero solution to the first derivate of the parabolic regression:

$$SGR = bT + cT^2 + a$$

where the optimal temperature is calculated as $T_{optSGR} = b / 2c$. Asymptotic S.E. of the mean for T_{optSGR} was calculated based on individual growth data. Geometric mean weight versus daily specific growth rate was analyzed using a two way Analysis of Covariance (ANCOVA). A significance level (α) of 0.05 was used if not stated otherwise.

Results
Effects of temperature on growth
There was no significant difference in initial mean weight between treatment groups (two way nested ANOVA, P = 0.27 and P = 0.89) and mean length within year groups (two way nested ANOVA, P =0.47 and P = 0.50, 2013 and 2014, respectively). The mean weights and lengths of fish in all treatment groups showed significant increase through the experimental period.

In 2014 fish were successively larger and displayed increasing growth rates from 4 through 13 °C from 15 May onwards. Growth rates of treatment groups 4 °C and ambient were significantly lower throughout the experimental period in comparison to all other groups (SNK-test, P < 0.05) in both trials (2013 and 2014, Fig. 3 and 4). In 2013 the two groups did not differ in mean weight until the final sampling when all groups displayed significantly different weights (SNK-test, P < 0.05) and

lengths (SNK-test, P < 0.05), while a significant difference in weight and length persisted among all groups until 10 June in the trial conducted in 2013 (SNK-test, P < 0.05). From this date, fish reared at 13 and 16 °C did not differ in average body weight nor body length, and no significant difference in overall specific growth rate between the two temperatures was observed (SNK-test, P < 0.05, Fig. 4). The overall specific growth rate for the entire experimental period showed a significant increase with increasing temperatures for all other treatments (SNK-test, P < 0.01, Fig. 3 and 4).

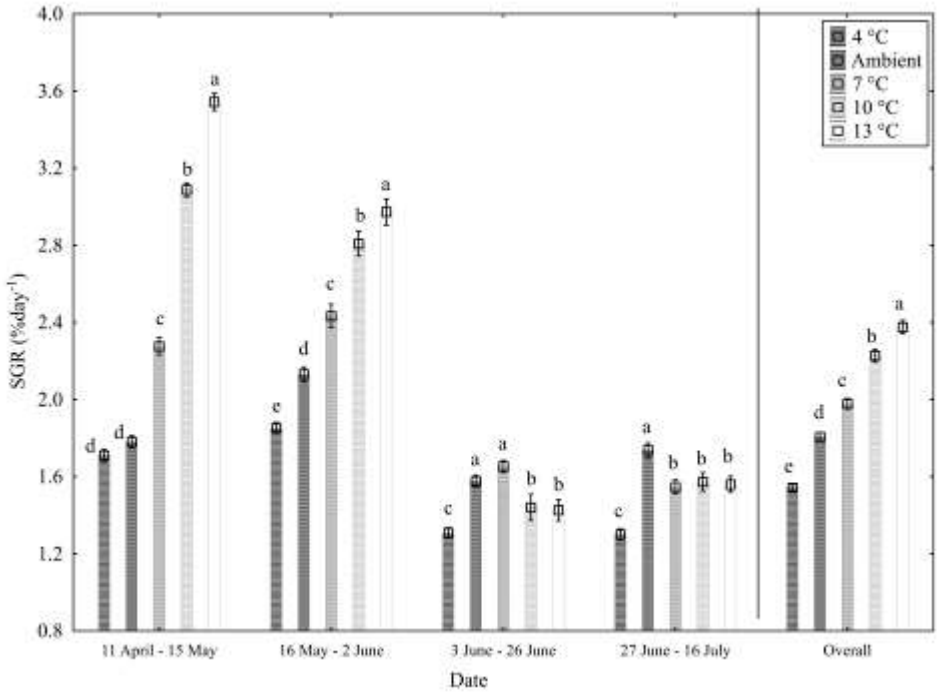


Fig. 3. *Specific growth rates (SGR% day^{-1} ± S.E., N = 68-72) for individually tagged juvenile lumpfish during the experimental period of experiment 1. Different letters indicate significant differences amongst treatments (SNK-test; P < 0.05).*

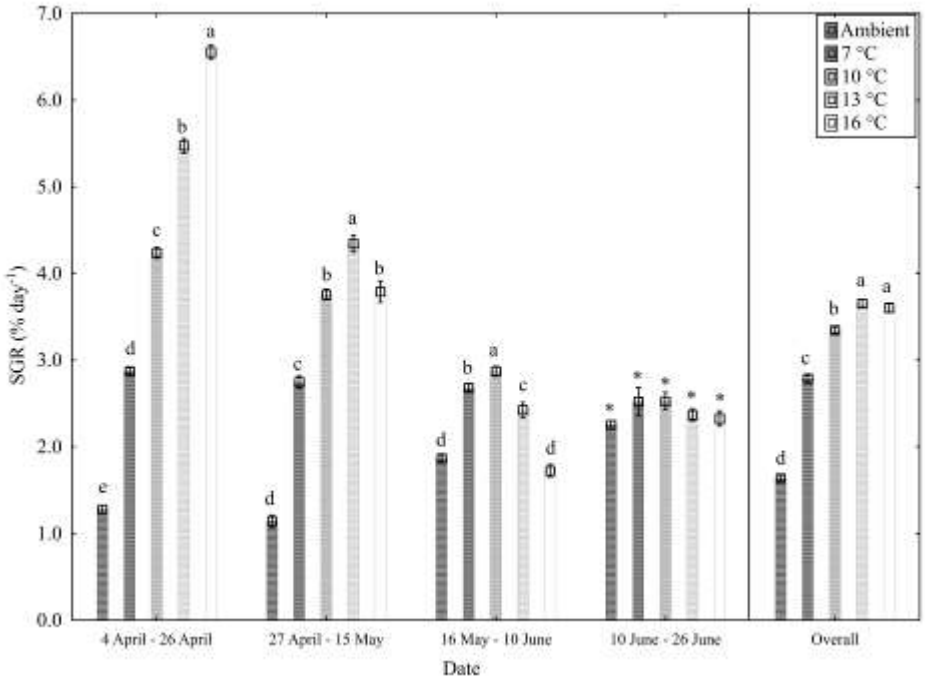


Fig. 4. *Specific growth rates (SGR% day-1 ± S.E., N = 54-60) for individually tagged juvenile lumpfish during the experimental period of experiment 2. Different letters indicate significant differences amongst treatments (SNK-test; P < 0.05).*

Q_{10} of overall specific growth rate for tagged individuals in Ex 1 was 3.16 between 4°C and ambient temperature, 1.48 between ambient and 7°C, 1.09 between 7 and 10°C and 1.15 between 10 and 13°C. In Ex. 2 Q_{10} was 5.10 between 7°C and ambient temperature, 1.90 between 7 and 10°C, 1.40 between 10 and 13°C and 0.95 between 13 and 16°C.

The effect of fish size on growth

Overall, there was a significant interaction between temperature and fish size in both experiments (ANCOVA, $P < 0.01$). Growth rates were plotted against temperature for six size classes of lumpfish (<6.5, 6.5 - 11.0, 11.0 - 20.0, 20.0 - 40.0, 100.0 -110.0 and 120.0 - 200.0 g) to produce parabolic regressions (Fig. 5). Temperature optimums for maximum specific growth rate (T_{optSGR}) declined with increasing body weight for all treatment groups, T_{optSGR} ± S.E was estimated to 15.7 ± 1.0°C for 11.0 - 20.0 g, 16.1 ± 1.4°C for 20.0 - 40.0 g, 13.1 ± 1.8°C for 100.0 - 110.0 g and 8.9 ± 0.1°C for 120.0 - 200.0 g. It was not possible to calculate T_{optSGR} for the two smallest groups (< 6.5 g and 6.5 - 11.0 g, Fig. 5) as maximum growth was not reached in the temperature range investigated.

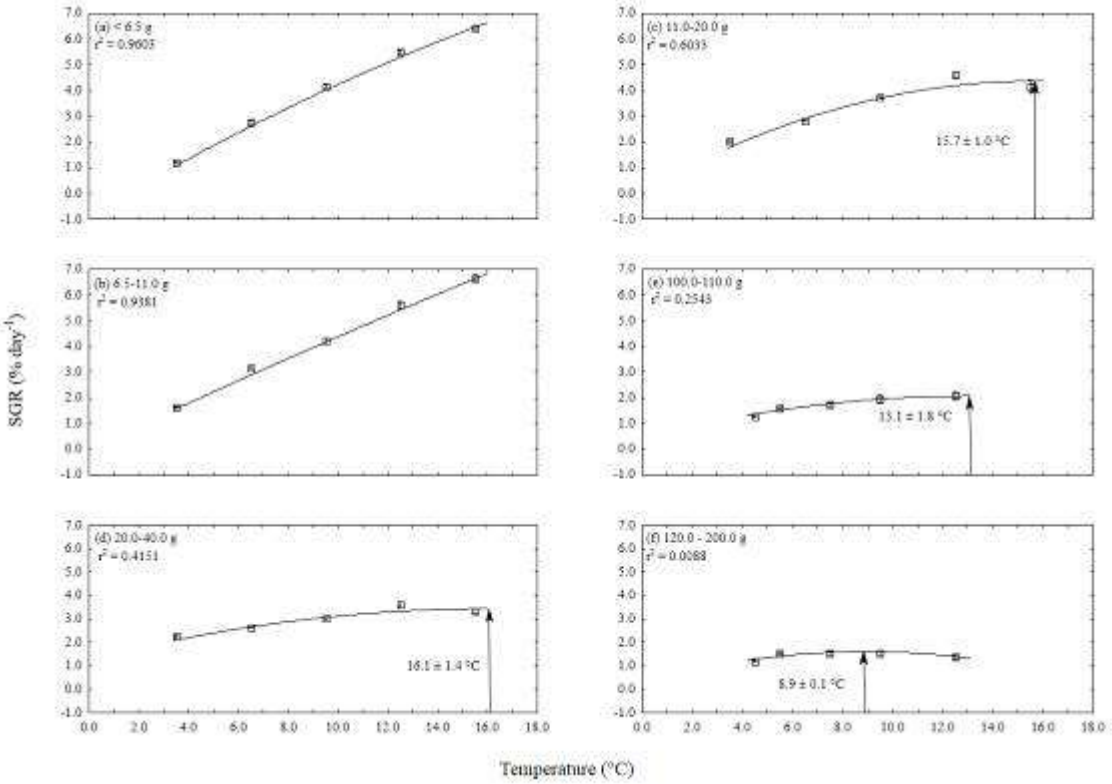


Fig. 5. *Changes in specific growth rate with temperature for six different size classes of juvenile lumpfish. Size classes a, b, c and d originate from Ex. 2 while size classes e and f are from Ex. 1. The lines represent the least-squares second order polynomial fit to the data: $SGR = aT + bT^2 + c$ where SGR = Specific growth rate, T = temperature, and a, b, and c are constants determined by the regression. When possible the optimum temperature for specific growth rate (T_{optSGR}, ± S.E) was calculated from the first order derivate of the parabolic regressions. Temperature optimums are indicated by arrows in the figure.*

Size ranking and size distribution

In Ex. 1 a significant size rank correlation (initial weight *v*. final weight) was maintained at all temperature regimes (Spearman rank correlation, $r_{Sp} > 0.50$ and SNK-test, $P < 0.05$). Size rank correlation was highest for the coldest treatment groups 4°C and ambient ($r_{Sp} = 0.95$ and $r_{Sp} = 0.93$, respectively) and lowest for the warmest treatment group 13°C ($r_{Sp} = 0.67$). Initial vs. final growth rates were only correlated for ambient temperature and correlation was negative for 4 and 13°C ($r_{Sp} = -0.05$ and $r_{Sp} = -0.002$).

In Ex. 2, there was no trend in terms of size rank correlation (initial weight *v*. final weight) with the exception of the 10°C group where a negative correlation ($r_{sp} = -0.277$, $P < 0.05$) was found.

Discussion

The results of the present study show that mean weights, lengths and growth rates of juvenile lumpfish are significantly influenced by temperature and fish size. Present data indicate very high growth potential for juvenile lumpfish reared at near optimum temperature for growth as juveniles can tenfold their mean weight in only 76 days. The overall highest growth rates were obtained at 13 and 16°C (3.65 and 3.60% day^{-1}), and decreased stepwise with declining temperature with the exception of treatments 13 and 16°C, which displayed insignificantly different overall results. The poor growth close to 4°C produced fish with a final mean weight 81% lower than fish reared at 13 and 16°C.

Juvenile lumpfish spend their early stages in the physically challenging intertidal zone where they are reported to grow rapidly before migrating to colder feeding grounds (Hedeholm et al., 2014) where they may better exploit the differences in temperature. Larger specimens are assumed to leave the temporal zone before smaller fish (Myrseth, 1971). Studies of both wild and cultured larval and juvenile lumpfish growth patterns show a rapid increase in growth rate from mid-July to August, before decreasing in August-September (Benfey and Methven, 1986; Moring, 2001; Ingolfsson and Kristjansson, 2002). This may be correlated to the declining T_{optSGR} and specific growth rates observed in the present study, and correlates to findings for other juvenile fish species (Fonds et al., 1992, Imsland and Jonassen, 2001).

According to the results from the present study, juvenile lumpfish display a large ontogenetic variation in optimum temperatures for growth with increasing body weight, demonstrated by high growth rates over a large temperature interval. Larger fish are seemingly less affected by temperature than smaller fish when reared close to T_{optSGR}, as the parabolic regression curve flattens out close to T_{optSGR} with increasing fish weight (Fig. 5).

Due to the rapid downwards shift in temperature preference with increasing size, the overall growth rate was insignificantly different between temperature groups 13 and 16 °C. This is in accordance with the general pattern of fish growth with increasing body size (Brett and Groves, 1979; Jobling, 2010). However it should be noted that the observed decrease in T_{optSGR} of 7°C from 11 to 200 g for lumpfish is somewhat higher than the presumption of a 1-2°C decrease in temperature preference for fish weighing 10-500 g as presented by Cuenco et al. (1985).

When reared at temperatures close to T_{optSGR}, the rapid growth of juvenile lumpfish produces a fish that is potentially large enough to participate in co-cultures with salmon within less than a year. Juvenile lumpfish persist to actively feed and grow at temperatures close to 4°C, providing a head start in comparison to the slower growing farmed wrasse at similar temperatures (Skiftesvik et al., 2013), and may have the potential to survive winters in net-pens with salmon, even in the northernmost localities due to the broad geographical distribution of the species in the wild (Cox and Anderson, 1922; Andrijasev, 1954; Blacker, 1983). Despite this, the lack of sub-optimal temperatures for size groups < 11.0 g causes biological nonsense in the model for the smallest fish. In order to establish models for temperature related growth for < 11.0 g juvenile lumpfish, further growth studies at temperatures above 16 °C must be undertaken.

The inclusion of the pilot study may cause inconclusive results as water temperatures for the groups reared at ambient conditions were different between the two years. This was corrected for as temperature, rather than group, is the dependent factor in this study, though little is known about maternal effects on growth in lumpfish.

An increasing occurrence of cataracts was observed in high-temperature treatments towards experiment termination may have been the cause for the observed mortality in the 13 and 16°C treatments. As temperature induced stress may increase the occurrence of cataracts (Bjerkås and

Sveier, 2004), this may have prevented the lumpfish from feeding, potentially affecting overall growth rate and causing mortality due to starvation.

Conclusions

Temperature optimum for growth in juvenile lumpfish decreased with increasing body weight, and a significant ontogenetic variation in optimum temperature for growth in juvenile lumpfish was observed. For size groups 11.0-20.0, 20.0-40.0, 100.0-110.0 and 120.0-200.0 g parabolic regressions suggested optimum temperatures for growth of 15.7, 16.1, 13.1 and 8.9°C, respectively. The temperature optimum for growth in size groups below 11 g was not reached in the investigated temperature interval (4-16°C), but an optimum temperature above 16°C is suggested. Growth rate declined with increasing size for all treatment groups, whereas temperature tolerance increased with increasing body size. In order to fully utilize the effect of temperature on growth in juvenile lumpfish, the utilization of "temperature steps", a reduction in temperature with increasing body size is suggested.

References (Task 1.2)

Andrijasev, A.P., 1954. Fishes of the northern seas of the U.S.S.R. Israel Program for Scientific Translations, Jerusalem.

Benfey, T.J., Methven, D.A., 1986. Pilot-scale rearing of larval and juvenile lumpfish (*Cyclopterus lumpus* L.), with some notes on early development. Aquaculture. 56, 301-306.

Bjerkås, E., Sveier, H., 2004. The influence of nutritional and environmental factors on osmoregulation and cataracts in Atlantic salmon (*Salmo sala*r L). Aquaculture. 235, 101-122.

Blacker, R.W., 1983. Pelagic records of the lumpsucker, *Cyclopterus lumpus* L. J. Fish Biol. 23, 405-417.

Brett, J.R., Groves, T.D.D., 1979. Physiological energetics. Fish Physiol. 8, 279-352.

Cox, P., Anderson, M., 1922. A study of the lumpfish (*Cyclopterus lumpus* L.). Contrib. Can. Biol. Fish. 1, 1-20.

Cuenco, M.L., Stickney, R.R., Grant, W.E., 1985. Fish bioenergetics and growth in aquaculture ponds. II: Effects of interactions among size, temperature, dissolved-oxygen, unionized ammonia and food on growth of individual fish. Ecol. Modell. 27, 191-206.

Fonds, M., Cronie, R., Vethaak, A.D., Vanderpuyl, P., 1992. Metabolism, food-consumption and growth of plaice (*Pleuronectes-Platessa*) and flounder (*Platichthys-Flesus*) in relation to fish size and temperature. Neth. J. Sea Res. 29, 127-143.

Hedeholm, R., Blicher, M.E., Grønkjær, P., 2014. First estimates of age and production of lumpsucker *Cyclopterus lumpus* in Greenland. Fish. Res. 149, 1-4.

Houde, E.D., Schekter, R.D., 1981. Growth rates, rations and cohort consumption of marine fish larvae in relation to prey concentrations. Rapp. p.-v. réun. - Cons. int. explor. mer 178, 441-453.

Imsland, A.K., Jonassen, T.M., 2001. Regulation of growth in turbot (*Scophthalmus maximus* Rafinesque) and Atlantic halibut (*Hippoglossus hippoglossus* L.): aspects of environment × genotype interactions. Rev. Fish Biol. Fisher. 11, 71-90.

Ingolfsson, A., Kristjansson, B.K., 2002. Diet of juvenile lumpsucker *Cyclopterus lumpus* (Cyclopteridae) in floating seaweed: Effects of ontogeny and prey availability. Copeia. 2002, 472-476.

Jobling, M., 2010. Are compensatory growth and catch-up growth two sides of the same coin? Aquacult. Int. 18, 501-510.

Moring, J.R., 2001. Intertidal growth of larval and juvenile lumpfish in Maine: A 20-year assessment. Northeast. Nat. . 8, 347-354.

Myrseth, B., 1971. Fekunditet, vekst, levevis og ernæring hos *Cyclopterus lumpus* L. . University of Bergen, Norway, pp. 113 bl.

Sayer, M.D.J., Reader, J.P., 1996. Exposure of goldsinny, rock cook and corkwing wrasse to low temperature and low salinity: survival, blood physiology and seasonal variation. J. Fish Biol. 49, 41-63.

Schmidt-Nielsen, K., 1990. Temperature effects. Cambridge University Press, New York.

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

Zar, J.H., 1984. Biostatistical analysis, 2nd edn. ed. Englewood Cliffs, N.J., New Jersey.

Task 1.3 Sperm quality parameters, short and long term (cryopreservation) storage of milt

Recent research has shown that lumpfish *Cyclopterus lumpus* (Linnaeus, 1758), a pelagic fish species naturally found in the North Atlantic Ocean, is an effective cleaner fish in combating infestation with sea lice *Lepeophtheirus salmonis* (Krøyer, 1838) and *Caligus elongates* (Nordmann, 1832) among salmonids in aquaculture, which is a growing problem in that industry [1, 2]. A few companies have, therefore, started commercial production of lumpfish juveniles in Norway. In the Faroe Islands,

Fiskaaling P/F has launched an R&D project aiming to establish year-round production of lumpfish juveniles.

An indication to see if a lumpfish male is mature is that they become reddish in their appearance, this is not always a guaranty that stripping will succeed. Therefore a check is done first by doing easy pressures on the fish stomach. If milt is appearing from the testis, the male is given an anaesthetic (benzocain) and when unconscious, the milt is collected using syringes while easy pressing the stomach area. Stripping most often results in small volumes of milt, from our own data (2014) stripping volume has ranged from 0.1 to 3.2 ml (n= 18). This is therefore a limitation when several females are ready for stripping, which needs to be initiated as soon as their bulge is swelling, otherwise they will let go of all the eggs into the water, and no artificial fertilization can take place.

Cryopreservation of lumpfish milt could solve the issues mentioned above, and ensuring a year-round supply of male gametes for a sustainable juvenile production. Therefore, an effective protocol has been established based on earlier cryopreservation protocols for other marine fish species [4-7], and from our earlier findings in the attempt to cryopreserve lumpfish milt.

Our protocol to cryopreserve lumpfish milt is based on tests with different combination of the chemical composition of Mounib sucrose- based medium as the diluent, along with DMSO as the cryoprotectant. Two freezing rates have been tested as well.

The motility percentage of fresh and cryopreserved milt (in triplicates), stripped from six lumpfish males has been determined by a computer assisted sperm analyser (CASA) system. This data demonstrates our results and by comparing fresh and cryopreserved milt using our developed protocol, cryopreserved milt has a 23.14 lesser motility percentage then fresh milt (Fig. 6).

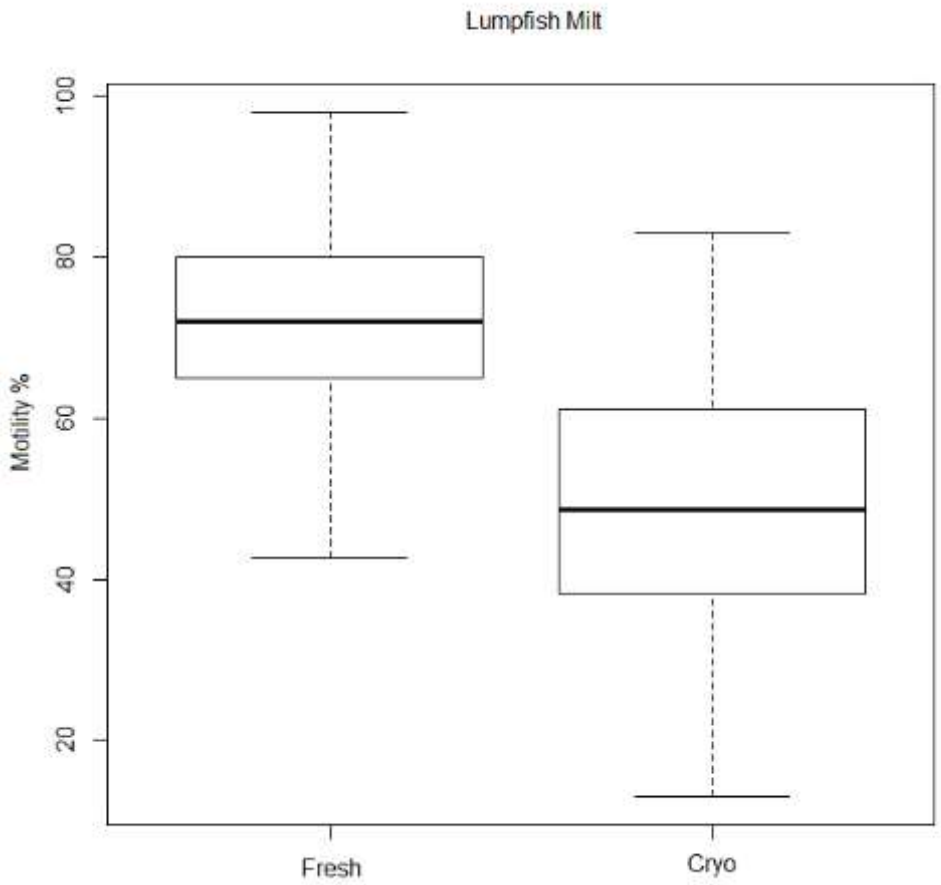


Fig. 6. *Shows data of the motility % of fresh and cryopreserved milt from six lumpfish, tested with type II Wald Chi-Squared statistical analysis, and revealing that cryopreserved milt has a 23 % lesser motility compared to fresh milt using our developed cryopreservation protocol.*

References (Task 1.3):

1. Willumsen, L., Fangst av rognkjeks (*Cyclopterus Lumpus* L.) og rognkjeks som lusespiser på laks. 2001, Gildeskål Forskningsstasjon a.s. GIFAS.

2. Vestvik, N.S., M, Forberedelse og bruk av en lusespiser i laksemerd. Rognkjeks ABC 1st Edition 2012.
3. Davenport, J., Synopsis of Biological Data on the Lumpsucker, *Cyclopterus Lumpus* (Linnaeus, 1758). 1985: Food & Agriculture Org.
4. Suquet, M., et al., Cryopreservation of sperm in marine fish. Aquaculture Research, 2001. 31(3): p. 231-243.
5. Rideout, R.M., E.A. Trippel, and M.K. Litvak, The development of haddock and Atlantic cod sperm cryopreservation techniques and the effect of sperm age on cryopreservation success. Journal of Fish Biology, 2004. 65(2): p. 299-311.
6. Ding, F., et al., Cryopreservation of sperm from Atlantic halibut (*Hippoglossus hippoglossus*, L.) for commercial application. Cryobiology, 2011.
7. Zhang, Y.Z., et al., Cryopreservation of flounder (*Paralichthys olivaceus*) sperm with a practical methodology. Theriogenology, 2003. 60(5): p. 989-996.

Work package number			2		**Start date or starting event:**		6				
Work package title			***Effective use of lumpfish for delousing salmon***								
Participant number	**1**	2	3	4	5	6	7				
Person-months per participant:	**5.5**	1.5	0.25	0.25	0.25	0	3				

Objectives

- Define the optimal size ratio between cleaner fish and target fish (Atlantic salmon) to induce effective biological delousing.
- Collect information on behaviour of lumpfish and Atlantic salmon when held mutually in tanks.
- Define attractive characteristics and establish protocols for genetically-assisted breeding programs for lumpfish.

Description of work

This Work Package consists of two Tasks:

Task 2.1 Feeding behaviour and population dynamics of lumpfish

A decisive factor for the use of lumpfish is to ensure that the fish become accustomed to eating lice from infested fish rather than eating other food sources such as formulated feeds delivered to the cages. Adaptation of lumpfish size in relation to the size of the salmon and pellets are crucial. It is therefore, important to develop protocols for the successful coexistence of lumpfish and Atlantic salmon under production conditions. This study will attempt to develop such protocols under semi-commercial conditions. The goal is to identify and quantify factors that affect feeding behaviour and population dynamics of lumpfish maintained in enclosed net pens. Further, studies will be undertaken to elucidate behavioural interactions between Atlantic salmon and lumpfish.

Task 2.2 Development of lumpfish breeding programme and search for QTLs

It is critical that maximum number of lumpfish actively eats lice of Atlantic salmon when reared together. Initial trials in Norway have shown that there is a large individual difference in sea lice grazing activity of lumpfish. In Task 2.2 these differences will be quantified and investigated to determine to what extent these differences are genetically based. By the use of published microsatellite markers and commercially available software an initial breeding programme for lumpfish will be developed. We will then screen for quantitative trait loci (QTL) in lumpfish by relating traits investigated in Task 1.1 (temperature tolerance) and Task 2.1 (sea lice grazing activity) in individual tagged fish. Similar approach has been used by the project group in other species (Imsland et al. 2011; Küttnet et al. 2011).

Role of the partners in WP2

Partner 1 (WP leader, APN) will be the participant with the main responsibility for WP2 but will cooperate with the industrial partners 3-5 in the tasks in WP2. Once established partner 1 (APN), partner 2 (FISKA) and partner 7 (HÓLAR) will use the feeding behaviour protocols for lumpfish developed in Task 2.1 for field tests at all industrial partners. Task 2.2 will be carried out by Partner 1 in cooperation with partners 2, 3 5, and 7.

Milestones

- M2.1. Protocols for the successful coexistence of lumpfish and Atlantic salmon (Month 12).
- M2.2. Optimal feeding regime for successful biological delousing of salmon (Month 24).
- M2.3. Lumpfish breeding programme and possible QTLs (Month 30).

WP2 Progress 2013-18

Task 2.1 Feeding behaviour and population dynamics of lumpfish (Akvaplan-niva)

The first part of this task i.e. studies to elucidate behavioural interactions between Atlantic salmon and lumpfish was performed during winter 2013-14. Next step was to investigate different sizes of lumpfish in relation to the size of the salmon and this was investigated in under semi commercial conditions during 2015 and is reported here (Trial 3). Below is the description of all three behavioural trials with Atlantic salmon and lumpfish.

Trial 1. Lumpfish behaviour with and without Atlantic salmon present (Akvaplan-niva)

Materials and methods

Atlantic salmon

The Atlantic salmon, *Salmo salar* L. Salmonidae, used in the study was 0+ 11G Atlantic salmon produced at Sundsfjord smolt AS and delivered to Gildeskål Research Station (GIFAS), Nordland, Norway in September 2012. The fish were transferred to small-scale sea pens in March 2013 and remained in those sea pens until the trial period. The salmon had an average initial mean (± SD) weight of 3500 g ± 350 g on 23 November 2013 when they were graded and 400 fish were distributed among four sea pens (5x5x5 m).

Lumpfish

Sexually mature wild lumpfish, *Cyclopterus lumpus* Cyclopteridae, were caught locally by Arctic Cleanerfish AS, Lofoten, Norway with nets during early spring, 2013. Natural spawning and fertilization occurred from 26 May onwards and first hatching occurred at around 240 day degrees. The juveniles were initially fed with natural plankton and Gemma micro starter feed (Skretting, Stavanger, Norway) by hand to ensure adequate feed intake. The lumpfish juveniles (age 0-group, mean weight (± SD) of 7.8 ± 1.5 g) were transferred from Arctic Cleanerfish to GIFAS in November 2011 and reared in one sea pen (5x5x5 m) at GIFAS site Langholmen, Nordland, Norway until the start of the trial (1 December 2013).

Experimental set-up

At the start of the trial (1 December 2013) the Atlantic salmon were counted and randomly distributed between four sea pens of 125 m^3 (5x5x5m) with mesh size of 10 mm, with 100 fish in each sea pen. Two sea pens of Atlantic salmon were further stocked with 40 juvenile lumpfish. In addition 40 lumpfish were reared without salmon in two sea pens of 125 m^3 (mesh size 10 mm) as control groups. The lumpfish had an average initial mean (± SD) weight of 11.2 ± 2.1 g. To investigate possible differences in sea lice infection two additional sea pens (5x5x5 m) were stocked with salmon only. The study lasted for 56 days and was terminated 25 January 2014. The fish in the sea cages were fed with a commercial salmon feed (7 mm, Biomar CPK, Aarhus, Denmark) to satiation by hand. One distinctive meal was fed each day and feed intake calculated on a daily basis. Some feed pellets were crushed on a daily basis to ensure that the lumpfish had a food source due to lack of natural food sources in winter.

Behavioural observations

Intra and interspecific behaviour was recorded. The behaviour of lumpfish in the sea cages was assessed by underwater camera technology (SM Remotes, Bergen, Norway) at regular intervals throughout the trial period. Behavioural observations commenced two weeks after the cages were established to allow for an acclimation period. Behaviour was classified (Table 1) by recording for 30 second intervals the principal activity of individual fish. Thirty fish were observed during each observation time period, giving a total of 30 activity records, on each observational time point. All lumpfish were individually tagged with a floy tag. Each floy tag had a unique number on the shaft and

this was used to identify each tagged fish. In addition eight different colours of tag were used to assist in individual identification (white, white with black strip, blue, green, yellow, red, black and orange) (five of each for eight fish). There was no algae growth on the tags so they were always visible. The tags were sited on the highest point of the back just beside the dorsal array. As each fish was identified either with camera or directly (if on or near the surface) the number was normally seen. If the number was not visible, then another fish would be observed until all 30 recordings were achieved by different fish. Behavioural observations were classified using behavioural indices as used by Tully et al. (1996).

Table 1. Behavorial types and detailed description of lumpfish behaviour in sea pens.

Behaviour type	Description
Feeding on net fouling	Observed feeding on side of nets
Feeding on free-swimming organisms	Observed feeding on organisms entering the sea pen
Actively competing for pellets	Observed competing with Atlantic salmon for pellets
Eating pellets outside salmon feeding zone	Observed eating pellets on the periphery of feeding Atlantic salmon
Eating from feeding stations	Observed eating from offered feed stations
Resting	Observed resting in folds of the sea pen net
Hovering	Near motionless in sheltered areas of the sea pen
Swimming along net side	Swim up or down at the sea pen net
Swimming at observer	Observing diver
Swimming in between Atlantic salmon	Observed swimming amongst Atlantic salmon
Shoaling	Groups of Lumpfish swimming together
Inspecting Atlantic salmon	Swimming along individual Atlantic salmon
Cleaning Atlantic salmon	Displaying cleaning of *L. salmonis* from Atlantic salmon
Other	Other behaviour

Assessment of sea lice infestation levels on salmon

At the start of the trial and every second week during the trial, a lice count was undertaken in the sea cages with salmon. Thirty salmon from each salmon cage were sedated and any lice present were recorded. Sea lice infestations were natural settlements and not experimental challenges.

Statistics

Differences in behaviour between the two experimental groups were tested using a chi-square test (Zar 1984) with lumpfish behaviour in the control cages as "expected" when performing the test. Possible differences in sea lice infections were tested with one-way analysis of variance (ANOVA). Significant differences revealed in ANOVA were followed by a Tukey's multiple range tests to determine differences among experimental groups. A significance level (α) of 0.05 was used if not stated otherwise.

Results

From the observations recorded over time, lumpfish exhibit a limited palette of behavioural traits. In the sea pens without Atlantic salmon (Fig. 7A) the majority of daylight time was spent actively foraging for food (over 50% of all recorded observations over time).

In the sea pens where lumpfish and Atlantic salmon were reared together (Fig. 7B) more variety of types of behaviour was recorded. However, the dominant types of behaviour observed were feeding related (approximately 60%) and no differences were seen in overall foraging behaviour ($\chi^2 < 2.0$, $df = 1$, $P > 0.20$) or in the time spent feeding on net fouling ($\chi^2_1 < 3.0$, $df = 1$, $P > 0.05$). If not feeding or foraging, the fish were found to be either resting within the floating seaweed within the cage or hovering just under the weed. The lumpfish spent more time resting when reared alone ($\chi^2 = 6.1$, $df = 1$, $P < 0.05$, Fig. 7B).

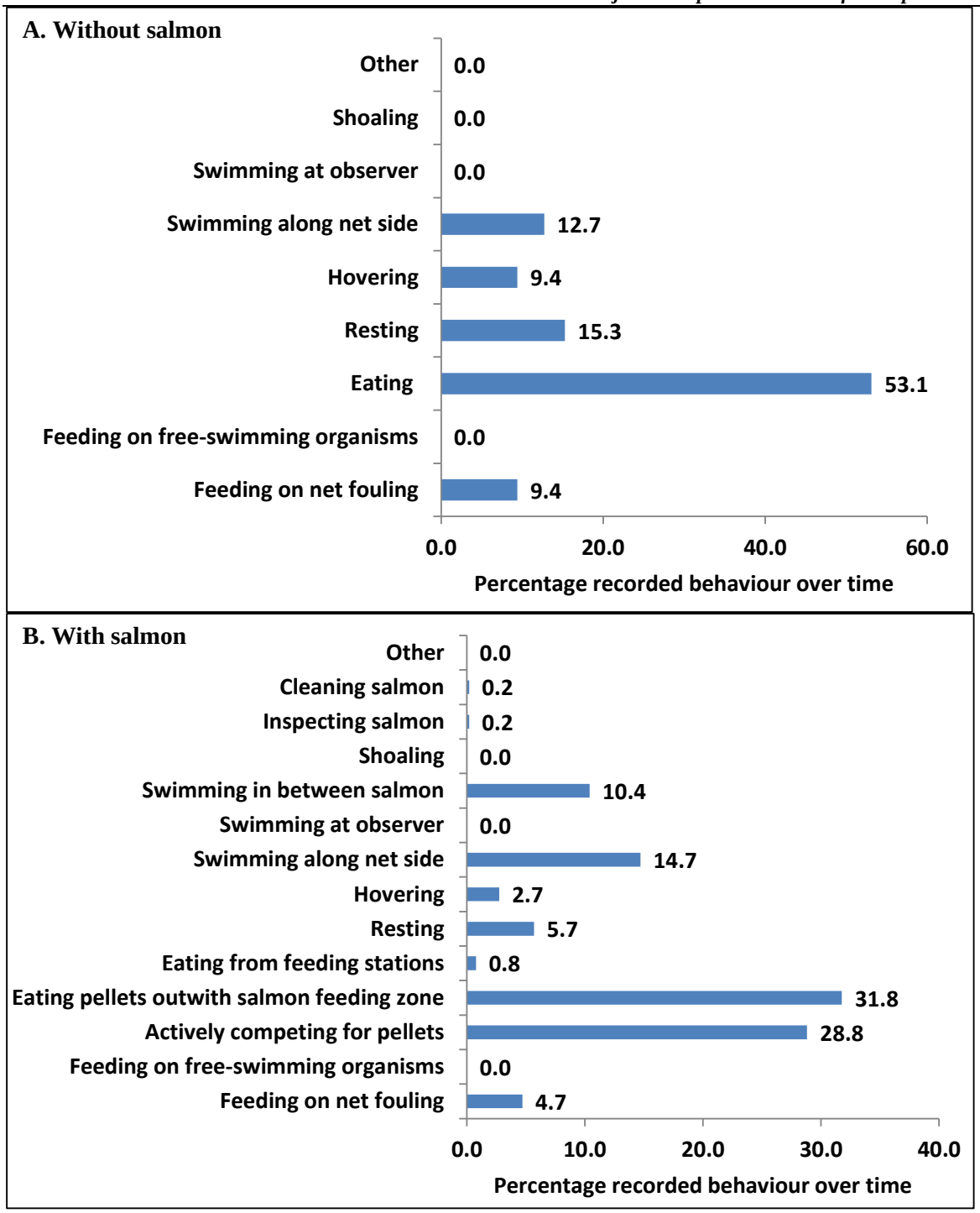


Fig 7. *(A) Behaviour of lumpfish in sea pens without (A) Atlantic salmon present and (B) with Atlantic salmon present*

Lumpfish were found to compete with the Atlantic salmon for access to pellets during feeding and observed swimming within the shoal of salmon when feeding commenced. Direct observations of lumpfish cleaning *L. salmonis* of Atlantic salmon were seldom (0.2% of the total time), but in spite of this differences were seen in Atlantic salmon sea lice infection levels during the trial period as significantly lower infection levels were seen in the sea cages with lumpfish in December and January ($F > 5.5$, $df = 58$, $P < 0.05$, Fig. 8).

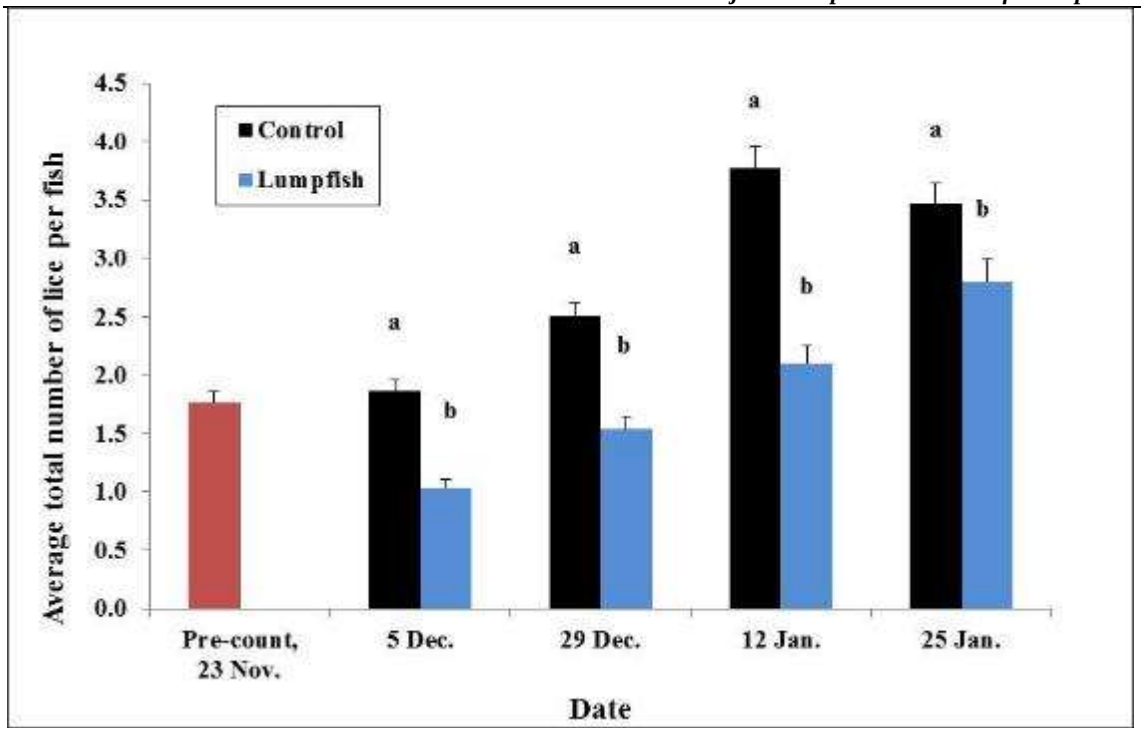


Fig. 8. *Average number of sea lice per salmon in sea cages with lumpfish present (lumpfish) and without lumpfish (control) during the trial period. Mean values which do not share a letter were found to be significantly different by ANOVA and by Tukey`s multiple range test*

Lumpfish showed opportunistic feeding behaviour and switch quickly from grazing from nets to feeding on free-swimming organisms to feeding on salmon pellets. Antagonistic behaviour between Atlantic salmon and lumpfish was not observed during the whole experimental period and no mortality was seen in either species.

Discussion

Based on present data the feeding behaviour in lumpfish in sea pens can be classified as strongly opportunistic and the fish do not restrict themselves or rely on a single food source if others are present. This omnivorous feeding behaviour has been reported with wild juvenile lumpfish (Ingólfsson and Kristjánsson 2002; Vandendriessche et al. 2007). Present data indicate that the majority of daylight time is spent foraging for food and if not feeding or foraging, the fish were found to be either resting within the floating seaweed within the cage or hovering just under the weed. In the absence of Atlantic salmon lumpfish spent more time resting.

Similar feeding behaviour as found in this study is seen in wild lumpfish. Killen et al. (2007) studied behaviour of wild juvenile lumpfish and found that the juvenile fish forage using one of two modes: they can actively search for prey while swimming or they can 'sit-and-wait' for prey while clinging to the substrate using a ventral adhesive disk. The study suggested that juvenile lumpfish forage in a manner that reduces activity and conserves space in their limited aerobic scope. The authors noted that this behavioural flexibility is of great benefit to this species, as it allows young individuals to divert energy towards growth as opposed to activity. Present data are in line with these observations.

Further, no antagonistic behaviour between the two species was observed and the two species seemed to co-exist along each other in the sea pens. Wild lumpfish and Atlantic salmon share feeding grounds and Sheedan et al. (2012) found large numbers of juvenile and adult lumpfish together with Atlantic salmon when sampling Atlantic salmon with surface trawl in the Northwest Atlantic (Labrador Sea). In the Northeast Atlantic (Norwegian Sea) lumpfish and Atlantic salmon inhabit much the same areas (Hansen and Jacobsen 2000, Bjelland and Holst 2004) feeding on similar prey items (Jacobsen and Hansen 2001; Vandendriessche et al. 2007). Holst (1993) investigated the geographic distribution of lumpfish in the Norwegian Sea and found that lumpfish is widely distributed throughout the Norwegian Sea, with the largest concentrations occurring in the areas close to the polar front i.e. north of 72°N. In their study of the spatial and temporal distribution of post-smolts of Atlantic salmon in the Norwegian Sea, Holm et al. (2000) found that post-smolts appear to follow the main surface currents

northwards into the Norwegian Sea where they spread in a fan-like distribution over an area covering most of the international waters between the exclusive economic zone (EEZ) of Norway, the Faroes and Iceland up to about 73–75°N i.e. similar to the geographic distribution of lumpfish (Holst 1993). The fact that lumpfish and Atlantic salmon share feeding grounds in the wild may help to explain the non-antagonistic behaviour seen between the two species in this study.

Cleaning behaviour is considered to be a classical example of mutualism. In the present trial lumpfish spent only limited amount of time eating *L. salmonis* off Atlantic salmon, but this was seemingly enough to influence the the sea lice infection levels as significantly lower sea lice infection levels was seen on Atlantic salmon when lumpfish was present. Imsland et al. (2014) investigated the efficacy of lumpfish grazing on attached *L. salmonis* on Atlantic salmon in sea pens during summer (June to August) when *L. salmonis* are more common on Atlantic salmon. There were clear signs of lumpfish grazing of *L. salmonis* with significantly lower average numbers of pre-adult, mature males and females stages of *L. salmonis* per Atlantic salmon. Results of gastric lavaging of lumpfish showed that 28% of all lumpfish were found to have ingested *L. salmonis* on the last sampling day (Imsland et al. 2014). Very limited information exists on the stomach content of wild lumpfish, but *L. salmonis* have been reported in the stomachs of wild juvenile lumpfish (Ingólfsson and Kristjánsson 2002) and other copepods are a substantial part of the diet of juvenile lumpfish (Ingólfsson and Kristjánsson 2002; Vandendriessche et al. 2007). Moreover, wild Atlantic salmon in the sea can have a large number of adult *L. salmonis* attached on their surface during winter (Jacobsen and Gaard 1997). Given the opportunity lumpfish will effectively graze on *L. salmonis* on Atlantic salmon (Imsland et al. 2014; present study) and it may be speculated that this parasite grazing behaviour has developed between the two species in a similar way as seen for parasite cleaning fish in the tropics (Grutter 1995; Clague et al. 2011).

References (Task 2.1)

Bjelland O, Holst JC (2004) Other fish species and fish communities. In: Skjoldal H (ed) The Norwegian Sea ecosystem. Tapir Academic Press, Trondheim, Norway, pp. 357-369

Clague GE, Cheney KL, Goldizen AW, McCormick MI, Waldie PA, Grutter AS (2011) Long-term cleaner fish presence affects growth of a coral reef fish. Biol Lett 7:863-865

Grutter AS (1995) Relationship between cleaning rates and ectoparasite loads in coral reef fishes. Mar Ecol Prog Ser 118:51-58

Hansen LP, Jacobsen JA (2000) Distribution and migration of Atlantic salmon (*Salmo salar* L.) in the sea. In: Mills D (ed) The ocean life of Atlantic salmon—environmental and biological factors influencing survival. (Mills, D., ed). Blackwell Science, Oxford, UK, pp. 75-87

Holm M, Holst JC, Hansen JP (2000) Spatial and temporal distribution of post-smolts of Atlantic salmon (*Salmo salar* L.) in the Norwegian Sea and adjacent areas. ICES J Mar Sci 57:955-964

Holst JC (1993) Observations on the distribution of lumpsucker (*Cyclopterus lumpus*, L.) in the Norwegian Sea. Fish Res 17:369-372

Ingólfsson A, Kristjánsson BK (2002) Diet of juvenile lumpsucker (*Cyclopterus lumpus*) in floating seaweed: effect of ontogeny and prey availability. Copeia 2:472-476

Imsland AK, Reynolds P, Eliassen G, Hangstad TA, Foss A, Vikingstad E, Elvegård TA (2014) The use of lumpfish (*Cyclopterus lumpus* L.) to control sea lice (*Lepeophtheirus salmonis* Krøyer) infestations in intensively farmed Atlantic salmon (*Salmo salar* L.). Aquaculture 424-425:18-23

Jacobsen JA, Gaard E (1997) Open ocean infestation by salmon lice (*Lepeophtheirus salmonis*): comparison of wild and escaped farmed Atlantic salmon (*Salmo salar* L.). ICES J Mar Sci 54:1113–1119

Jacobsen JA, Hansen LP (2001) Feeding habits of wild and escaped farmed Atlantic salmon *Salmo salar* L., in the Northeast Atlantic. ICES J Mar Sci 58:916-933

Killen SS, Brown JA, Gamperl AK (2007) The effect of prey density on foraging mode selection in juvenile lumpfish: balancing food intake with the metabolic cost of foraging. J. Anim Ecol 76:814-825

Sheedan TF, Reddin DG, Chaput G, Renkawitz MD (2012) SALSEA North America: a pelagic ecosystem survey targeting Atlantic salmon in the Northwest Atlantic. ICES J Mar Sci 69:1580-1588

Tully O, Daly P, Lysaght S, Deady S, Varian SJA (1996) Use of cleaner-wrasse (*Centrolubrus exoletus* (L.) and *Ctenolabrus rupestris* (L.)) to control infestations of *Caligus elongatus* Nordmann on farmed Atlantic salmon. Aquaculture 142:11-24.

Vandendriessche S, Messiaen M, O'Flynn S, Vincx M, Degraer S (2007) Hiding and feeding in floating seaweed: Floating seaweed clumps as possible refuges or feeding grounds for fishes. Estuarine Coast Shelf Sci 71: 691-703

Zar JH (1984) Biostatistical analysis. Prentice-Hall, New Jersey, 718 pp.

Trial 2. Cleaning behaviour in lumpfish (Fiskaaling)

Summary

In a 5 m diameter tank study carried out a Fiskaaling, indications are that lumpfish actively search among salmon for lice. Cleaners were willing to travel away from their sea weed shelter and approach salmon. However, conditions in a commercial salmon farm involve greater distances and proximity is likely to have an impact on the observed behaviour.

Activity levels in lumpfish and salmon were correlated, though a causative factor could not be established in this study. However, presence or absence of lice on the salmon affected the behaviour of both species, with the fish having lower activity levels when no lice were present.

One might conclude, then, that lumpfish do not cause stress or heighten activity levels in salmon, which could result in lowered growth rates. However, observations also indicate that each individual nip at the salmon by a lumpfish, be it aimed at salmon lice or salmon fins causes a startle reaction in the salmon. A structured study observing individual cleaning events over the space of a few months would allow us to determine whether salmon habituate to being cleaned so this reaction is lessened or becomes extinct.

Methods

Three cylindrical tanks (diameter 5 m, depth 1.5 m) were stocked with 135 salmon in each. Salmon were regularly (weekly) infected with copepodid stage salmon lice and regular weekly louse counting of 10 salmon per tank commenced one month post initial infection. Lumpfish (15% stocking density) were added on a schedule according to louse burden: 1) lumpfish added on the same day as the salmon, 2) lumpfish added once mobile lice were observed, and 3) lumpfish were added once the number of lice exceeded the delousing treatment threshold set by the Faroese Health Authorities (HFS).

Behaviour within the tanks was observed using a submersible camera with a recording timer set to record 5 times per day for 10 minutes each time. Salmon activity (number of salmon swimming out of view within a set time) and lumpfish activity (total amount of time that a lumpfish was seen actively swimming within a set time period) were recorded. Number of observed cleaning events were also recorded. Each tank was observed 4 times in this manner with observations being spaced over the duration of the trial. This ensured that there were lumpfish-free observations, low louse burden observations and high louse burden observations before and after the addition of lumpfish.

Results

There was a positive correlation between salmon activity and lumpfish activity (Pearson corr coeff: 0.904, $t = 4.24$, $df = 4$, $P = 0.013$). Causation could not be established. However, salmon activity levels were higher when adult lice were present regardless of presence of lumpfish ($F_{1,65}=20.76$, $P < 0.001$).

Though not quantified, the behaviour observed indicated that lumpfish would approach salmon and salmon showed a startle reaction when cleaned or nipped at by lumpfish.

Louse numbers in the tanks indicated that lumpfish would primarily consume adult lice. In figure 9 the adult lice trends in each tank indicate a rapid increase in adult lice in the tank with no lumpfish (green tank). In figure 10, such a trend is not apparent.

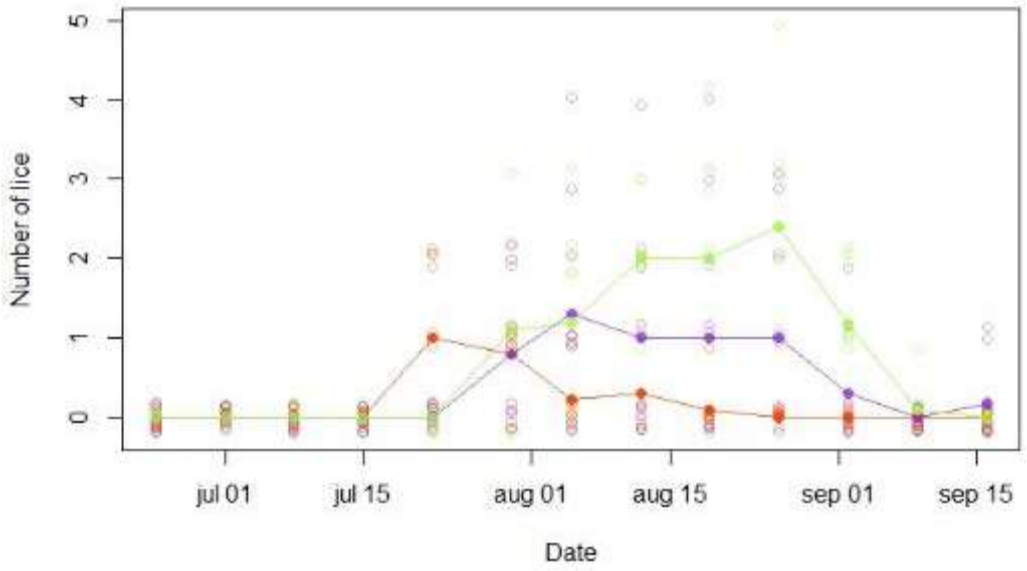


Fig. 9. *Numbers of adult lice throughout the experimental period. Open points are counts on individual salmon and filled points are means per tank. Red points are tank 1, purple points tank 2 and, green points tank 3. Lumpfish were added on 08-07-2014 in tank 2 and 26-08-2014 in tank 3.*

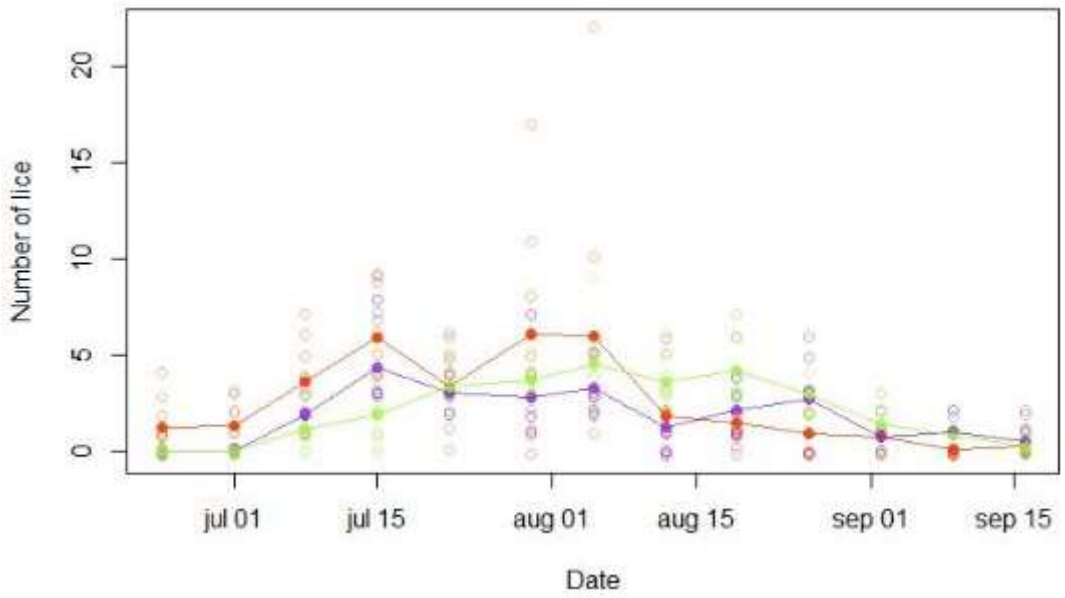


Fig. 10. *Counts of mobile lice over time. Open circles represent individual counts and filled circles are means per tank. Red circles represent tank 1, purple circles are tank 2 and green circles are tank 3.*

Conclusions

Lumpfish affect adult louse numbers and are willing to approach salmon to forage for lice. Though activity levels in lumpfish and salmon are positively correlated, causation was difficult to establish in this study. There is an indication that adult salmon lice may cause increased activity in salmon and that the correlation between lumpfish activity and salmon activity is related to the presence of palatable lice on the salmon. A controlled study could disentangle these effects.

Trial 3. Effects of lumpfish size on foraging behaviour and co-existence with sea lice infected Atlantic salmon in sea cages (Akvaplan-niva)

Materials and Methods

Atlantic salmon

The Atlantic salmon (N_{total}=1200) used in the study were 0+ 11G Atlantic salmon produced at Sundsfjord smolt AS and delivered to Gildeskål Research Station (GIFAS), Nordland, Norway in October 2014. The salmon were from the Aqua Gen strain and they were vaccinated with Pentium Forte Plus (Novartis Aqua, Oslo, Norway). All salmon originated from the same group of fish and shared the same genetic and environmental background. From October 2014 to January 2015 the salmon were reared at a sea pen facility at Langholmen, Nordland, Norway where the study was performed. The salmon were transferred to small-scale sea cages (5x5x5 m) in January 2015 and remained in those sea cages during the trial period. The salmon had an average initial mean (± SD) weight of 538 ± 14 g on 25 January 2015 when they were graded and the 1200 salmon were distributed among 8 sea cages (150 salmon in each sea cage). During the study period the salmon were fed a standard commercial diet (CPK 100, Biomar, Århus, Denmark) twice daily. A lice count was undertaken on the Atlantic salmon prior to transfer into the trial cage to assess the lice burden prior to treatment and thereafter every two to four weeks during the trial period. At each occasion 30 salmon in each sea cage (N_{total} = 240) were sedated and any lice present were recorded. Lice were registered in 5 categories: i) *Lepeophtheirus salmonis*: Adult female; ii) *Lepeophtheirus salmonis*: Adult male; iii) *Lepeophtheirus salmonis*: Pre-adult; iv) *Lepeophtheirus salmonis*: *Chalimus*; v) *Caligus elongatus*. As number of naturally accuring se lice during the trial period was low comparisons were made on the combined total of all categories. In order to minimize inter-observer variation the same person categorized lice at each sampling.

Lumpfish

Sexually mature wild lumpfish (8 males and 7 females) were caught by gill nets in Sandnessundet outside Kraknes, Troms County, Norway during April-May 2014. Eggs were stripped, fertilized and incubated at 9–10°C at Akvaplan-niva research station at Kraknes (APN-K) outside Tromsø, Norway. The juveniles were initially fed with Gemma Micro (150 -500 µm, Skretting, Norway). After 30 days the juveniles were fed with 500-800 µm dry feed pellets (Gemma Wean Diamond, Skretting, Norway). On 7 October 2014 the juveniles (mean weight 7 g) were transferred from APN-K to GIFAS. On 7 December 2014 all lumpfish were anaesthetized (Benzoak® 80 mg l^{-1}) and tagged intraperitoneally with a Trovan® Passive Integrated Transponder. In addition a Floy tag was inserted slightly off centre at the highest vertal point of the dorsal array. A different coloured and/or numbered Floy tag was used for each lumpfish from each duplicate (two lumpfish with the same tag). All lumpfish were vaccinated with ALPHA MARINE micro 4 (Pharmaq AS, Oslo, Norway) on 7 December 2014.

Experimental set-up

At the start of the trial (25 January 2015), 1200 Atlantic salmon were individually weighed, counted and randomly (by using a systematic random assignment starting with a random number (between 1 and 8) and then take each individual salmon and put it into sea cage 4, then 5, 6, 7, 8, 1, 2, 3, and then repeat) distributed between eight cages of 125 m^3 (5x5x5m), with 150 salmon in each cage. The experimental groups were thereafter assigned randomly (assigned by using random numbers) among predetermined duplicate distributions of the cages. There was one final weighing for Atlantic salmon in all eight cages at the end of the study period. Without prior starvation, all salmon in all cages were counted and individually weighed.

On 25 January three size classes of lumpfish were established by size grading with an initial mean (±SD) weight of 22.6 ± 0.7 g, 77.4 ± 3.6 g and 113.5 ± 2.1 g. These three groups are termed small, medium and large hereafter. Six sea cages were stocked with 15 lumpfish each (10% stocking density), with two sea cages for each size group and two cages without lumpfish as control group. Two submerged substrates of black 10 mm polyethylene (PE) plates (80x80 cm) (Helgeland Plast, Mo i Rana, Norway, see Imsland et al., 2015b) were sited in each of the eight cages prior to the lumpfish being transferred in order to provide shelters for the lumpfish. The study lasted for 159 days and was terminated on the 5 July, 2015. Daily mean temperature in the sea cages increased from 4.5°C on the

25 January to 10.8°C on the 5 July. Salinity ranged from 29.6 ppt. to 34.1 ppt. throughout the study period. Dissolved oxygen ranged between 9.0 mg l^{-1} and 12.4 mg l^{-1} during the trial period. Secchi depth in the sea cages varied from between 5 and 10 m. Individual pit-tag ID, weights and lengths of all the lumpfish were registered at the same dates when gastric lavage was performed.

Specific growth rate (SGR) of individual lumpfish and salmon was calculated according to the formula of Houde and Schekter (1981):

$$SGR = (e^{g}-1) \times 100$$

where $g = (\ln (W_2)-\ln (W_1) / (t_2-t_1)$ and W_2 and W_1 are weights on days t_2 and t_1, respectively.

Gastric lavage of lumpfish

During the trial period gastric lavage was performed at two to four week intervals to assess the feeding preferences of individual lumpfish. All samplings started at the same time in the morning. On each occasion, 20 lumpfish from each size group (i.e. 10 lumpfish from each sea cage) were anaesthetized (Benzoak® 80 mg l^{-1}) and a silicon tube (15 cm long and diameter of 4 mm) connected to a syringe filled with seawater was carefully inserted into the stomach cavity of the sedated lumpfish. Water was expelled from the syringe into the stomach cavity and the gut content was allowed to flow out of the stomach and into a container. After each lavage, the stomach contents were transferred to a clean Petri dish and identified under a dissecting scope. After sampling, the lumpfish were placed into a recovery tank containing aerated seawater and allowed to recover before being placed back into their specific cages.

Behavioural observations

Intra- and interspecific behaviour of lumpfish in the sea cages was assessed by underwater camera technology (SM Remotes, Bergen, Norway) 2-4 times per week throughout the trial period. Behavioural observations commenced two weeks after trial start to allow for an acclimation period. Behaviour was classified by recording for 30 second intervals the principal activity of individual lumpfish. Ten lumpfish from each size class from all six cages were observed giving a total of 20 activity records per size group, on each observation time point (N_{total} = 60). All lumpfish were individually tagged with a Floy tag. Each Floy tag had a unique number on the shaft and this was used to identify each tagged lumpfish. Only lumpfish with identifiable Floy tags were assessed. There was no algae growth on the tags so they were always visible. As each lumpfish was identified either directly or with camera (if on or near the surface) the number was normally seen. Behavioural observations were classified using behavioural indices as used by Tully et al. (1996).

Statistics

All statistical analyses were conducted using Statistica™ 12.0 software. A Kolmogorov-Smirnov test (Zar, 1984) was used to assess for normality of distributions. The homogeneity of variances was tested using the Levene's F test (Zar, 1984). Possible difference between the lumpfish size groups mean weights, feeding preferences and behaviour were tested with two-way nested analysis of variance (ANOVA), where replicates were nested within treatments. This test was also applied to test for possible differences in Atlantic salmon growth in each experimental group and for possible differences in growth between lumpfish sampled for gastric lavage and those not sampled for gastric lavage. Significant differences revealed in ANOVA were followed by Student-Newman-Keuls (SNK) post hoc test to determine differences among experimental groups. A 95% confidence interval (CI) was calculated for the mean total lice burden of each experimental group applying

$$\bar{X} \pm 1.96\, SE$$

where $\bar{X}$ is the mean lice burden, 1.96 is that standard normal deviate for the 97.5 percentile point for the standard normal probability distribution and SE the standard error of the mean.

Results

Growth and mortality of salmon

Mortality of salmon was low (2.6%) during the 159 day experimental period and did not differ between the experimental groups. There were no significant differences in mean weights between the groups at the start of the study with an initial mean (±SD) weight of 538.4 ± 13.9 (two-way nested ANOVA, $P > 0.05$). However, at the end of the study period significant differences in mean weight were found between the treatments (SNK test, $P < 0.01$) with higher mean weights for salmon reared together with small (1553.7±54.9) and medium (1587.9±36.0) sized lumpfish compared to the control group (1336.2±45.8) and salmon reared with large (1399.1±32.1) lumpfish. No difference was seen in feed consumption of the salmon in the eight experimental cages ranging from 128 to 134 kg.

Consumed sea lice levels

No negative effect on growth of lumpfish was seen when comparing growth after gastric lavage with lumpfish that were not sampled for gastric lavage (two way nested ANOVA, $P > 0.75$). Although the sea lice infestation rate was low in the study (<0.5 lice salmon^{-1}, all stages combined, Fig. 11), the percentage of lumpfish found to have consumed all stages of sea lice varied between three size classes (SNK post hoc test, $P < 0.05$, Fig. 12). No ingested sea lice were found at days 21, 56, 85 and 97. At day 35, 25% of lumpfish sampled from the small size class were found to have consumed sea lice, whereas no lumpfish from the other two size classes were found to have consumed sea lice. Similarly, at day 141, 15% of lumpfish sampled from the smaller size class were found to have ingested sea lice, though no ingested sea lice were found from lumpfish from the medium and large size classes. At the termination of the trial the total lice burden was 40% lower in the small lumpfish group (95% CI, 0.18-0.30) compared to the control group (95% CI, 0.40-0.48, Fig. 11).

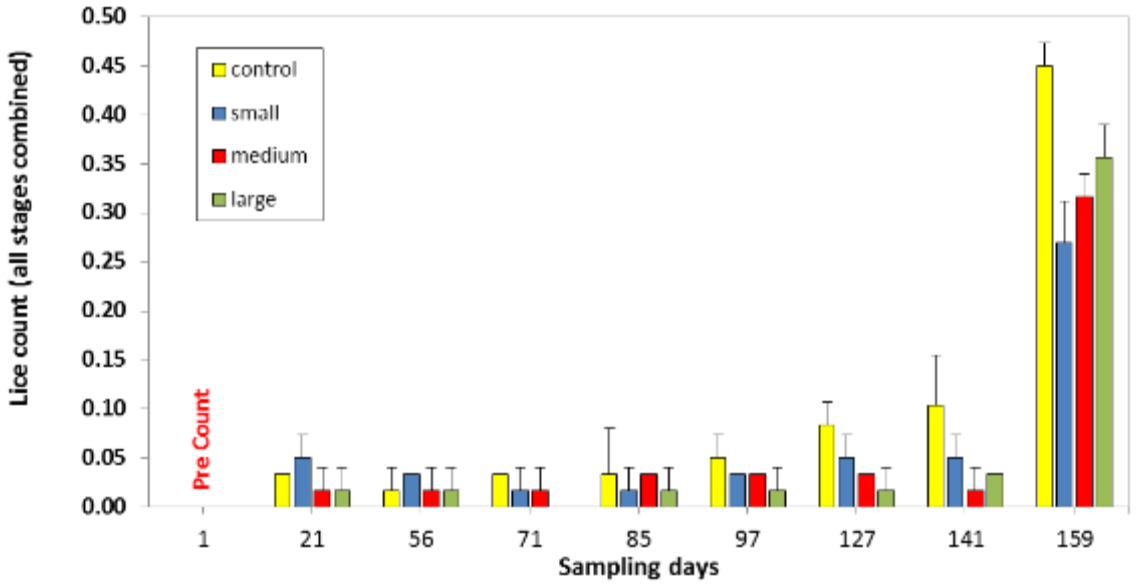


Fig. 11. *Mean (± SE) overall lice counts (all stages combined) on the Atlantic salmon in the four experimental groups throughout the experimental period.*

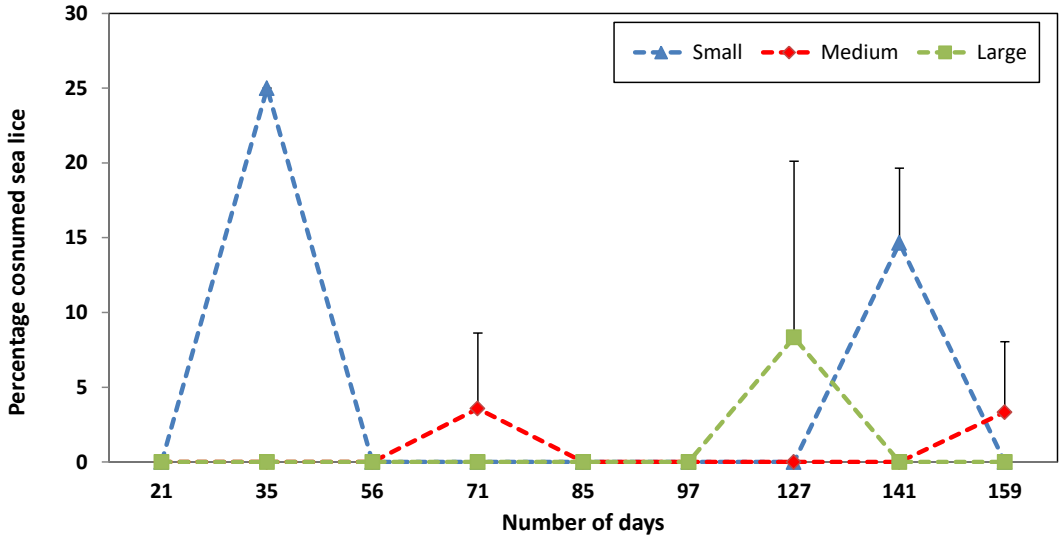


Fig. 12. *Percentage values of food choices for three sizes of lumpfish sampled at each sampling time point. Values are presented as means ± SD. A: sea lice (all stages of L. salmonis and Caligus elongatus).*

Behaviour of lumpfish

There were significant differences in the frequency of three behavioural types, i.e. lumpfish feeding on net fouling, lumpfish swimming between salmon and lumpfish actively competing for feed pellets with Atlantic salmon, between the three size groups throughout the study period (two-way ANOVA, $P < 0.05$, Fig. 13).

The small and medium sized lumpfish were feeding on net fouling (4.3 and 2.1% respectively) while large lumpfish were not (two-way nested ANOVA, $P < 0.01$, Fig. 13). The percentage of lumpfish from each size group observed swimming between salmon varied with a higher percentage for the medium (3.2%, Fig. 13B) and large lumpfish (4.6%, Fig. 13C) compared to the smallest lumpfish (1.4%, Fig. 13A) (two-way nested ANOVA, $P < 0.01$) throughout the study period. The difference in feeding competition with salmon was small, but still varied significantly between size classes of lumpfish (Fig. 5A, B and C, SNK post hoc test, $P < 0.05$), where the two larger size classes showed more feeding competition with salmon (large: 43.2% and medium: 41.2%) than the small size class (38.2%). For all other behaviour classes no significant differences were seen between the three size groups ($P > 0.05$, Fig. 13).

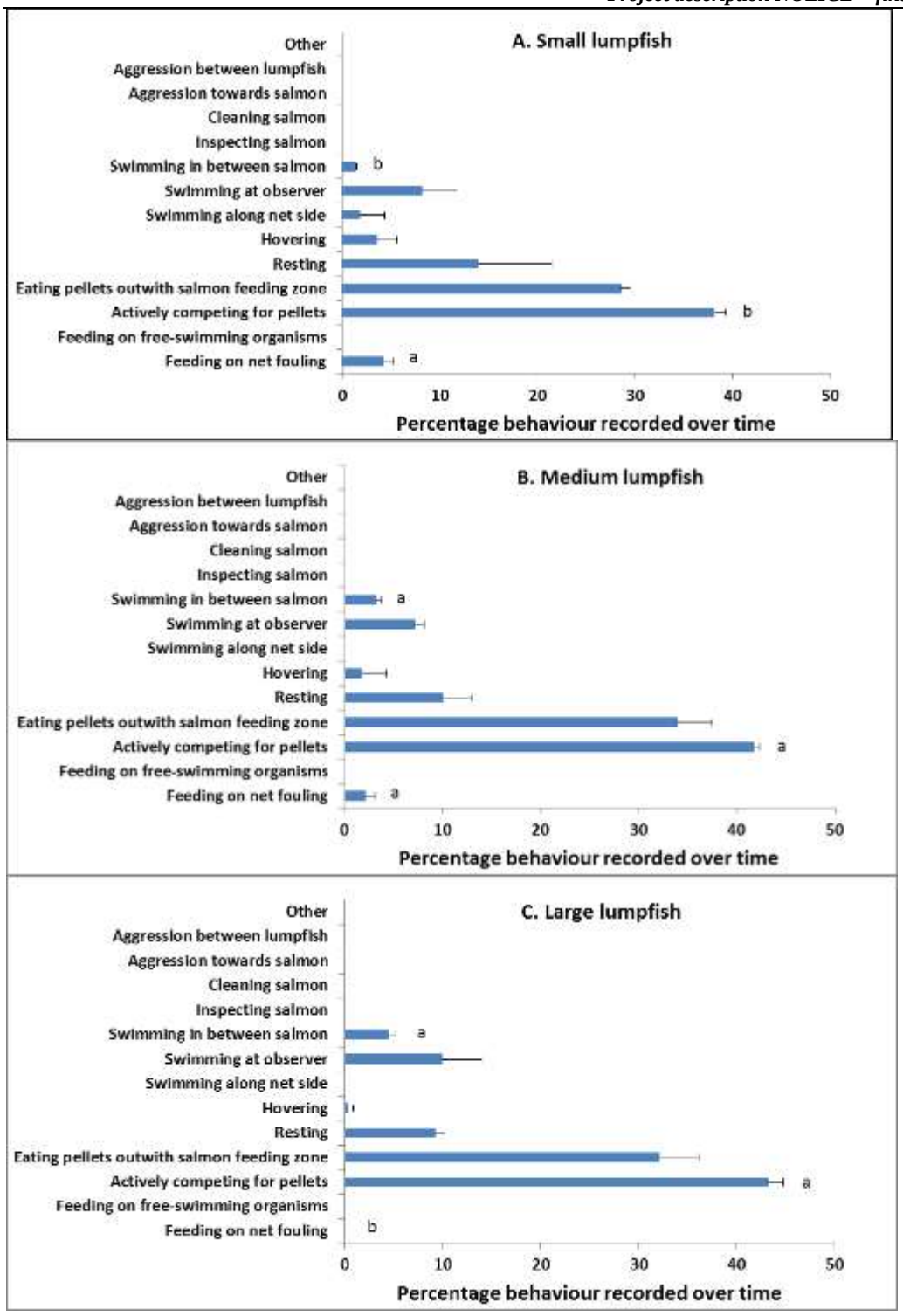


Fig. 13. *Behaviour of three size groups of lumpfish throughout the experimental period. Values are presented as means ± SD. Different letters indicate significant differences (SNK test, $P < 0.05$) in behaviour between the size groups.*

Discussion

Salmon growth and mortality

There were no indications of increased mortality in Atlantic salmon as a direct result of the presence of lumpfish. Growth rates calculated for the salmon were comparable to what would be expected given the size of the salmon and water temperature (Austreng et al., 1987; Folkedal et al., 2012). Lice burden was low in the control group, but increased towards the end of the trial. There was a slight but significant difference in growth of salmon as growth was higher for salmon reared along with small and medium size lumpfish than for salmon reared alone or with large lumpfish. A plausible explanation for the observed salmon growth stimulation may be the higher lice grazing activity, particularly in cages with the smallest lumpfish, which may increase the salmon welfare and hence increase appetite and growth. However, such effects are difficult to confirm, and in previous trials with lumpfish smaller (Imsland et al. 2014a, c) and larger (169.4 g, unpublished data) than in the current experiment, no effects of lumpfish on the growth of salmon has been observed. Alternatively, a plausible explanation of the growth differences may be linked to the fact that higher proportion of male sexual maturation was seen in the medium and large size lumpfish groups. It has been shown that male lumpfish become increasingly aggressive and territorial (Gaulet et al., 1986). This aggressive behaviour may have interfered with the feeding behaviour of the salmon and in fact there was a slightly higher proportion of feeding competition with the salmon in the medium and the large size groups.

Gastric lavage and sea lice infestation levels

Present results of gastric lavaging of lumpfish showed that there were few lumpfish which were found to have ingested *L. salmonis* and/or *C. elongatus* at each of the sampling points. This may be explained the low infestation levels present in all of the cages throughout the study period. However, there were differences in the proportion of consumed sea lice between the size classes. There was a higher proportion of the smaller lumpfish found with ingested sea lice compared to the other two groups at days 35 and 141 although the percentage of lumpfish found to have ingested sea lice was lower compared to previous studies (Imsland et al., 2014a). Ongoing trials in full scale production units (Nytrø et al., unpublished data) indicate similar trend i.e. reduced sea lize grazing for lumpfish larger than 200-300 g. The lower sea lice grazing seen in the present study can probably be explained by much lower incidence of sea lice in this study (< 0.5 sea lice salmon^{-1} in the control group) compared to 2.4-4.0 sea lice salmon^{-1} in the control group in Imsland et al. (2014a).

Behaviour

Lumpfish from the smaller size class were more predisposed in choosing to eat natural food sources as compared to feed pellets (observed feeing and/or swimming alongside the nets). Foraging on natural food sources required more energy expenditure compared to eating feed pellets which are more energy dense, easier to catch and more readily available. Small lumpfish were also resting more frequently compared to the other two size groups. Such energy saving behaviour may contribute to higher growth. A previous study has shown that wild juvenile lumpfish forage using one of two modes: they can actively search for prey while swimming or they can 'sit and- wait' for prey while clinging to the substrate using a ventral adhesive disk (Killen et al., 2007). The study suggested that juvenile lumpfish forage in a manner that reduces activity and conserves space in their limited aerobic scope. The authors noted that this behavioural flexibility is of great benefit to this species, as it allows young individuals to divert energy towards growth as opposed to activity. The present data derived for the small size group are in line with these observations. Further, smaller lumpfish generally exhibit higher growth rates compared to larger lumpfish (Nytrø et al., 2014) and modifying feeding and foraging behaviour may be regulated to optimize feed intake and growth.

Conclusions

Lumpfish from the smallest size class had a higher preference for sea lice, compared to the other two size classes. Moderate growth stimulation was seen in the medium and small size groups. Final lice burden was 40% lower in salmon groups stocked with small lumpfish compared to the control group without lumpfish.

References (Trial 3)

Austreng, E., Storebakken, T., Åsgård, T., 1987. Growth rate estimates for cultured Atlantic salmon and rainbow trout. Aquaculture 60, 157–160.
Folkedal, O., Stien, L.H., Nilsson, J., Torgersen, T., Fosseidengen, J.E., Oppdal, F., 2012. Sea caged Atlantic salmon display size-dependent swimming depth. Aquat. Living Resour. 25, 143–149.
Houde, E.D., Schekter, R.C., 1981. Growth rates, rations and cohort consumption of marine fish larvae in relation to prey concentrations. Rapp. P.-v. Réun. Cons. Int. Explor. Mer. 178, 441-453.
Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Foss, A., Vikingstad, E., Elvegård, T.A., 2014a. The use of lumpfish (*Cyclopterus lumpus* L.) to control sea lice (*Lepeophtheirus salmonis* Krøyer) infestations in intensively farmed Atlantic salmon (*Salmo sala*r L.). Aquaculture 425-426, 18-23.
Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Nytrø, A.V., Foss, A., Vikingstad, E., Elvegård, T.A., 2014b. Notes on behaviour of lumpfish in sea pens with and without Atlantic salmon. Journal of Ethology 32, 117-122.
Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Nytrø, A.V., Foss, A., Vikingstad, E., Elvegård, T.A., 2014c. Assessment of growth and sea lice infection levels in Atlantic salmon stocked in small-scale cages with lumpfish. Aquaculture 433, 137-142.
Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Nytrø, A.V., Foss, A., Vikingstad, E., Elvegård, T.A., 2015a. Feeding preferences of lumpfish (*Cyclopterus lumpus* L.) maintained in open net-pens with Atlantic salmon (*Salmo salar* L.) Aquaculture 436, 47-51.
Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Nytrø, A.V., Foss, A., Vikingstad, E., Elvegård, T.A., 2015b. Assessment of suitable substrates for lumpfish in sea pens. Aquaculture International 23, 639-645.
Killen, S.S., Brown, J.A., Gamperl, A.K., 2007. The effect of prey density on foraging mode selection in juvenile lumpfish: balancing food intake with the metabolic cost of foraging. J. Anim. Ecol. 76, 814-825.
Lein, I., Noble, C., Nergaard, S., Helland, S., 2013. Evaluation of grazing efficiency of Ballan wrasse in sea pens with large salmon. Nofima report, Tromsø Norway, ISBN 978-82-8296 (in Norwegain with abstract in English).
Nytrø, A.V., Vikingstad, E., Foss, A., Hangstad, T.A., Reynolds, P., Eliassen, G., Elvegård, T.A., Falk-Petersen, I.B., Imsland, A.K. 2014. The effect of temperature and fish size on growth of juvenile lumpfish (*Cyclopterus lumpus* L.). Aquaculture 434, 296-302.
Skiftesvik, A.B., Bjelland, R., Durif, C.M.F., Johansen, I.S., Browman, H.I., 2013. Delousing of Atlantic salmon (*Salmo salar*) by cultured vs. wild ballan wrasse (*Labrus bergylta*). Aquaculture 402-403, 113-118.
Tully, O., Daly, P., Lysaght, S., Deady, S., Varian, S.J.A., 1996. Use of cleaner-wrasse (*Centrolubrus exoletus* (L.) and *Ctenolabrus rupestris* (L.)) to control infestations of *Caligus elongatus* Nordmann on farmed Atlantic salmon. Aquaculture 142, 11-24.
Zar, J.H., 1984. Biostatistical Analysis, 2nd edition, Prentice-Hall, Inc., Englewood Cliffs, N.J., 718 pp.

Task 2.2 Development of lumpfish breeding programme and search for QTLs

This Task was performed during spring and summer of 2015. Sea lice grazing of 9 different families of lumpfish was investigated and initial findings are reported below.
Further work involving the screening of quantitative trait loci (QTL) in lumpfish by relating traits individual sea lice grazing with individual genetic composition will be performed in the final year of the project.

Trial 1. Is cleaning behaviour in lumpfish (Cyclopterus lumpus) parentally controlled? (Akvaplan-niva, Nordlaks)

Materials and Methods

Atlantic salmon

The Atlantic salmon (N_{total}=3600) used in the study were underyearling (0+) 11G (eleventh generation of the Norwegian breeding program for Atlantic salmon) produced at Sundsfjord Smolt AS (Nordland, Norway) and delivered to Gildeskål Research Station (GIFAS), Nordland, Norway in March 2015. The fish were from the Aqua Gen strain and they were vaccinated with Pentium Forte Plus (Novartis Aqua, Oslo, Norway). All fish originated from the same group of fish and shared the same genetic and environmental background. From March to May 2015 the fish were reared at a sea pen facility at Langholmen, Nordland, Norway where the study was performed. The fish were transferred to small-scale sea pens (5x5x5 m) in March 2015 and remained in those sea pens during the trial period. The salmon had an average initial mean (± SD) weight of 123.3 ± 12.3 g on 25 May 2015 when they were graded and 400 fish were distributed among 9 sea pens. During the study period the salmon were fed a standard commercial diet (CPK 75, Biomar, Århus, Denmark) twice daily. A lice count was undertaken on the Atlantic salmon prior to transfer into the trial cage to assess the lice burden prior to treatment and thereafter every two weeks during the trial period in accordance with Norwegian legislation. On each occasion 30 fish were sedated and any lice present were recorded. Lice were registered in 5 categories: i) *Lepeophtheirus salmonis*: Adult female; ii) *Lepeophtheirus salmonis*: Adult male; iii) *Lepeophtheirus salmonis*: Pre-adult; iv) *Lepeophtheirus salmonis*: *Chalimus*; v) *Caligus elongatus*.

Lumpfish

Sexually mature wild lumpfish (6 males and 8 females) were caught by Akvaplan-niva staff in gill nets in Sandnessundet outside Kraknes, Troms County, Norway during April-May 2014. Eggs were stripped, fertilized and incubated at 9–10°C at Akvaplan-niva research station at Kraknes (APN-K) and later transferred to Nofima's Center for marine aquaculture at Kraknes, Troms County, Norway where they hatched between 20-24 June (Table 1). Nine different families (6 half-sib and 3 full-sib) of lumpfish were used in the study obtained by crossing the different males and females (Table 1). The juveniles from each family were reared in replicate tanks (230 L), from hatching to tagging. They were initially fed with Aglo Norse 200-300 (Tromsø Fiskeindustri AS, Tromsø, Norway) and Artemia by hand to ensure adequate feed intake. On 2 December 2014 all lumpfish were anaesthetized (benzoak 80 mg l^{-1}) and tagged intraperitoneally with a Trovan® Passive Integrated Transponder. All lumpfish were vaccinated with ALPHA Marin micro 4 (Pharmaq AS, Oslo, Norway) on 15 December 2014. The same day, 40 fish from each family (N_{total} = 360) were mixed together and transferred to APN-K and kept there in an 11 m^3 tank at ambient water temperature until transfer to GIFAS. The fish were transferred to GIFAS on 7 May 2015 and held under quarantine in four 3 m^3 tanks until the start of the experiment (25 May).

Experimental set-up

At the start of the trial (25 May 2015), 3600 Atlantic salmon were individually weighed, counted and randomly distributed between nine cages of 125 m^3 (5x5x5m), with 400 fish in each cage. To minimize the effects of water quality and current, experimental groups were assigned randomly among predetermined duplicate distributions of the cages. There was one final weighing for Atlantic salmon in all nine cages at the end of the study period. Without prior starvation, all fish in all cages were counted and individually weighed. All the nine cages were stocked with 40 lumpfish (10% stocking density). All lumpfish from each family were tagged with a separate colour external Floy tag at the highest ventral point of the dorsal array. For each family, 20 lumpfish were stocked into one of nine

5x5x5m cages and 20 into another cage thus establishing duplicate treatments for each genetic family giving two families stocked per cage. All lumpfish were identified by scanning each fish for their pit tag ID prior to placement. The study lasted for 78 days and was terminated on the 11 August, 2015. Daily mean temperature in the sea pens increased from 7.1°C on the 25 May to 13.2°C on the 11 August. Salinity ranged from 29.6 ppt. to 34.1 ppt. throughout the study period. Dissolved oxygen ranged between 8.9 mg l^{-1} and 11.1 mg l^{-1} during the trial period. Secchi depth in the sea pens varied from between 5 and 10 m. Individual weights and lengths of all the lumpfish were registered at the same dates when gastric lavage was performed. The lumpfish were not offered supplemenatary feed during the trial period as they readily ate the salmon diet fed to the salmon in the sea cages along with the organism naturally present in the sea cages.

Specific growth rate (SGR) of individual lumpfish and salmon was calculated according to the formula of Houde and Schekter (1981):

$$SGR = (e^{g}-1) \times 100$$

where $g = (\ln (W_2)-\ln (W_1) / (t_2-t_1)$ and W_2 and W_1 are weights on days t_2 and t_1, respectively.

Gastric lavage of lumpfish

During the trial period gastric lavage was performed every two weeks to assess the feeding preferences of individual lumpfish. All samplings started at the same time in the morning. On each occasion, all lumpfish were anaesthetized (Benzoak® 80 mg l^{-1}) and a silicon tube (15 cm long and diameter of 4 mm) connected to a syringe filled with seawater was carefully inserted into the stomach cavity of the sedated fish. Water was expelled from the syringe into the stomach cavity and the gut content was allowed to flow out of the stomach and into a container. After each lavage, the stomach contents were transferred to a clean Petri dish and identified under a dissecting scope. The food items identified by gastric lavage were categorised as: a) sea lice (all stages of *L. salmonis* and *Caligus elongatus*) b) formulated feed fragments, c) crustacean species (e.g. Caprella spp.), d) hydrozoan species, e) unidentified material or no contents found. After sampling, the fish were placed into a recovery tank containing aerated seawater and allowed to recover before being placed back into their specific cages.

Statistics

All statistical analyses were conducted using Statistica™ 12.0 software. A Kolmogorov-Smirnov test (Zar, 1984) was used to assess for normality of distributions. The homogeneity of variances was tested using the Levene's F test (Zar, 1984). Possible differences between the lumpfish families mean weights, feeding preferences and behaviour were tested with two-way nested analysis of variance (ANOVA), where replicates were nested within families. Significant differences revealed in ANOVA were followed by Student-Newman-Keuls (SNK) post hoc test to determine differences among experimental groups. A significance level (α) of 0.05 was used if not stated otherwise.

The contribution of the different variables (i.e. different families, maternal ID, paternal ID) for feeding preferences was estimated using the VEPAC (Variance Estimation and Precision) program in STATISTICA™ (www.statsoft.com). In short, the program will estimate the fixed effects design and random effects design separately, but incorporate the variance of random effects in the tests of the parameters of the linear model, and related statistics (Searle et al., 1992; Demidenko, 2004).
The model equation of the mixed linear model had the form:

$$\boldsymbol{y} = X\boldsymbol{\beta} + Z\boldsymbol{\gamma} + \boldsymbol{\varepsilon}$$

where $\boldsymbol{y}$ is the vector of feeding preferences of the lumpfish,
X is a design matrix that accounts for the family effect (set as fixed effect) and
$\boldsymbol{\beta}$ is the unknown vector of parameter estimates for fixed family effects;
Z is a design matrix that accounts for all random effects (here maternal and paternal ID) and
$\boldsymbol{\gamma}$ is the unknown vector of parameter estimates for random effects; and
$\boldsymbol{\varepsilon}$ is the vector of unknown random error which is no longer required to be independent or homogenous.
In this model the variance components for both the random and fixed effects are estimated with a Restricted Maximum Likelihood Estimate (REML) procedure (Searle et al., 1992; Demidenko, 2004).

Results

Consumed sea lice levels

Although the sea lice infestation rate was low in the study (Fig. 14), the percentage of lumpfish found to have consumed pre-adult and mature female stages of *L. salmonis* and/or *C. elongatus* varied

between the families. No consumption of sea lice was found for families 3, 5, 7, 8 and 9, whereas incidence of consumed sea lice for family 2 increased throughout the study and was significantly higher (SNK test, $P < 0.05$, Fig. 14A) at day 78. This coincided with the period with the lowest infestation rates in cages 402 and 408 containing family 2 (Fig 15), being 43-92% lower than in the other cages. In family 1, 5% of the lumpfish were found with ingested sea lice at day 16, 56 and 78. The REML based variance component analysis (VEPAC) of sea lice grazing showed significant paternal ($F_{5, 45} = 6.9$, $P < 0.01$) and maternal ($F_{3, 45} = 4.0$, $P < 0.05$) effect on sea lice grazing.

Despite low sea lice infestation rates there was a high delousing effect of lumpfish in cages 402 and 408 particularly, and both contained family 2 (Fig. 15). This is demonstrated by a more than 70% reduction of infestation from the maximum infestation (sum of Chalimus stages, pre-adult and mature males and females) at day 42 (rate of approx. 0.17) to a stable minimum infestation rate of less than 0.05 from day 56 onwards.

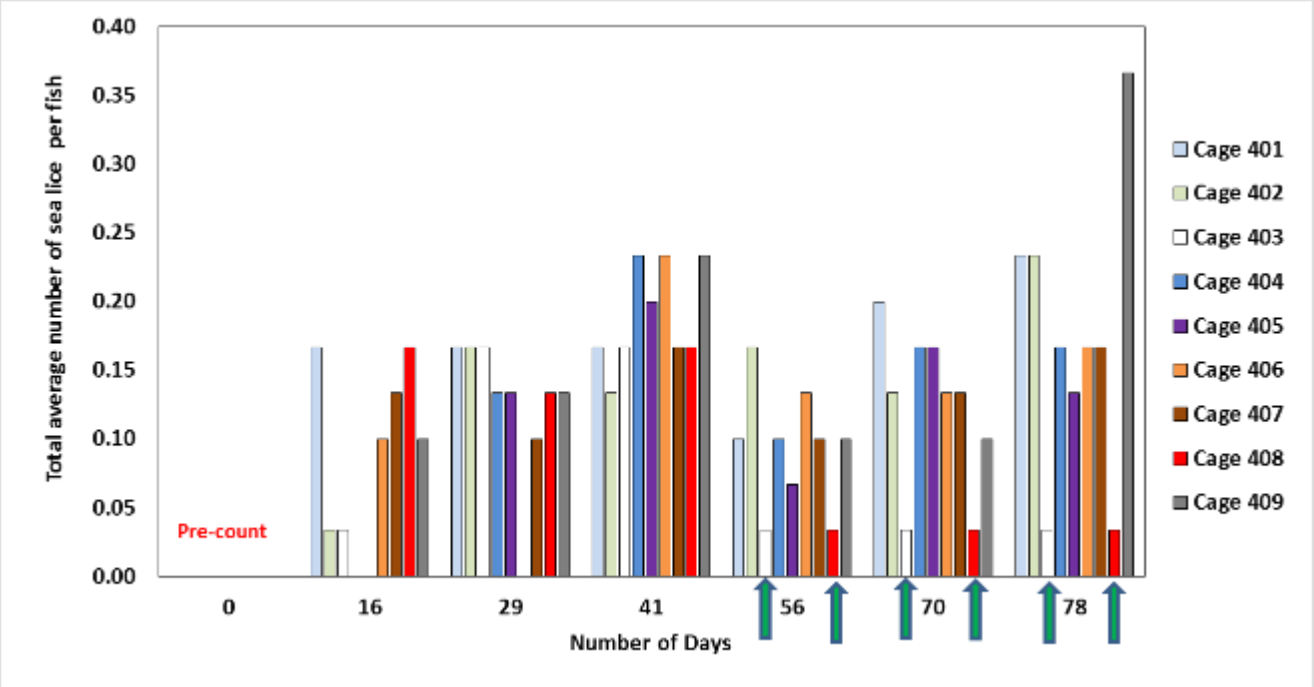


Fig. 14. *Total average number of sea lice (all stages L. salmonis and Caligus elongatus) in each experimental sea pen throughout the experimental period. Arrows indicate the sea pens where family 2 was reared.*

Formulated salmon pellets

The percentage of lumpfish found to have ingested salmon feed pellets on each sampling day varied between the different families (two-way nested ANOVA, $F_{8, 101} = 6.7$, $P < 0.01$, Fig. 15B). Significant differences were seen at all dates apart from the last sampling day. At all the other sampling days the percentage with ingested feed pellets was highest (80-95%) in family 5 and lowest (35-55%) in family 2. The VEPAC analysis showed significant maternal effect ($F_{3, 45} = 7.0$, $P < 0.05$) on ingestion of formulated salmon pellets.

Crustaceans

The percentage of lumpfish in the different families found with ingested crustacean species was significantly different (two-way ANOVA, $F_{8, 101} = 4.7$, $P < 0.05$, Fig. 15C) at all sampling points apart from the final sampling. In general the consumption of crustaceans was highest in family 2 (SNK test, $P < 0.05$, day 29, 41, 56 and 70, Fig. 14C) and lowest in family 5. Family 1 had the highest consumption of crustaceans at the first sampling point (day 16, SNK test, $P < 0.05$). The VEPAC analysis showed significant maternal effect ($F_{3, 45} = 4.8$, $P < 0.01$) on ingestion of crustaceans.

Hydrozoans

The consumption of hydrozoans varied between the families at day 16, 29 and 56 (two-way ANOVA, $F_{8, 101} > 5.3$, $P < 0.05$, Fig. 15D). No consumption of hydrozoans was found at any date in family 5,

whereas the consumption was significantly (SNK test, $P < 0.05$) higher in families 1, 2 and 3 at day 16, in family 3 at day 29 and in family 2 at day 56. No maternal or paternal effect on consumption of hydrozoans was found (VEPAC model, $P > 0.10$).

Unidentified or no stomach content

Apart from day 29 no differences in unidentified/empty stomach were found. Lower (SNK test, $P < 0.05$, Fig. 15e) amount and unidentified stomach content (5%) was seen in family 2 at day 29. In general the amount of unidentified ingested food items or empty stomach was similar in all families at all sampling dates and no consistent trend was seen. However, there was an increase in other/empty stomachs as temperature increased towards the end of the experiment. No maternal or paternal effect on content of unidentified/empty stomach was found (VEPAC model, $P > 0.55$).

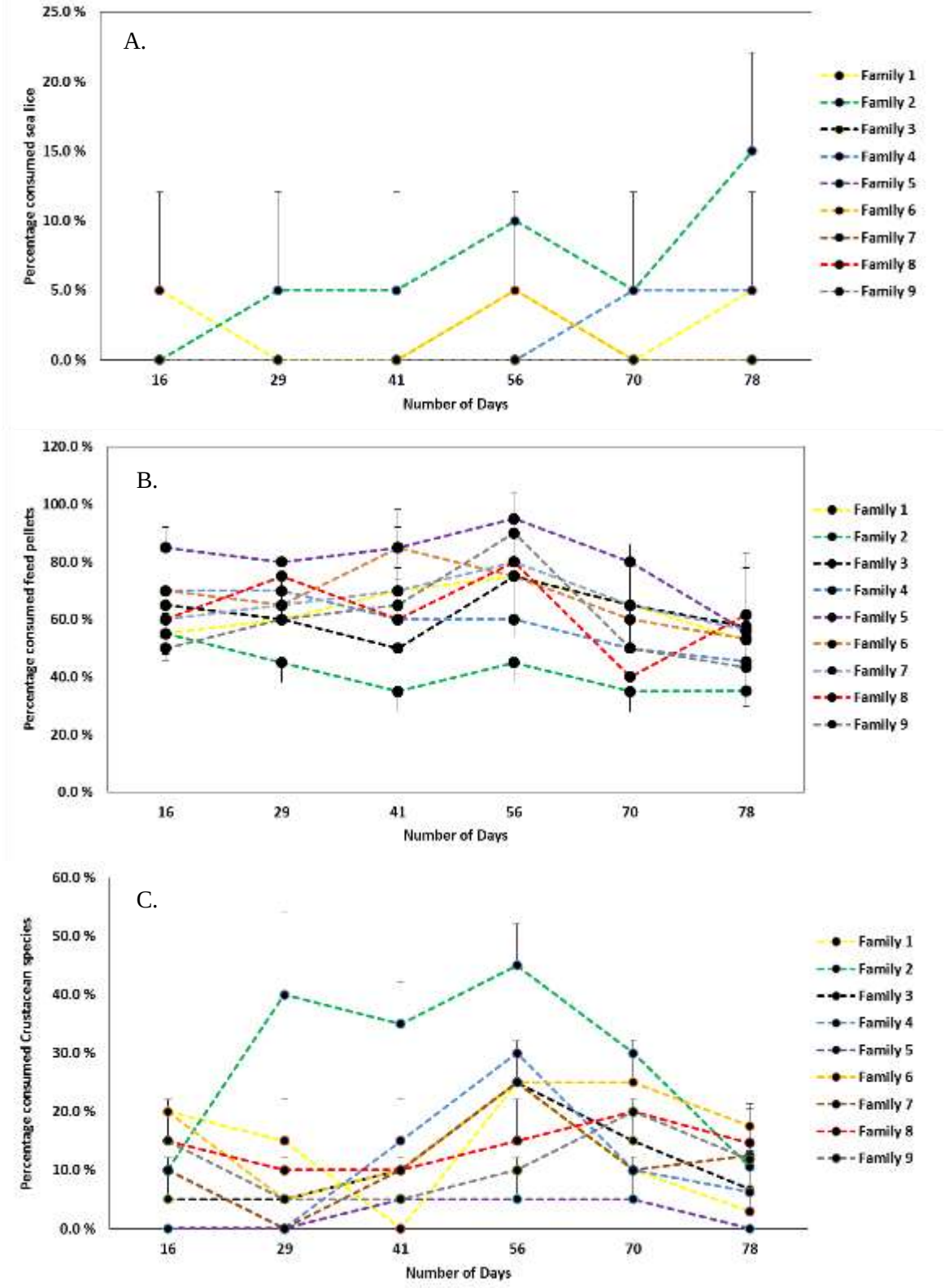


Fig. 15. *Percentage values of food choices for lumpfish of the nine lumpfish families sampled at each sampling time point. Values are presented as means ± S.D. A: sea lice; B: salmon feed pellets; C: crustacean species.*

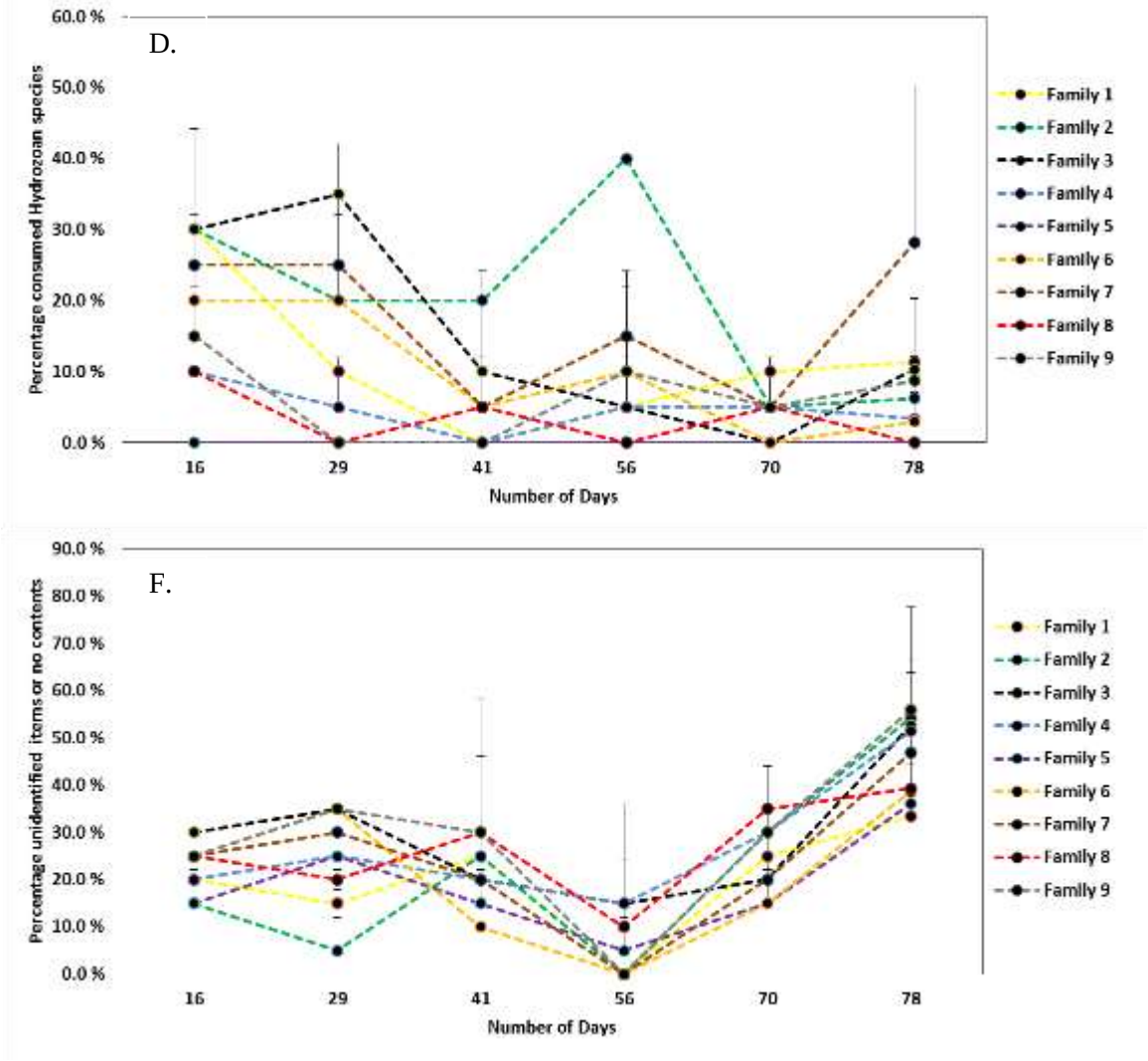


Fig. 15. *Percentage values of food choices for lumpfish of the nine lumpfish families sampled at each sampling time point. Values are presented as means ± S.D. D: hydrozoans; E: other/unidentified food items.*

Discussion

Gastric lavage and sea lice infestation levels

Efficient delousing capability at low sea lice infestation rates is an extremely important trait for ecological control with sea lice in salmon farming. This delousing efficiency was particularly linked to family 2, where up to 15% of the fish had registered consumed sea lice. Fish from families 3, 5, 7 and 8 had no registrations of consumed sea lice throughout the study period whereas fish from family 1, 4, 6 and 9 showed low and inconsistent content of sea lice in their stomach compared to family 2. This is also seen when looking at the total average of sea lice found on the salmon as this was 43-92% lower in the sea pens with family 2. Previous studies have shown that up to 36% of lumpfish populations (Imsland et al., 2014a) were found to have consumed sea lice, but this was at much higher infestation levels (0.5-3.0) on the salmon. The lumpfish used in those studies were smaller (intial size 54 g) compared to the fish in this study. Imsland et al. (2014 c) found that sea lice grazing was negatively size dependent (54 g vs. 360 g lumpfish) which may, together with general lower infestation levels, explain the low sea lice grazing seen in the present study.

The most common food item identified in the stomachs of lumpfish throughout the study period was fragments of salmon pellets. However, this is not surprising given that pellets are nutritious, energy dense and readily available to these fish, and at no time are there spatial and/or temporal fluctuations in the supply of this particular food source compared to other types of food items (Imsland et al., 2014a, 2015b). There were significant differences in the frequency of feed pellets being consumed between families with fish from family 5 having a higher incidence of ingested feed pellets compared to all other families but particularly more compared to fish from family 2. There were differences between families with fish from family 2 having a higher incidence of consumed crustaceans species compared to all other families whereas fish from family 5 had the least amount of consumed crustaceans. Similar patterns of food choice were recorded for both hydrozoan species and mussel spat. Previous studies have shown that lumpfish seem to switch natural food choice to whatever becomes available to them within their environment (Imsland et al., 2015b). Hydrozoan species were found in the stomachs of fish throughout the study period whereas mussel spat was found from day 41 onwards. This coincides with the increase in the colonization of *M. edulis* seen on the nets. Ingólfsson and Kristjánsson (2002) found only a limited proportion of juvenile lumpfish had eaten molluscs, but the proportion could increase if molluscs were available. The present data shows temporal changes in feed choice throughout the period seemingly linked with food availability. This omnivorous feeding behaviour has been reported with wild juvenile lumpfish (Ingólfsson and Kristjánsson, 2002; Vandendriessche et al., 2007). Although the nutritious and energy dense feed pellets are equally available for all families, and the energy demand is well covered, family 2 still has a preference for other less energy dense food organisms compared with the other families, particularly sea lice. This confirms with recent theory (West-Eberhard, 2003; Sih et al., 2004a, b) that behaviour might not be as plastic as previously assumed, and the genetic influence may be strong.

Given the differences recorded in consumption of natural food sources between family 2 and the other families, these fish may be more predisposed in actively seeking out natural food sources as compared to feed pellets and this behaviour may well have a genetic basis. If so, the genetic composition for family 2 needs further elucidation. It is well known that behavioural traits respond to both natural and sexual selection. Fish from families 1 and 2 shared the same father but had different mothers and given the differences in consumption of natural food sources between these two families and given that these differences have a degree of genetic influence then it would appear more likely that this difference is passed through maternal rather than paternal lines. Recent studies have indicated both maternal (Royle et al., 2012) and paternal (McGhee and Bell, 2014) effect on offspring behaviour via epigenetic alterations to the genome.

Both maternal and paternal effect was found for sea lice grazing. In this context we define maternal and paternal effects as the causal influence of the maternal and paternal genotype or phenotype on the offspring phenotype (Wolf and Wade, 2009; Curley et al., 2011). This definition leads to a simple statement of the evolutionary importance of parental effects—evolutionary changes in the distribution of parental traits (i.e. genotypes or phenotypes) will cause evolutionary changes in some offspring traits due to the causal influence of those parental traits on those offspring traits (here sea lice grazing in the different families tested). The unstated assumption is that all of the resemblance between offspring and parents was due to genetics and is additive. The most important cause of non-

genetic resemblance between parents and offspring is a maternal effect only, because mothers often have profound effects on their offspring through provisioning of the seed or egg in most plants and animals, gestation and lactation in mammals, and direct care of the young in many animal species (Mousseau and Fox, 1998). This is not the case for the present data on sea lice grazing as the REML based variance component analysis on sea lize grazing implied effect from both parents. There being both a paternal and a maternal effect on lice grazing activity suggests that this family effect is likely a genetic effect that can be selected for in future selection programmes for lumpfish behaviour.

References (Task 2.2)

Curley, J.P., Mashoodh, R., Champagne, F.A., 2011. Epigenetics and the origins of paternal effects. Horm. Behav. 59, 306-314.

Demidenko, E., 2004. Mixed models – theory and applications. Wiley, New York, 530 pp.

Hansen, L.P., Jacobsen, J.A., 2000. Distribution and migration of Atlantic salmon (*Salmo salar* L.) in the sea. In: Mills, D. (Ed.), The ocean life of Atlantic salmon—environmental and biological factors influencing survival. Blackwell Science, Oxford, UK, pp. 75-87.

Houde, E.D., Schekter, R.C., 1981. Growth rates, rations and cohort consumption of marine fish larvae in relation to prey concentrations. Rapp. P.-v. Réun. Cons. Int. Explor. Mer. 178, 441-453.

Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Foss, A., Vikingstad, E., Elvegård, T.A., 2014a. The use of lumpfish (*Cyclopterus lumpus* L.) to control sea lice (*Lepeophtheirus salmonis* Krøyer) infestations in intensively farmed Atlantic salmon (*Salmo sala*r L.). Aquaculture 425-426, 18-23.

Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Nytrø, A.V., Foss, A., Vikingstad, E., Elvegård, T.A., 2014b. Notes on behaviour of lumpfish in sea pens with and without Atlantic salmon. Journal of Ethology 32, 117-122.

Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Nytrø, A.V., Foss, A., Vikingstad, E., Elvegård, T.A., 2014c. Assessment of growth and sea lice infection levels in Atlantic salmon stocked in small-scale cages with lumpfish. Aquaculture 433, 137-142.

Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Nytrø, A.V., Foss, A., Vikingstad, E., Elvegård, T.A., 2015a. Feeding preferences of lumpfish (*Cyclopterus lumpus* L.) maintained in open net-pens with Atlantic salmon (*Salmo salar* L.) Aquaculture 436, 47-51.

Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Nytrø, A.V., Foss, A., Vikingstad, E., Elvegård, T.A., 2015b. Assessment of suitable substrates for lumpfish in sea pens. Aquaculture International 23, 639-645.

Ingólfsson, A., Kristjánsson, B.K., 2002. Diet of juvenile lumpsucker (*Cyclopterus lumpus*) in floating seaweed: effect of ontogeny and prey availability. Copeia 2, 472-476.

Jacobsen, J.A., Hansen, L.P., 2001. Feeding habits of wild and escaped farmed Atlantic salmon *Salmo salar* L., in the Northeast Atlantic. ICES J. Mar. Sci. 58, 916-933.

McGhee, K.E., Bell, A.M., 2014. Paternal care in a fish: epigenetics and fitness enhancing effects on offspring anxiety. Proc. R. Soc. B 281, 1146-1151.

Mousseau, T., Fox, C., 1998. Maternal effects as adaptations. Oxford Univ Press, NY., 369 pp.

Royle, N.J., Smiseth, P.T., Kolliker, M., Champagne, F.A., Curley, J.M., 2012. Genetics and epigenetics of parental care. *In*: Royle, N.J., Smiseth, P.T., Kolliker, M. (Eds.), The evolution of parental care. Oxford University Press, Oxford, UK., pp. 304–324.

Searle, S.R., Casella, G., McCullock, C.E., 1992. Variance components. Wiley, New York, 497 pp.

Sih, A., Bell, A.M., Johnson, J.C., 2004a. Behavioral syndromes: an ecological and evolutionary overview. Trends Ecol. Evol. 19, 372-378.

Sih, A., Bell, A.M., Johnson, J.C., Ziemba, R., 2004b. Behavioral syndromes: an integrative overview. Quart. Rev. Biol. 79, 241-277.

Vandendriessche, S., Messiaen, M., O'Flynn, S., Vincx, M., Degraer, S., 2007. Hiding and feeding in floating seaweed: Floating seaweed clumps as possible refuges or feeding grounds for fishes. Estuarine Coast Shelf Sci. 71, 691-703.

Wolf, J.B., Wade, M.J., 2009. What are maternal effects (and what are they not)? Phil. Trans. R. Soc. B 364, 1107-1115.

Zar, J.H., 1984. Biostatistical Analysis, 2nd edition, Prentice-Hall, Inc., Englewood Cliffs, N.J., 718 pp.

Work package number			3		**Start date or starting event:**		9						
Work package title			***Commercial scale protocols for biological delousing***										
Participant number	1	2	3	4	5	6	**7**						
Person-months per participant:	3	0	1	0	2	0.5	**2**						

Objectives

- To develop biological and technical operational protocols for the use of lumpfish as delousing agents in open net pens with farmed Atlantic salmon.
- Define the optimal size ratio between lumpfish and Atlantic salmon to induce effective biological delousing.
- To develop operation protocols for delousing under different densities and temperature conditions.
- To develop commercial scale protocols for successful biological delousing of Atlantic salmon in sea pens.

In WP 3 the optimized production protocols developed in WP 1-2 will be tested in semi-commercial and commercial scale where lumpfish will be reared together with Atlantic salmon through large part of their production cycle in sea pens. This WP is divided into two tasks:

- *Task 3.1 Optimise lumpfish density for effective lice control*
- *Task 3.2 Optimizing the effectiveness of farmed lumpfish for biological delousing of salmon*

Description of work

Task 3.1 Optimise lumpfish density for effective lice control

This task will seek to quantify grazing effect by carrying out sea lice counts and by identifying the different developmental stages of lice from salmon in cages with and without lumpfish. A comparison would be made of lice numbers where lumpfish are present and where medicinal treatments are used.

There is no information on the optimum stocking ratio of lumpfish to salmon in cages. This will be tested in trials with salmon and lumpfish at different densities in cages, from 5:100 to 15:100.

Task 3.2 Optimizing the effectiveness of farmed lumpfish for biological delousing of salmon

Focus will be on the biology of farmed lumpfish in polyculture with salmon and the sea lice grazing behaviour of lumpfish under normal production routines. The effects of different seasons and size of salmon on sea lice grazing will be studies in detail. These grazing interactions have not been studies previously, but have a crucial impact on further development and success of biological delousing use for aquaculture in the Nordic region. Different rearing installations will be tested from large on-shore tanks (> 8 m^3), sea-pens of different sizes (12x12 m, 24x24 m) and finally in full scale sea-pens (60 and 90 m circumference). Growth of all species will be studied along with, feeding efficiency. Number of sea lice (all developmental stages) will be studied in targeted sub-group of salmon at start and then every 2-3 weeks during the experimental period.

Role of the partners in WP3

Partners 7 (WP leader, HÓLAR), 1 and 2 will be the participants with the main responsibility for WP3 and will cooperate with all industrial partners during the implement of eco-friendly biological delousing with lumpfish under large scale production conditions.

Milestones

- M3.1. Effect of salmon size and lumpfish density for biological delousing (Month 30).
- M3.2. Biological and technical production protocols for biological delousing in sea pens (Month 36).

WP3 Progress 2015-18

Planning of activity in WP3 was started in autumn of 2014 and the trials started in the spring of 2015 and are ongoing. Final trials were performed from 2016-18.

Task 3.1 Optimise lumpfish density for effective lice control (Innblandingsprosent av rognkjeks og kombinasjonsbruk med rognkjeks og luseskjørt)

Partner 1 (APN) performed this trial in cooperation with Partner 3 (Nordlaks) during 2015-17. The trials focus on optimizing the use of lumpfish during the whole production cycle of Atlantic salmon in sea pens. Final results are reported in this report. This report is in Norwegian.

Task 3.2. Optimizing the effectiveness of farmed lumpfish for biological delousing of salmon

Large scale trials with lumpfish were performed in 2017-18 at the production sites of Fjarðarlax (which is now owned by Arnarlax hf). Findings are reported in this final report.

Task 3.1 Innblandingsprosent av rognkjeks og kombinasjonsbruk med rognkjeks og luseskjørt (Nordlaks og Akvaplan-niva)

Gjennomføring

Del 1:

Oppstart juni 2015. Totalt seks merder à 130 diameter med spissnot inngikk i forsøket, hvor to forskjellige innblandingsprosenter av rognkjeks, 3,75 %, 7,5 % ble undersøkt i replikat. I tillegg var det to kontrollmerder med laks uten rognkjeks (0 % innblanding). Startvekt for rognkjeks og laks var hhv. 13 g og 80-180 g. Hver merd hadde totalt 150.000-165.000 smolt ved utsett. Alle merder hadde luseskjørt fra forsøksstart.

Del 2:

Oppstart Juni 2016 (Fig. 16) på samme lokalitet som i del 1 av forsøket etter splitting. Totalt seks merder med spissnot (130 m) inngikk i forsøket, hvor to forskjellige innblandingsprosenter av rognkjeks, hhv. 7,5, og 5,0 innblanding, samt kontrollmerder uten rognkjeks (0% innblanding) ble undersøkt i replikat. Ved oppstart var laksen ca. 2,2 kg og antall laks per merd var 110.000 – 140.0000. Startvekt for rognkjeks ca. 70 g. Det ble foretatt månedlig oppfølgning av fisken fra juli 2016 til oktober 2016.

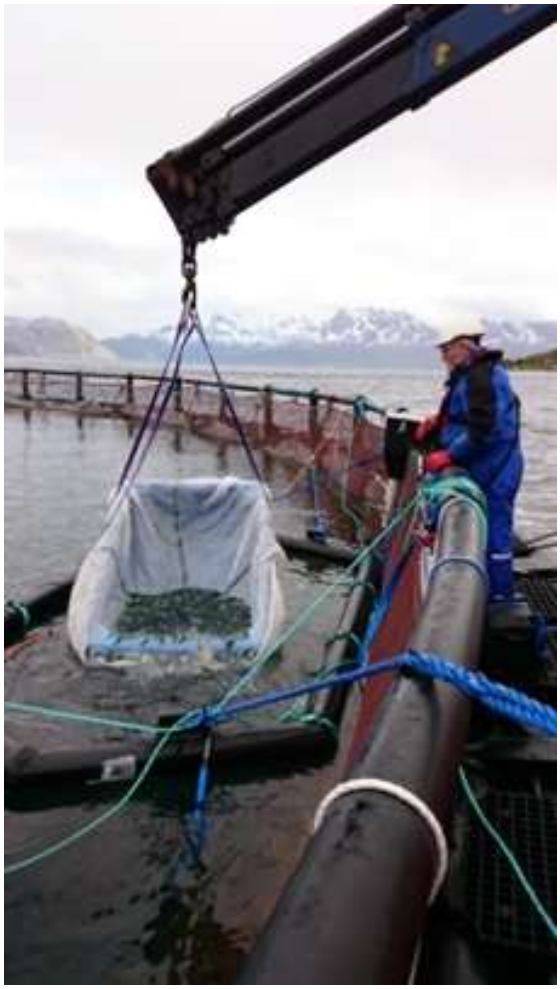


Fig. 16. Utsett av rognkjeks i merd (foto: Ane V. Nytrø, Akvaplan-niva).

Avlusning av laks

I uke 42 ble laksen avluset med SLICE på grunn av det høye påslaget av skottelus i anlegget. Det ble ikke påvist noen dødelighet på rognkjeksen i anlegget som følge av dette. Mengden skottelus i mage

hos rognkjeksen ble betydelig redusert etter dette, men andelen lusespisere i merdene økte deretter på tross av lave nivå av både lakselus og skottelus.
Del 2 av forsøket måtte termineres tidlig på grunn av høye lusetall. Behandling med hydrogenperoksid ble gjennomført.

Resultater

Utsett:

Transport av rognkjeks ble gjennomført i henhold til anbefalinger. Kun vann fra transporttankene fra bil og oksygenering ble benyttet i den snaut 20 min sekundærtransporten i transportkar på båt ut til lokalitet Skøyen (Nordlaks). Under transporten var det problemer med regulering av oksygen som ført til overmetning under ett av de to utsettene sommeren 2016. All rognkjeksen ble satt ut direkte i skjul.

Gjenfangst:

Det var ikke mulig å hente ut rognkjeksen i forbindelse med pumping av laks. Det ble derfor benyttet håv løftet med kran. Rognkjeks ble deretter avlivet ved bruk av Finquel eller Benzoak.
Hos Lerøy ble det estimert en gjenfangstprosent på om lag 40 etter intensiv utfisking våren 2016. Et hovedproblem under gjenfangsten var at rognkjeksen ikke oppholdt seg i nærheten av skjulene sannsynligvis pga. størrelsen og var spredt sammen med laksen i merden. Store størrelsesforskjeller hos rognkjeksen samt behov for rask håndtering av laks i forbindelse med trenging og pumping gjorde at en ikke fikk tak i rognkjeksen i denne operasjonene. Utfiskingsmetoden på begge lokaliteter ble derfor nitidig utfisking ved bruk av krantrukket håv eller gjenfangst ved bruk av hånd-holdt håv. Begge metodene er svært tidkrevende og tunge.

Del 1. Dødelighet og sykdom på rognkjeks, Nordlaks Oppdrett, Skøyen

Høy dødelighet på rognkjeksen i perioden før lusepåslag medførte at startverdiene for innblandingsprosenten for rognkjeks ble redusert til hhv. 2.5% og 5.5% (Fig. 17).

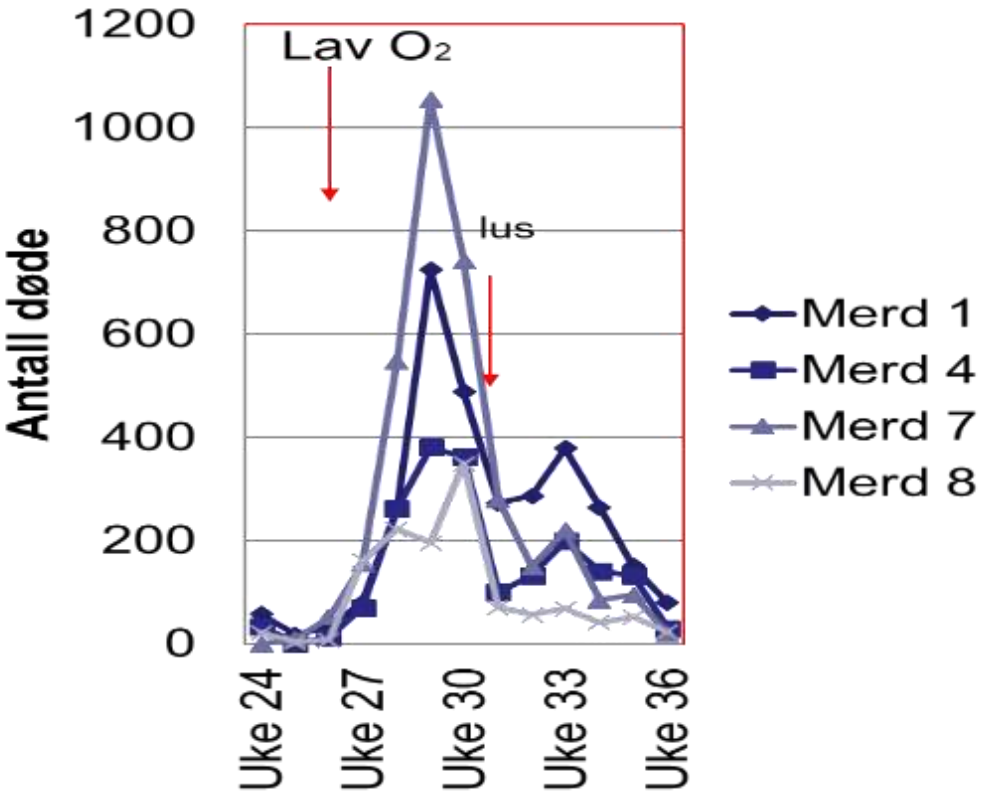


Fig. 17. Dødelighet (antall per uke) hos rognkjeks i Del 1 forsøket hos Nordlaks (Skøyen).

Da dødeligheten avtok før lusepåslaget på Skøyen ble innblandingsprosenten beregnet på det tidspunktet (hhv. 5,5% og 2,7%) benyttet for resten av forsøksperioden. Analyser av død rognkjeks viste trichodina (*Trichodina, Tenacibaccullum)* og gjellebetennelser på fisken, men ingen av disse ble antatt å være hovedpatogener. Det var også mistanke om *Pasteurellla*, men dette ble ikke bekreftet. Nye prøveuttak påviste sopphyfer i milt, nyre og hjerte, nekroser i milt og nyre samt myokarditt i hjerte fra fisk i en merd. I en annen merd var sykdomsbildet svært annerledes med funn av miljøbakeriene *Vibrio logei* og *Vibrio splendidus* som ikke antas å være hovedpatogener. Det var også sett lever- og nyrenekroser og multifokale blødninger i muskulatur. Forkalkninger i nyre ble også observert, sammen med funn av en hittil ukjent bakterie på rognkjeks: *Photobacterium*. Det ble også gjort funn av gjellebetennelse hos noe fisk. Det ble observert mye skottelus på en del rognkjeks i forsøket (Fig. 18) samt øyeskader av diverse slag (Fig. 19) og soppinfeksjoner (Fig. 20).

Fig. 18. Skottelus på rognkjeks. På dette individet ble det telt over 70 skottelus. (Foto: Ane V. Nytrø, APN).

Fig. 19. Øyeskader av diverse slag var en tilbakevendende problemstilling i forsøksperioden (Foto: Ane V. Nytrø, APN).

Fig. 20. Soppinfeksjoner observert på rognkjeks. (Foto: Ane V. Nytrø, APN)

Del 1. Mageinnhold, Nordlaks (Skøyen)

Det ble observert økende antall rognkjeks med lus i magen fra juni til september 2015 (Fig. 21). Dette var både skottelus og lakselus. I tillegg hadde nesten 100% av fisk raudåte (Fig. 22-23). Andel rognkjeks som spiste pellet (Fig. 24) økte også gjennom forsøket og var rundt 40% mot slutten. Det ble observert lite laksefôr i rognkjeksmagene.

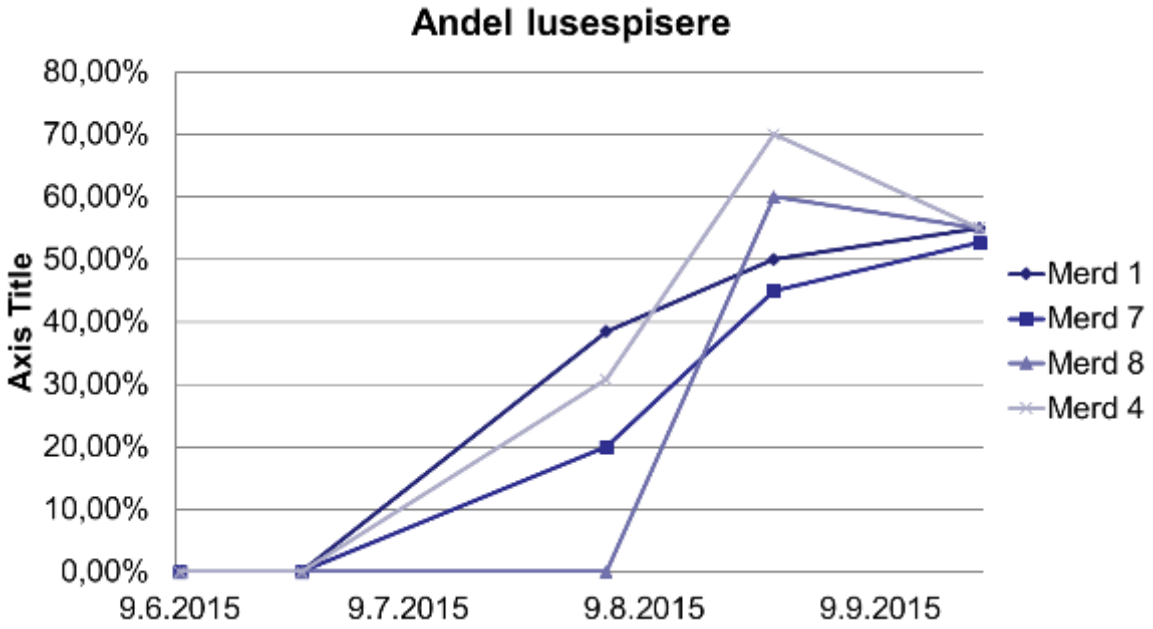


Fig. 21. Andel lusespisere (skottelus og lakselus) blant rognkjeks i Del 1 forsøk hos Nordlaks.

Fig. 22. Raudåte fra rognkjeksmage i Del 1 forsøk hos Nordlaks.

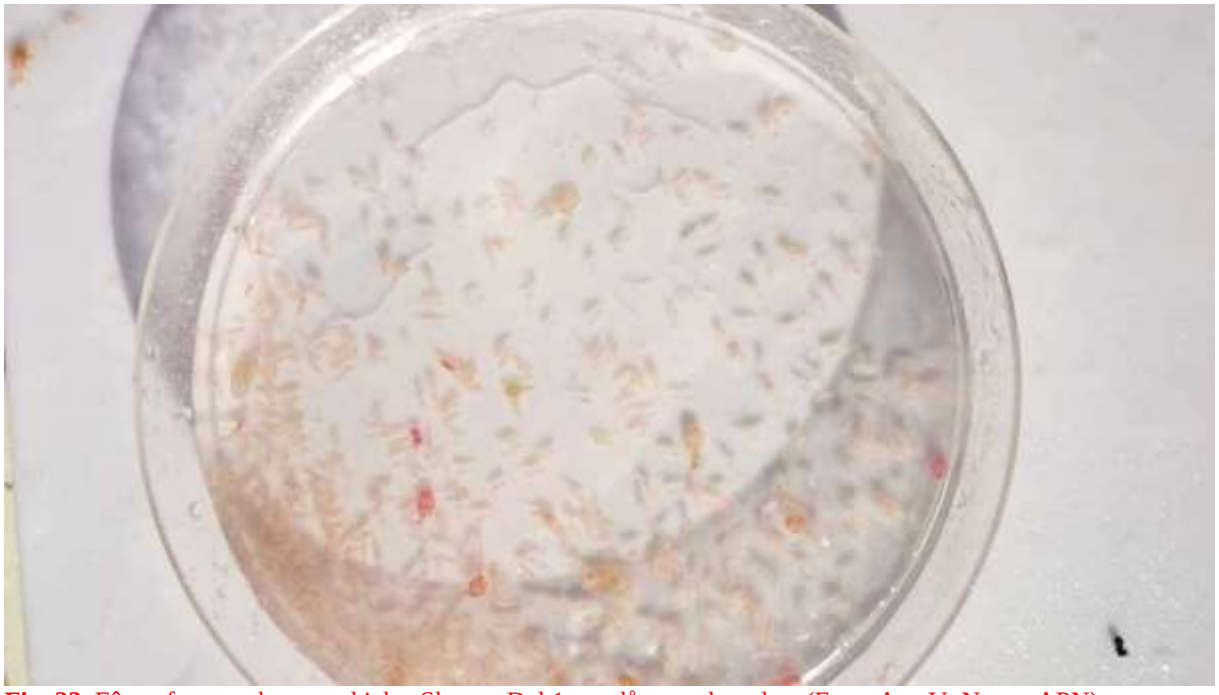


Fig. 23. Fôrpreferanser hos rognkjeks, Skøyen Del 1: raudåte og skottelus. (Foto: Ane V. Nytrø, APN).

Fig. 24.. Fôrpreferanser hos rognkjeks, Skøyen Del 1: marinpellet og lus. (Foto: Ane V. Nytrø, APN).

Andel rognkjeks som spiste glassmaneter økte med økende fiskestørrelse.

Del 1. Utvikling i lus på laks, Nordlaks, Skøyen
Utvikling av lakselus på laks på lokalitet Skøyen er vist på Figur 24 (alle stadier lakselus). Generelt ble det funnet lite lus i forsøket og total mengde lus lå under eller rundt tiltaksgrensen i begge rognkjeksgruppene. Antall lus i kontrollgruppen (uten rognkjeks) var signifikant høyere (ANOVA, $P < 0.05$, Fig. 25) mellom uke 38 og 40 og mellom uke 45 og 47 i 2015.
Svært lite lus ble funnet i alle tre forsøksgrupper fra uke 49 i 2015 og til forsøksslutt i uke 24 i 2016 (Fig. 25).

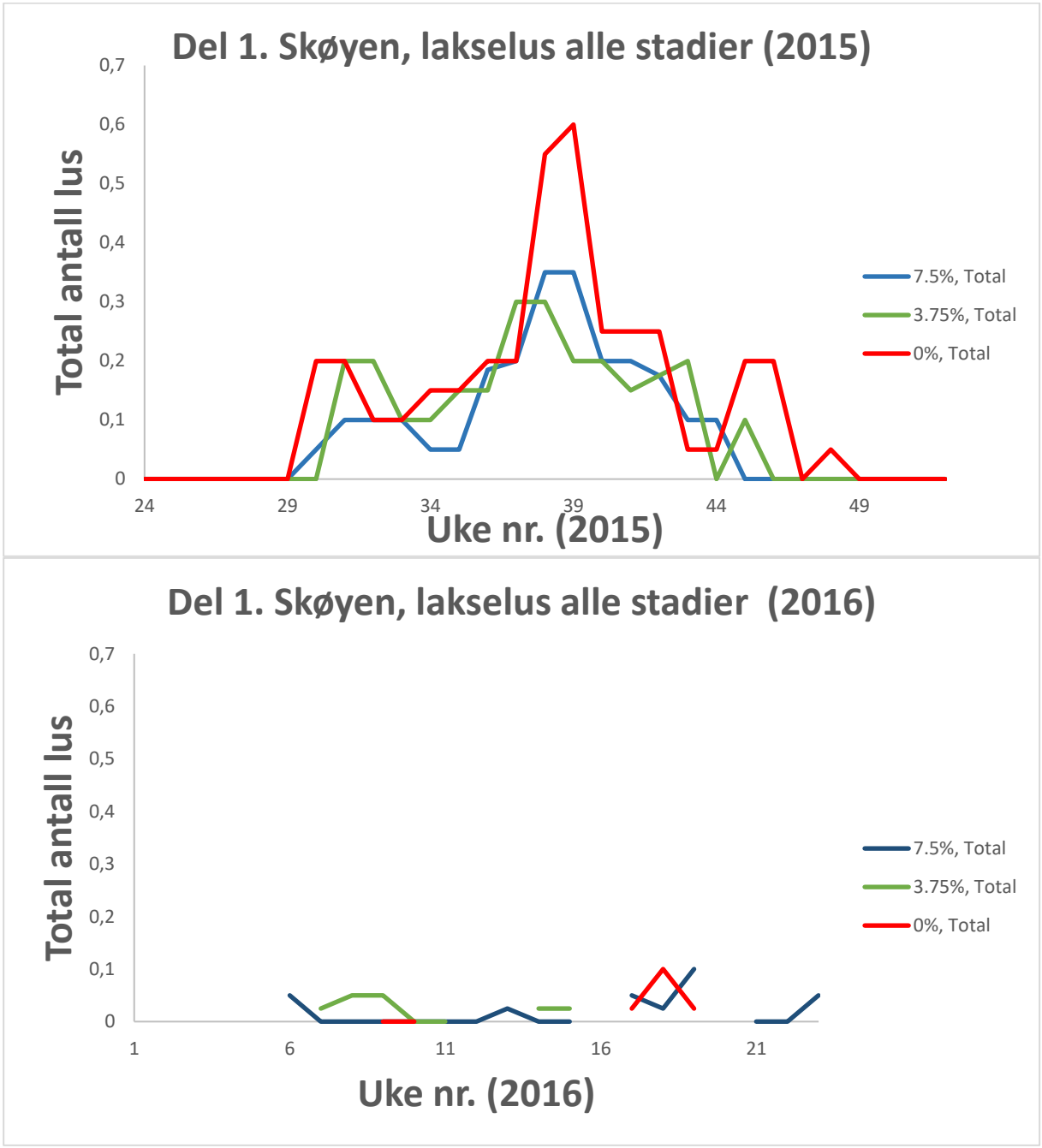


Fig. 25. Utvikling i lusetall ved lokalitet Skøyen i 2015 og 2016 (Del 1).

Del 1, Hovedkonklusjoner fra Skøyen 2015 (første år i sjø).

- Mye skottelus i rognkjeksmager – raudåte en utfordring. Rognkjeks er opportunist og spiser det den måtte finne i merden. Raudåte overtok som hovedføde når den var på topp i området (tilsvarende observasjoner er gjort tidligere i forsøk hos Engesund Fiskeoppdrett). En hypotese er at rognkjeksen blir mindre interessert i lus når det er mye raudåte i sjøen.
- Mye marinpellet i mageprøvene - i tillegg til alle andre næringsorganismer. Økende innslag av glassmaneter ettersom fisken var større. En hypotese er at rognkjeksen blir mindre interessert i lus når tilgangen på andre næringsorganismer er god. Glassmaneter er vanlig å finne i magene til vill rognkjeks i norske fjorder.
- En fant ingen effektiv måte å få ut stor rognkjeks på fra merden etter forsøksperioden. Rognkjeksen benytter skjulene mindre når den blir større.
- Rognkjeksen benytter hele merden.

Del 2. Dødelighet, Nordlaks Oppdrett, Skøyen

I del 2 av forsøket (etter splitting av laks juni 2016) ble det bestemt at de opprinnelige innblandingsprosentene for forsøket skulle være gjeldende (dvs. 3,75% og 7,5%). Etter utsett av rognkjeksen ble det observert høy dødelighet, og tilsvarende problemer med sykdom som i del 1 av forsøket. Ca. 3000 rognkjeks døde de første 3-4 ukene etter utsett (Fig. 26). Hardest gikk det ut over merd nr. 8 som var en av merdene med lavest innblandingsprosent og lavest antall laks. Her gikk innblandingsprosenten fra 3,8 % til om lag 1% etter dødelighetsperioden før en stabilisering, og i replikatgruppen,som også hadde en startvekt på 47,8 g 3,75%, falt innblandingsprosenten til 1,4 % i samme periode. Det ble påvist *Tricodina* og *Tenacibacculum* på fisken.

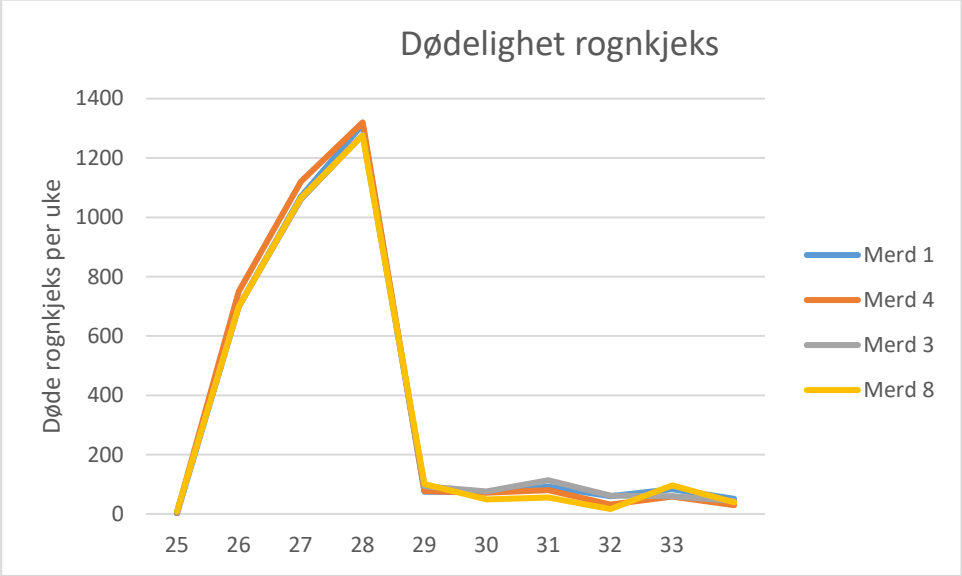


Fig. 26. Dødelighet (antall per uke) hos rognkjeks i Del 2 forsøket hos Nordlaks (Skøyen).

Del 2. Mageinnhold, Nordlaks, Skøyen

Lite lus ble funnet i rognkjeksmagene i del 2, og det var indikasjoner på at rognkjeksen foretrakk glassmaneter. I samme periode var det høye temperaturer i sjøen (12,5-13,0°C) og rognkjeksen doblet vekten i løpet av august.

Del 2. Utvikling i lus på laks, Nordlaks, Skøyen

Utvikling i lusetall 2016 (del 2) på Skøyen er vist på Figur 27. Ingen forskjeller ble funnet i antall fastsittende eller kjønnsmodne hunnlus mellom forsøksgrunne. Bevegelig lus var høyere i kontrollgruppen (uten rognkjeks) fra uke 31 til 33. Fra uke 34 økte antall bevegelig lus (data ikke vist på figur) i alle tre gruppen og laksen ble behandlet med Alphamax i uke 40 da forsøket ble avsluttet.

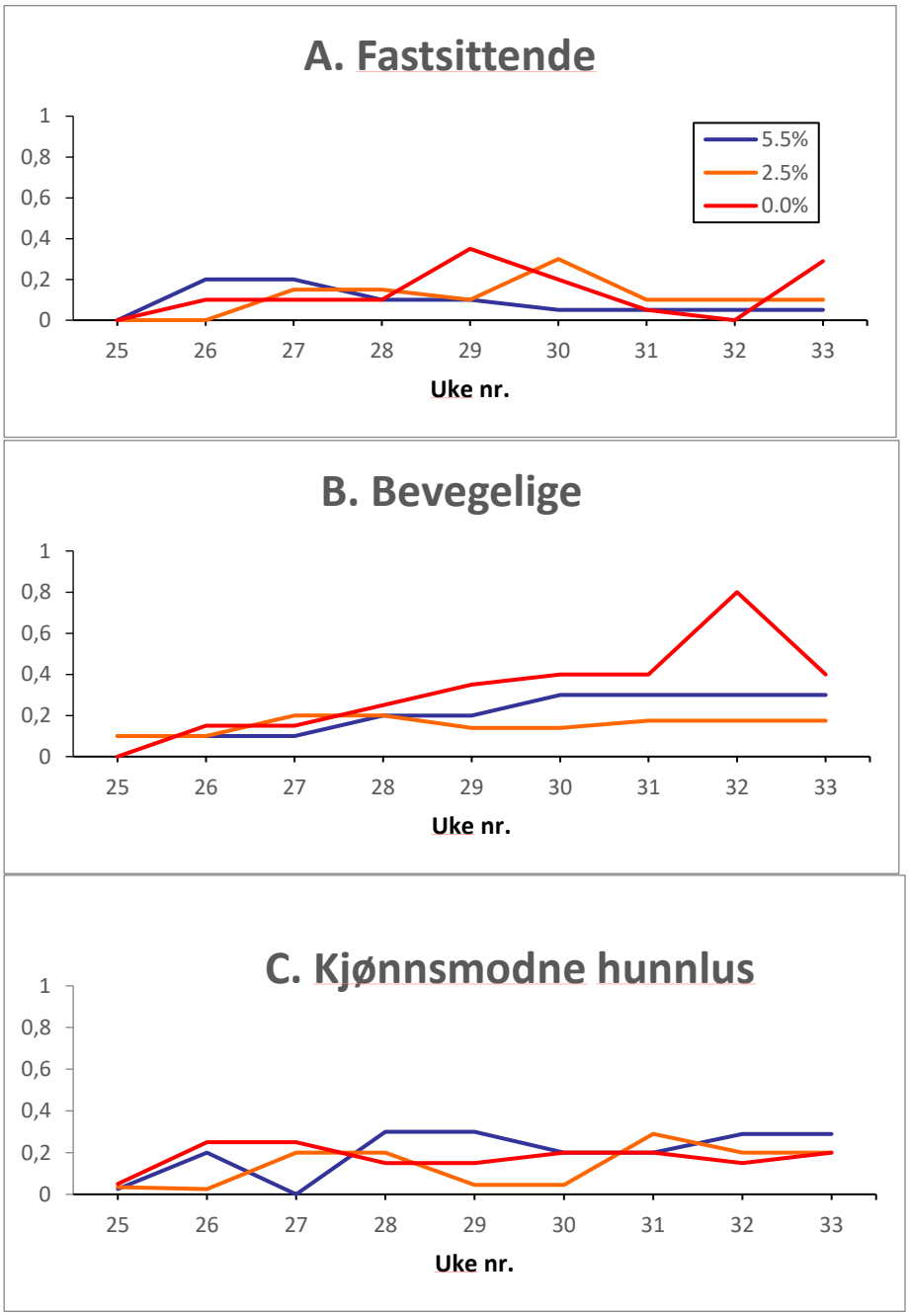


Fig. 27. Utvikling i lusetall ved lokalitet Skøyen fra uke 25 til 33 i 2016 (Del 2).

Del 2, Hovedkonklusjoner fra forsøk hos Nordlaks (andre år i sjøen)

- Generelt lave lusetall i perioden.
- Høyere innslag av bevegelige lus enn året før.
- Rask vekst på rognkjeksen juli-august (fra 70 g til 120-150 g på ca. en mnd.)
- Tilsynelatende lite lusespising fra uke 38 førte til behov for avlusing.
- Nesten utelukkende funn av glassmaneter i rognkjeksmager
- I motsetning til del 1 spiste rognkjeks i del 2 lite lus og pellet

Task 3.2. Optimizing the effectiveness of farmed lumpfish for biological delousing of salmon (Fjarðalax/Arnarlax and Akvaplan-niva)

Materials and methods

Large scale trials with lumpfish were performed in 2017-18 at the production sites of Fjarðarlax (which is now owned by Arnarlax hf). Lumpfish were put into sea in september (week 38) of 2017 at the production site of Haganes in Arnarfjörður (Vestfirðir, Fig. 28). The neigbouring site of Steinarnes was used as control (no lumpfish in sea pens, but all sea pens had lice skirt).

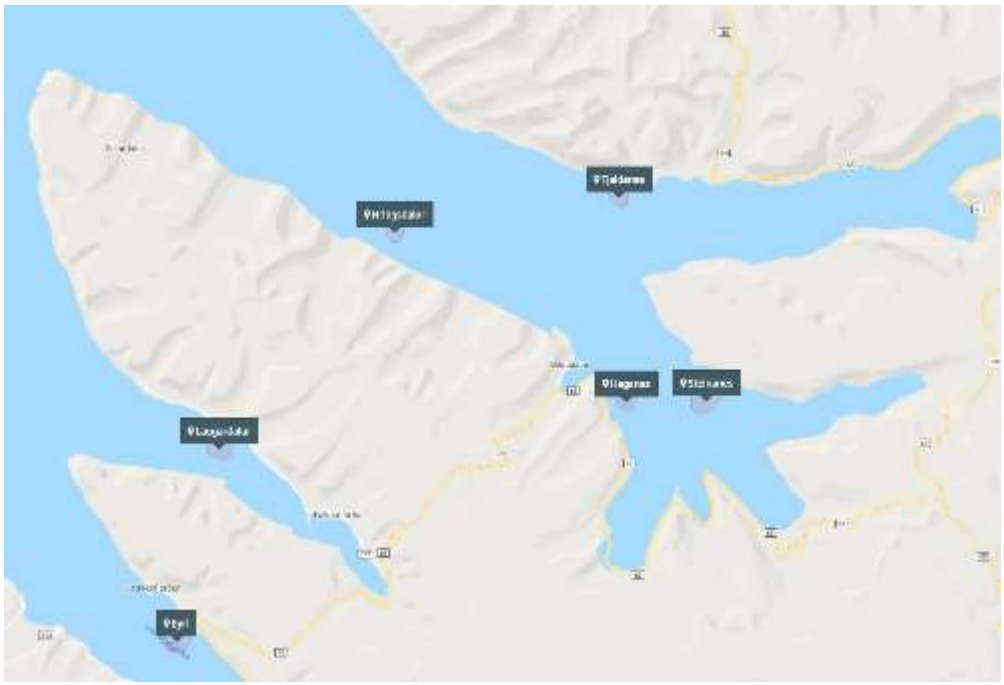


Fig. 24. Production sites of Fjarðarlax/Arnarlax in 2017-18. The site with lumpfish is Haganes (second furthest to the right) and the control site is Steinarnes (no lumpfish, furthest to the right).

A total of six sea pens were used in the trial, each with 120 m diameter and rearing volume of 15300 m^3. The density of lumpfish at start varied between 8.4 to 12.4% (Table 2). Startweight for lumpfish was 25 g and for salmon 873.

Table 2. Number of Atlantic salmon and lumpfish at the production site of Haganes at start of the trial in September 2017.

	Sea pen 1	Sea pen 2	Sea pen 3	Sea pen 4	Sea pen 5	Sea pen 6
# Salmon:	80.459	82.116	83.431	83.471	89.140	79.231
# Lumpfish	10.000	7.500	7.500	10.000	7.500	7.500
Density of lumpfish:	12.4%	9.1%	9.0%	12.0%	8.4%	9.5%

In August 2018 50000 lumpfish (mean size 25) were added to the Haganes sea pens. At this time the mean size of the salmon had increased to 3.2 kg.
Sea lice on salmon were counted once a week from week 38 to week 51 and from week 18 onwards in 2018. The sea temperature was below 4°C from late December to late April 2018 and during this time it was not possible to monitor sea lice on the salmon.

Results

Levels of adult female sea lice (Fig. 25) increased at both production sites during 2017. No lice could be observed from January to April 2018 but once counted again in late April the levels of sea lice were lower in the lumpfish sea pens at Haganes, whereas the sea lice levels rose to over 1 in June 2018 at the Steinarnes location. Accordingly the salmon at Steinarnes had to be treated with Alphamax in week 26. At that time the sea lice levels was three times higher at Steinarnes (0.99) compared to Haganes (0.33).

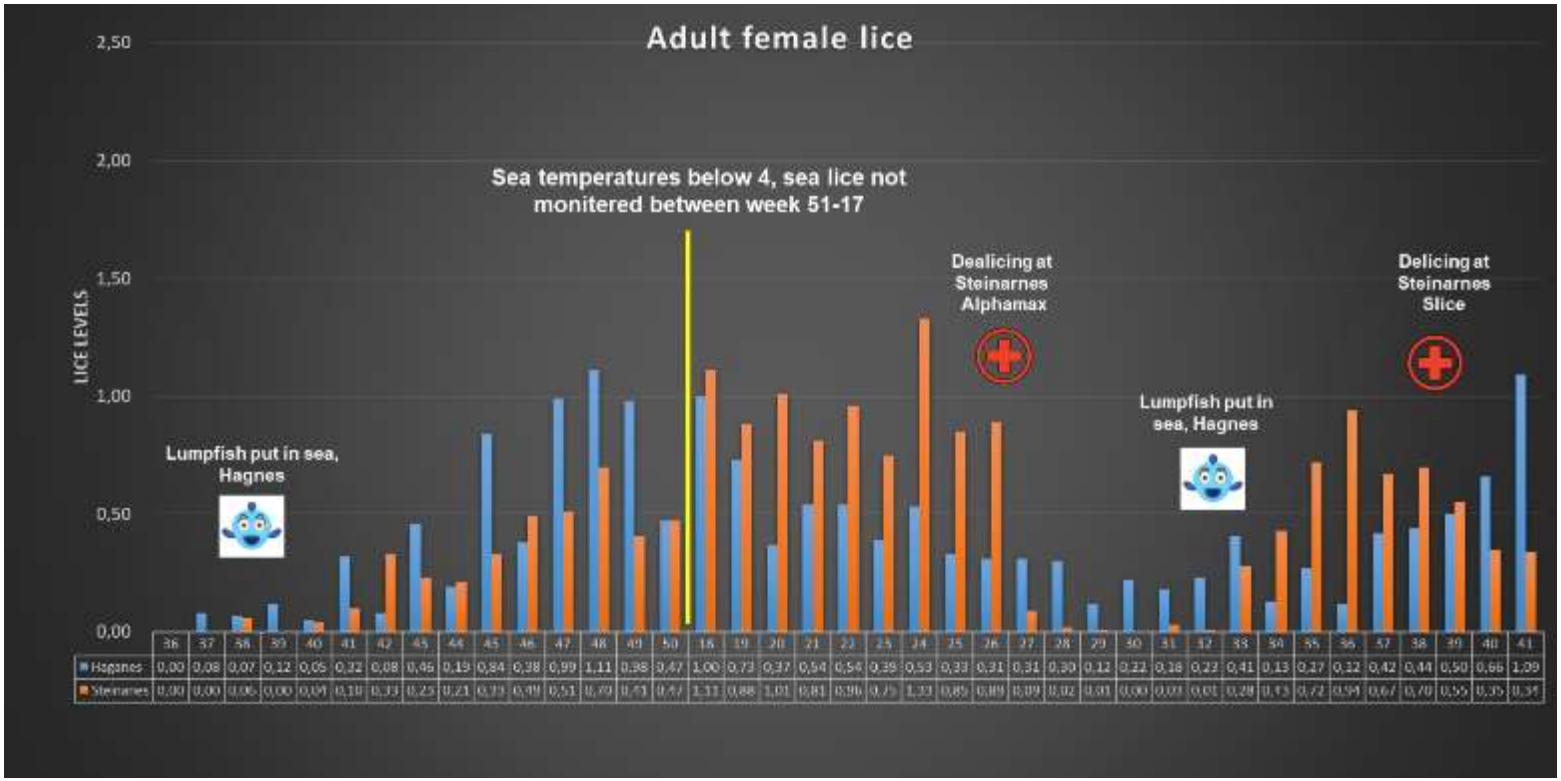


Fig. 25. Sea lice levels on Atlantic salmon at the production sites of Haganes (with lumpfish) and Steinarnes (no lumpfish) in 2017-18.

Discussion

Lower levels of sea lice were seen in sea pens with lumpfish compared to sea pens without lumpfish. Of particular interest is the observation that lumpfish graze efficiently on sea lice during the winter months when sea temperatures at the production sites was below 4°C. There was strong indication of sea lice grazing from late January onwards. The average numbers of mature female lice decreased in the Haganes group from December to April. During January to March the average levels of adult female lice per fish had increased to 0.9-1.1 per fish in the control group, whereas the lumpfish group had between two and three times lower levels (0.33-0.52). This is similar to what has been found under small scale testing in previous trials (Imsland et al., 2014a-b; 2015). The low average numbers of mature female lice found in the lumpfish group during the latter half of the study period suggest that this stage is actively selected by lumpfish as a preferred prey item as suggested by Imsland et al. (2014a). If lumpfish are preferentially selecting the larger mature females, then the potential for re-infestation is greatly diminished. It has been shown that mature females can live for up to 7 months and produce approximately 6 to 10 new egg strings during this time (Mustafa et al., 2010). Removing or reducing mature female lice has the greatest effect in reducing the size of the sea lice population (Boxaspen, 2006).

No antagonistic behaviour between the two species was observed and the two species seemed to co-exist along each other in the sea pens. Wild lumpfish and Atlantic salmon share feeding grounds and Sheedan et al. (2012) found large numbers of juvenile and adult lumpfish together with Atlantic salmon when sampling Atlantic salmon with surface trawl in the Northwest Atlantic (Labrador Sea). In the Northeast Atlantic (Norwegian Sea) lumpfish and Atlantic salmon inhabit much the same areas (Hansen and Jacobsen, 2000, Bjelland and Holst, 2004) feeding on similar prey items (Jacobsen and Hansen, 2001; Vandendriessche et al., 2007). The fact that lumpfish and Atlantic salmon share feeding grounds in the wild may help to explain the non-antagonistic behaviour seen between the two species in the present study as well as in previous studies (Imsland et al., 2014a-c).

References

Bjelland, O. and Holst, J. C. (2004). Other fish species and fish communities. In The Norwegian Sea ecosystem (ed. H. Skjoldal), pp. 357-369. Tapir Academic Press, Trondheim, Norway.

Boxaspen, K. (2006). A review of the biology and genetics of sea lice. *ICES J. Sea Res.* 63, 1304-1316.

Hansen, L. P. and Jacobsen, J. A. (2000). Distribution and migration of Atlantic salmon (Salmo salar L.) in the sea. In The ocean life of Atlantic salmon—environmental and biological factors influencing survival (ed. D. Mills), pp. 75-87. Blackwell Science, Oxford, UK.

Imsland, A. K., Reynolds, P., Eliassen, G., Hangstad, T. A., Foss, A., Vikingstad, E. and Elvegård, T. A. (2014a). The use of lumpfish (*Cyclopterus lumpus* L.) to control sea lice (*Lepeophtheirus salmonis* Krøyer) infestations in intensively farmed Atlantic salmon (*Salmo sala*r L.). *Aquaculture* 425-426, 18-23.

Imsland, A. K., Reynolds, P., Eliassen, G., Hangstad, T. A., Nytrø, A. V., Foss, A., Vikingstad, E. and Elvegård, T. A. (2014b). Notes on behaviour of lumpfish in sea pens with and without Atlantic salmon. *J. Ethol.*32, 117-122.

Imsland, A. K., Reynolds, P., Eliassen, G., Hangstad, T. A., Nytrø, A. V., Foss, A., Vikingstad, E. and Elvegård, T. A. (2014c). Assessment of growth and sea lice infection levels in Atlantic salmon stocked in small-scale cages with lumpfish. Aquaculture 433, 137-142.

Imsland, A. K., Reynolds, P., Eliassen, G., Hangstad, T. A., Nytrø, A. V., Foss, A., Vikingstad, E. and Elvegård, T. A. (2015a). Feeding preferences of lumpfish (*Cyclopterus lumpus* L.) maintained in open net-pens with Atlantic salmon (*Salmo salar* L.) Aquaculture 436, 47-51.

Jacobsen, J. A. and Hansen, L. P. (2001). Feeding habits of wild and escaped farmed Atlantic salmon Salmo salar L., in the Northeast Atlantic. ICES J. Sea Res. 58, 916-933.

Mustafa, A., Conboy, G. A. and Burka, J. F. (2000). Lifespan and reproductive capacity of sea lice, *Lepeophtheirus salmonis*, under laboratory conditions. *Aquac. Assoc. Can., Spec. Publ.* 4, 113-114.

Sheedan, T. F., Reddin, D. G., Chaput, G. and Renkawitz, M. D. (2012). SALSEA North America: a pelagic ecosystem survey targeting Atlantic salmon in the Northwest Atlantic. ICES J. Mar. Res. 69, 1580-1588.

Vandendriessche, S., Messiaen, M., O'Flynn, S., Vincx, M. and Degraer, S. (2007). Hiding and feeding in floating seaweed: Floating seaweed clumps as possible refuges or feeding grounds for fishes. Est. Coast, Shelf. Sci. 71, 691-703.

Work package number			4	**Start date or starting event:**			1				
Work package title			***Management, dissemination and exploitation***								
Participant number	**1**	2	3	4	5	6	7				
Person-months per participant:	**1.5**	0.5	0.25	0.25	0.25	0.5	1				

Objectives

- To co-ordinate the overall development of the project, to assess the accomplishment of tasks allocated to each participant, to discuss the results obtained during different phases of the project.
- Exploitation of results, including the implementation of new rearing methods and technology by training employees and management at the premises of the SMEs.

Description of work

This Work Package consist of three Tasks:

- *Task 4.1 Project coordination, consortium meetings*
- *Task 4.2 Training of SMEs*
- *Task 4.3 Exploitation of project results, publications, conferences, workshops*

Task 4.1 Project coordination, consortium meetings

The project coordinator (Prof. Albert K. Imsland) will monitor and ensure the fulfilment of the objectives envisaged in the proposal as well as keeping deadlines and quality standards required. Prof. Albert Imsland has extensive management experience in administration of several European, Norwegian, Icelandic and Nordic scientific projects (see attached CV). There will be a Phase-Meeting held at the onset of the project, after one year and in the end of year two, with all participants to plan and assess the accomplishment of each task.

Task 4.2 Training of SMEs

The SMEs will be offered a training plan on implementation of lumpfish in normal year round production of Atlantic salmon, and this method can be used as a management tool to monitor and safeguard welfare of salmon. The training will also be carried out at the premises of the SMEs. Both employees and management will be involved.

Task 4.3 Exploitation of project results

In order to ensure that the industrial partners are able to assimilate and exploit the results of the project, a number of steps are foreseen. Emphasis will be on introducing the new biological delousing method within the NORA region, including presentations at conferences, workshops as well as peer-review publications.

Role of partners

Partners 1 (WP leader, APN) and all other partners will cooperate on the implementation of dissemination (Task 4.1 and 4.3, led by APN) and training (Task 4.2, led by FISKA).

WP4 Progress 2013-18

The kick-of meeting in the NOLICE project was held in Reykjavik 14 November 2013 with partners from Iceland, Norway and the Faroes present. At this meeting the experimental work for the first two years was discussed. We plan a consortium meeting with all project partners in early spring 2016 in the Faroes.
In spring 2014 it was decided to send one technician from Hólaskóli (Arnþór Gústavsson) to Akvaplan-niva research station (Troms Marin Yngel) near Tromsø, Norway to participate in the hatchery work there in order to transfer the knowledge from Norway to Iceland. TMY has been involved in lumpfish rearing since 2011. This practise will be repeated in year 2 of the project with 1-2 technicians from Hólaskóli travelling to Tromsø. Personnel from Fiskaaling were also stationed at TMY to learn the hatchery techniques developed for lumpfish.
During spring 2015 a second visit from Hólaskóli (researcher Soizic Le Deuff) to Akvaplan-niva research station (Troms Marin Yngel) near Tromsø, Norway to participate in the hatchery work there was performed. As a result the production of juveniles at Hólaskóli increased during both 2015 and 2016.

Results from all WPs of the project have been published in peer review scientific journals and are listed below.

List of publications from the project work of NOLICE

WP1

Nytrø, A.V., Vikingstad, E., Foss, A., Hangstad, T.A., Reynolds, P., Eliassen, G., Elvegård, T.A., Falk-Petersen, I.B and Imsland, A.K. 2014. The effect of temperature and fish size on growth of juvenile lumpfish (*Cyclopterus lumpus* L.). *Aquaculture* 434, 296-302

Nordberg, G., Johannesen, A., Arge, R., 2015 Cryopreservation of lumpfish *Cyclopterus lumpus* (Linnaeus, 1758) milt. PEERJ 3, Article Number: e1003, DOI: 10.7717/peerj.1003

WP2

Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Nytrø, A.V., Foss, A., Vikingstad, E., Elvegård, T.A. 2014. Notes on behaviour of lumpfish in sea pens with and without Atlantic salmon. *Journal of Ethology* 32, 117-122.

Imsland, A.K., Reynolds, P., Nytrø, A.V., Eliassen, G., Hangstad, T.A., Jónsdóttir, Ó.D.B., Emaus, P.A., Elvegård, T.A., Lemmens, S.C.A., Rydland, R., Jonassen, T.M. 2016. Effects of lumpfish size on foraging behaviour and co-existence with sea lice infected Atlantic salmon in sea cages. *Aquaculture* 465, 19-27.

Imsland, A.K., Reynolds, P., Eliassen, G., Mortensen, A., Hansen, Ø.J., Puvanendran, V., Hangstad, T.A., Jónsdóttir, Ó.D.B., Emaus, P.A., Elvegård, T.A., Lemmens, S.C.A., Rydland, R., Nytrø, A.V., Jonassen, T.M. 2016. Is cleaning behavior in lumpfish (*Cyclopterus lumpus*) parentally controlled? *Aquaculture* 459, 156-165.

WP3

Imsland, A.K., Hanssen, A., Reynolds, P., Nytrø, A.V., Jonassen, T.M., Hangstad, T.A., Elvegård, T.A., Urskog, T.C., Mikalsen, B. 2018. It works! Lumpfish can significantly lower sea lice infections in large scale salmon farming *Biology Open* 7, 7, bio036301. doi:10.1242/bio.036301

Conferences

Results from Task 1.1 (larval and juvenile rearing) and 1.2 (temperature trial) were presented at the European Aquaculture Society conference in San Sebastián, Spain, 14-17 October 2014.
Results from Task 2.1 (Feeding behaviour and population dynamics of lumpfish) and Task 2.2 (Development of lumpfish breeding programme and search for QTLs) have been presented at the 10th International Sea Lice Conference 2014 in Portland Maine USA, 1-5 September 2014 (poster and oral), FHF cleanerfish conference, Gardermoen, Oslo, 8-9 February 2016 (oral) and at Aquaculture Europe 16, Edinburgh, Scotland, 21-23 September 2016 (oral).

The partners allowed the free use of scientific results obtained through the proposed project after their presentation through publications, international conferences and open workshops. These results are now available to all interested in Atlantic salmon farmers in the NORA region to facilitate the use of lumpfish for biological delousing of salmon.

3.2 Project management structure

The main project management functions are

- Administration, Contractual and Financial Matters;
- Scientific and Technical Co-ordination and Monitoring;
- Dissemination and Communication;
- Exploitation and Impact Measures

Administration, Contractual and Financial Matters;
The role of the Project Coordinator (PC) is to assume responsibility for the overall management and co-ordination of the Project including dealing with REA and organising consortium meetings.

The coordinator will appoint a financial controller from within their organisation. The Financial Controller (FC) should exercise control of the budget and assist WP Leaders in resource and cost monitoring and reporting.

Exploitation and Impact Measures
A representative from the participating SMEs will be appointed as the Exploitation and Impact Co-ordinator (IC). The implementation of the outputs of each work package should be the responsibility of the WP Leader but would be co-ordinated at the Project level by the IC.

Project administration-decision making
The split of the overall project into work packages and tasks will allow each *partner* to be responsible for activities close to its expertise so that parallel progress can be achieved in a number of tasks. Task assignment and responsibility will be based on the expertise and the interest expressed and the facilities available by the *partner* and will be managed by the group leader of the *partner*.

Tasks within the same work package will also be co-ordinated by the work package co-ordinator who will be selected based on expertise and express of interest. *A Task Leader* will be nominated among the partners involved in a task (the Task Group) for the detailed co-ordination, planning, monitoring and reporting of the task and for the detailed co-ordination of the task with the other tasks in the project.

3.3 Importance for the aquaculture sector

By the end of the project, the aquaculture sector in the NORA region should have all the necessary biological and technological knowledge that will allow for large scale production of lumpfish and its use for biological delousing. This knowledge will lead to significantly reduced use of chemotherapeutics in food production thereby ensuring optimal welfare and sustainability during farming of Atlantic salmon. The participating salmon farmers will be able to benefit from the improvements immediately. The scientific and production oriented findings of the project will be used both by the industrial partners and the researchers to evolve and support a continuous research effort for further improving on-growing methods and technology beyond the end of the project. The new equipment and on-growing methods are expected to be available in the market within a maximum period of **two years** after the termination of the project.

3.4 Form of project results

For the four work packages we have defined the following 9 milestones or achievements and indicate subsequent dates for reaching these goals.

Milestone num	Milestones Title	WP number	Nature	Delivery date
1	Protocols for brood-fish management and spawning of lumpfish	**1**	**R**	**12**
2	Protocols for the successful coexistence of lumpfish and Atlantic salmon	**2**	**R**	**12**
3	Optimal rearing protocol of juvenile lumpfish	**1**	**R**	**24**
4	Protocols for cryopreserving lumpfish sperm and optimal artificial insemination protocol of lumpfish	**1**	**R**	**30**
5	Optimal feeding regime for successful biological delousing of salmon	**2**	**R**	**30**
6	Effect of salmon size and lumpfish density for biological delousing	**3**	**R**	**30**
7	Lumpfish breeding programme and possible QTLs	**2**	**R**	**36**
8	Biological and technical production protocols for biological delousing in sea pens	**3**	**R**	**36**
9	Dissemination activity report	**4**	**R**	**36**

[ii]Nature of deliverables related to milestones:

Please indicate the nature of the deliverable using one of the following codes:

R = Report.

4. Partners and their role

4.1 Project partners

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4.2 Partners profiles

The consortium is made up of participants from the three countries in the NORA region, Norway, Faroe Islands and Iceland. They represent the three most important countries in farming of Atlantic salmon in the area. Each participating country contributes in one or more of three important areas needed in order to achieve the purpose of *NOLICE*, namely:

- Experts in propagation of new species, including lumpfish (APN, FISKA, HÓLAR)
- Experts in up-scaling technologies in order to reach a proper commercialisation (APN, FISKA, HÓLAR, NIVA);
- Fish farmers and enterprises involved in fish production (end-users of the technology) (NLAKS, FFFA, FLAX)
- Producers of lumpfish (APN, FISKA).

Coordinator, Akvaplan-niva (APN), Iceland

Akvaplan-niva AS (www.akvaplan.niva.no) will provide expertise in lumpfish culture, technical assistance, rearing technology, data analysis and interpretation and trouble shoot any problems. APN is actively involved in several aquaculture R&D projects. This allows APN to rapidly utilize new technology and management strategies in operational fish farming. The strength of APN lies in the up-scaling of experimental set-ups and strategies to commercial aquaculture production. APN is able to rapidly test new technology and management strategies in a realistic production environment.

APN has a subsidiary company (Troms Marin Yngel AS) that is a marine hatchery founded in 1995 and is based in Tromsø, Norway. Its main area of specialization is aquaculture production and research in new technology. The TMY marine hatchery has all necessary laboratory facilities to perform rearing studies with lumpfish as outlined in the proposal. TMY comprises an extensive range of high quality rearing facilities, including tanks, incubators and temperature control rooms. Water temperatures may be set between 3 and 20°C and light is controlled from a computer simulating any photoperiod regime.

Principal research personnel involved

Professor Albert K. Imsland has been working with marine species in culture since 1991 mainly focusing on environmental and genetical regulation of growth mechanisms and fish maturation. Prof. Imsland has since 1995 authored and co-authored over 120 scientific papers published in peer-review journals. Prof. Imsland has extensive management experience in administration of several European, Norwegian, Icelandic and Nordic scientific projects. He was the coordinator in two CRAFT projects in the 6 FP (TURPRO, 508070 and RACEWAYS, 016869) and is the coordinator of one R4SME project in the 7 FP (MAXIMUS, 286200).

Dr. Atle Foss has been working with aquaculture of marine fish species since 1995. He holds a Ph.D. in water quality optimization in intensive culture of marine fish and has authored and co-authored over 60 scientific papers published in peer-review journals on the topic of marine finfish aquaculture.

Dr. Erik Vikingstad has been working with aquaculture of marine species and salmonids since 1995, focusing on the environmental and endocrine control of reproduction.

Drs. Snorri Gunnarsson has over 20 years of experience from the aquaculture industry.

Dr. Ólöf Dóra Bartels Jónsdóttir has a PhD degree in population genetics and will be involved in Task 2.2.

Msc. Thor Arne Hangstad has 15 years of experience with hatchery production of marine aquaculture species. He will lead the juvenile lumpfish production at TMY.

Fiskaaling, Faroe Islands

Fiskaaling (www.fiskaaling.fo) is the only company of its kind on the Faroe Islands working exclusively with research and development within aquaculture. The company emphasises researching within fish and animals that are, or may become of interest to the farming industry

in the Faroes. The company´s vision is to create an international research community that will expand the knowledge base through research and development for the purpose of further increasing quality in aquaculture.
The research efforts are organised into the following three fields: Technology and environment, production development and bio-technology. Emphasis is also placed on collaborative research. Fiskaaling is currently involved in research co-operations with several research institutes on the Faroes and abroad.
Fiskaaling has for many years worked within R & D of marine fry production of different species. Our Marine Research Centre located in Nesvík has good facilities for such research as well as for production of natural feed organisms and reared rotifers and Artemia. Recently, projects aiming at providing farmers with better tools in management of sea lice problems have played a central role within Fiskaaling. This encompasses sea lice resistance to chemotherapeutants, spreading of sea lice and cleanerfishes.
Principal research personnel involved
Msc Regin Arge, fish nutrition, breeding and manager of R&D in production.
Msc Gunnvør Joensen, developer of cryopreservation of lumpfish sperm.
Dr. Ása Johannesen, specialist in fish behaviour studies.
Msc. Ása Jacobsen, genetic studies.

Nordlaks Oppdrett AS (NLAKS), Norway
Nordlaks is an integrated aquaculture company with own broodfish, juvenile production and on-growing facilities in Troms, Northern Norway. The company was established in 1989 and is today fully integrated company that farms, sells and process Atlantic salmon and rainbow trout. Main markets are in the EU, Russia and USA.
Personnel involved
CEO Tor Anders Elvegård and **farming manager Tommy Hansen** will be responsible for the NORD tasks in the project. Both have extensive experience in farming of Atlantic salmon. In addition several technical staff will be involved in the experimental work to be carried out.

The Faroese Fish Farmer Association, Faroe Islands
Havbúnaðarfelagið is a branch organisation for the Faroese aquaculture industry. The organisation was established in 1980. All fish farmers in the Faroes are members of the organisation. Thus the association represents 100% the fish farming industry on the Faroes. During the past decade or so, significant structural changes have taken place in the fish farming industry, with a clear trend towards fewer companies controlling an increasing share of farming licenses. Most of the companies are vertically integrated controlling the process from egg to customer.
The partners in this project are: Bakkafrost, Marine Harvest Faroes, Hiddenfjord and Faroe Farming.

Bakkafrost.
Bakkafrost is the largest producer of salmon and salmon products from the Faroe Islands and is listed on Oslo Stock Exchange Market. Bakkafrost controls an extended value chain, from feed to finished value added products. Bakkafrost holds 50% of total farming licences and was the production in 2012 more than 45.000 tonnes gutted weight. Based on the company´s experience and history, biological security is acknowledged to play an important part in the production of salmon to achieve cost efficiency and focus on biological security. Through its experience from many years of salmon farming in the Faroe Islands and the results from veterinary and biological best practices, Bakkafrost aims to produce salmon products through balancing the production volumes between economies of scale and biological capacities.

Marine Harvest, Faroes.

Marine Harvest Faroes is a daughter company of Marine Harvest group with an annual local production of 5000-7000 tonnes gutted weight. The mother company is present in all major salmon farming regions in the world and is the biggest producer of farmed salmon. In addition to fresh and frozen salmon, Marine Harvest offers a wide range of value added products. The company´s farming practices aim at rearing fish under conditions that suit their biological needs and use husbandry techniques that minimise stress, aggression and injury. In sea lice management within Marine Harvest, biological control with cleanerfishes (wrasse) developed early in the 1990's and has the company established own production facilities for wrasse in Norway. Recently, the company also started cooperation with Norwegian lumpsucker producers to use this species for biological sealice control.

Hiddenfjord.

Hiddenfjord or Luna is the only 100% Faroese owned salmon farming company founded back in 1929 as processing white fish, and is one of the pioneers when the fish farming started in the Faroe Islands in the 1970'ies. It runs a land based smolt station and sea sites. Their annual production is 8000-10000 tonnes. The focus of the company is on high quality products and in 2011 they created the HiddenFjord brand of premium salmon. The company is well aware of the challenges concerning sealice and is currently in the front when expanding the fish farming industry into more open waters in a sustainable manner. The company has long time experience from fish farming operations in exposed areas.

Faroe Farming.

Faroe Farming is located on the southernmost island of the Faroes. Faroe Farming has 3 farming sites on the Island, which gives the company the possibility of producing approximately 6000 tons/year. From starting with farming of salmon only, Faroe Farming has developed into a vertical integrated salmon farming, harvesting, portion production and sales company. Although Faroe Farming may be the smallest farming company in the Faroe Islands, Faroe Farming sites recently were able to produce the best biological production results in the Faroes.

Fjarðalax hf (FLAX), Iceland

Fjarðalax hf is a commercial salmon farm with production localities in Tálknafjörður, Patreksfjörður and Arnarfjörður in Northwest Iceland. The company has a production allowance for 3000 mt of Atlantic salmon and aims at increasing the production up to 10.000 mt in 2018/19. The company slaughtered about 300 mt in 2011.

Personnel involved

Farming manager Jónatan Þórðarson and **area manager Jón Örn Pálsson** will be responsible for activity at Fjarðalax in *NOLICE*. They will be assisted by own technological staff.

Norsk Institutt for vannforskning (NIVA), Norway

The Norwegian Institute for Water Research (NIVA) (http://www.niva.no/), is the largest centre focusing on water-related research in Norway. In addition to water quality and toxicology, NIVA has also extensive research experience in aquaculture. In particular, NIVA works to promote sustainable aquaculture through close cooperation with the aquaculture industry, public administration agencies and other research environments nationally and internationally. NIVA has now established a new research strategy for the period up to 2020, with a main focus on "*Research for a sustainable future*", in which the development of sustainable aquaculture and introduction of new marine species is a priority area.

Personnel included

The work of NIVA (Task 1.3) will be led by **Dr. Anne-Laure Groison** who is a researcher within the Fish and Aquaculture group at NIVA. She has a PhD in the field of fish reproductive biology, and for her thesis work she investigated the potential of a new aquaculture species in Norway, the European hake. Throughout her PhD, Dr. Groison worked in close collaboration with the aquaculture industry in Norway, as well as a private company, to build up a viable hake broodstock. She has gained valuable experience working with fish sperm, sperm cryopreservation techniques and sperm quality assessment.

Hólar University Collage (HÓLAR), Iceland

Hólar University Collage (www.holar.is) has extensive experience in research and developmental work in aquaculture. The Department of Aquaculture and Fish Biology has conducted research on a number of fish species including Arctic charr, Seabass, Atlantic cod, Atlantic halibut and turbot. The main research focus in HÓLAR bas been on growth of fish, genetics and breeding, water quality, feed development, fish biology and fish physiology. At the department, there is also an active research group in fish behaviour, ecology and evolution in the department. HÓLAR has excellent facilities for growth experiments with environmental control (temperature, salinity, oxygen, photoperdiod) and behavioural studies in tanks of varying sizes. The research facilities of the department will be used in experiments conducted by *NOLICE* and departmental staff will contribute to the project. HÓLAR is the centre for aquaculture education in Iceland both at undergraduate and graduate level. A large number of graduate students have conducted their research under the supervision of departmental staff. Graduate students from HOLAR will actively participate in the research work of *NOLICE*.

Personnel included

Prof. Helgi Thorarensen will be in charge of the team from Hólar. He has nearly thirty year experience in conducting trials with fish in culture, especially focusing on growth, oxygen demand, salinity tolerance and photoperiod manipulation. Helgi has a PhD degree in fish physiology. **Prof. Bjarni K. Kristjánsson,** will be in charge of behaviour studies at Hólar University College. Bjarni has a PhD degree in fish evolutionary ecology. Bjarni has extensive experience with research on fish, including studies on Lumpfish. **Msc. Arnþór Gústavsson** will be in charge of the day-to-day management of the trials conducted at Hólaskóli. He has extensive experience in culture of several fish species including Atlantic salmon and lumpfish.

5. Project budget

Overview of costs and financing plan for three years project (all numbers in thousand DKK)

Participants	Description of costs			Total	Applied from NORA	Nordic national funding	Own contribution
	Personnel	Durable equipm.	Travel				
APN	1112	50	110	1282	850	170	262
FISKA	407	50	110	567	200	140	227
NLAKS	162	150	27	339	0	100	239
FFFA	162	150	27	339	0	100	239
FLAX	88	150	27	265	0	100	165
NIVA	422	50	55	527	150	0	377
HOLAR	454	50	110	614	300	100	214
Total	*2807*	*650*	*466*	*3923*	*1500*	*710*	*1713*

Budget details

The budget below is based on the assumption that parts of the experiments will be financed by and performed at the facilities of the industrial partners (NLAKS, FFFA and FLAX). Included in the travel costs are costs related to 3 project meetings (all partners) and additional field trips (APN, FISKA, NIVA and HOLAR). All numbers are thousand DKK.

	Durable equipment	
APN	Experimental running (tank rental fish etc.)	50
FISKA	Experimental running (tank rental fish etc.)	50
NLAKS	Experimental running (tank rental fish etc.)	150
FFFA	Experimental running (tank rental fish etc.)	150
FLAX	Experimental running (tank rental fish etc.)	150
NIVA	Experimental running (artificial insemination/sperm quality)	50
HOLAR	Experimental running (tank rental fish etc.)	50
	Total durable equipment costs	650

	Personnel costs	
APN	Akvaplan-niva 13.5 man months, at 563 DKK/time	1112
FISKA	Fiskaaling 5.0 man months, at 563 DKK/time	407
NLAKS	Nordlaks 1.75 man months, at 620 DKK/time	162
FFFA	Havbúnaðarfelagið 1.75 man months, at 620 DKK/time	162
FLAX	Fjarðarlax 1.75 man months, at 620 DKK/time	88
NIVA	NIVA 5 man months, at 563 DKK/time	422
HOLAR	Holar 9 man months, at 337 DKK/time	454
	Total personnel costs	2807

	Travel costs	
APN	Akvaplan niva, project meetings and field trips	110
FISKA	Fiskaaling, project meetings and field trips	110
NLAKS	Nordlaks, project meetings	27
FFFA	Havbunaðarfelag, project meetings	27
FLAX	Fjarðarlax, project meetings	27
NIVA	NIVA, project meetings and field trips	55
HOLAR	HOLAR, project meetings and field trips	110
	Total travel costs	466
	Total cost	3923

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6. Project progress schedule

Tasks/Timeline (month)	0	6	12	18	24	30	36
WP 1 – Domestication of lumpfish							
T 1.1 Domestication of lumpfish: broodstock management, larval rearing and juvenile production			**M1.1**				
T 1.2 Thermal optima and thermal tolerance of juvenile lumpfish					**M1.2**		
T 1.3 Protocols for cryopreservation of lumpfish sperm and artificial insemination						**M1.3**	
WP 2 – Effective use of lumpfish for delousing salmon							
T 2.1. Feeding behaviour and population dynamics of lumpfish			**M2.1**		**M2.2**		
T 2.2. Development and implementing lumpfish breeding programme in commercial-scale systems						**M2.3**	
WP 3 – Commercial scale protocols for biological delousing							
T 3.1 Optimise lumpfish density for effective lice control					**M3.1**		
T 3.2 Optimizing the effectiveness of farmed lumpfish for biological delousing of salmon							**M3.2**
WP 4 – Dissemination and exploitation							
T 4.1 Dissemination activities			**PR**		**PR**		**FR**
T 4.2 Training of industrial partners			**T**		**T**		
WP 5 – Management activities							
T 5.1 Consortium meetings and project coordination	**PM**		**PM**		**PM**		**PM**

Abbreviations: M = milestones; PR = Periodal report; FR = Final report; T = Training activity;