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BASECOD - Stable and safe production of high quality cod larvae and juveniles

- The importance of cod farming for the Nordic countries
- Improving the quality of cod larvae and juveniles
- Novel approaches for production of high quality cod larvae and juveniles

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Abstract: Stable supplies of high quality cod larvae and juveniles are urgently needed for the development of successful cod farming industries in countries around the North Atlantic. The project is a collaboration between key actors from the cod farming industry and the research sector in Iceland, Norway and the Faroe Islands, with involvement of additional key actors and stakeholders within the Nordic countries and Canada. The overall project aim was to join forces of key actors for identifying the current status and the main problems encountered in intensive production of cod larvae and juveniles and describe novel approaches for production of high quality cod larvae and juveniles, thereby promoting not only the cod farming industry but also the fish processing industry and related industries within the Nordic countries.		
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Executive summary

The aim of the project was:

• to join forces of key actors involved in intensive production and research aiming for improved products qualities, with the common aim to promote the quality and success in the production of cod larvae and juveniles within the Nordic countries.
• to create a foundation for a network of excellence within this field, by involving other Nordic as well as international key actors.
• to define the current status in intensive production of cod and describe an innovative approach for production of high quality cod larvae and juveniles within the Nordic countries.

The aims were achieved through:

- an analysis of the current status in intensive production of cod and a description of the main problems encountered in production of cod larvae and juveniles.
- developing a joint strategic action plan describing innovative approaches for a stable and safe production of high quality cod larvae and juveniles within the Nordic countries.
- two meetings held within the project, with the involvement of additional and international key actors that helped outline the current status, define objectives and describe a strategic action plan for production of high quality cod larvae and juveniles with focus on innovative approaches.

Method

The project´s participants were selected based on their knowledge and engagement in the production of high quality cod larvae and juveniles within the Nordic countries and Canada. Together with other enterprises engaged in production of cod larvae and juveniles, the participating enterprises therefore represent the main target group for dissemination of project results and the stakeholders to be involved in future collaboration. Cod farming initiatives within the Nordic countries were also identified as key actors and stakeholders to be involved in further work. National research and innovation funds furthermore have central role in contributing with financing common initiatives and were therefore identified as key actors for dissemination of main conclusions drawn by the project consortium.

Main results – the current status

A SWOT analysis is a useful tool for incorporation into a strategic planning model. Such an analysis helps address individual items for the objective-driven strategic planning. An analysis was carried out, identifying and describing the **S**trengths (attributes of the production that are helpful to achieving the objective), **W**eaknesses (attributes of the production that are harmful to achieving the objective), **O**pportunities (external conditions that are helpful to achieving the objective) and **T**hreats (external conditions which could do damage to the objective) in todays production of cod larvae and juveniles within in the Nordic countries.

The main strengths include already established markets for products made from cod with extensive human resources, knowledge and expertise of processing and co-product utilization

existing within the Nordic countries. Developed technologies and analytical tools are furthermore available and the use of similar equipment for cod and salmon farming also facilitate the development of industrial production of cod larvae and juveniles. The fact that at farmed cod is mostly free of parasites and is low in environmental contaminants also represent strengths.

The main weaknesses identified include the lack of performance of cod juveniles produced today, resulting in high fluctuations in supply and demand for cod juveniles. Effective vaccines against the main diseases encountered in cod are furthermore needed and early maturation represents a considerable problem during the on-growing phase.

The main opportunities consist in possibilities to lower the production costs of farmed cod through exploitation of new technologies. The increased general health awareness of consumers and the possibility to offer stable supplies of farmed cod to the fresh-fish markets within Europe also represent numerous opportunities. Processing of farmed is furthermore more efficient as compared to wild cod and numerous opportunities consist in the use of co-products from processing of farmed cod.

The main threats consist in high production costs, the lack of effective vaccines and the current disease threats. The risk of changed consumer behaviour, with products from other and cheaper species of white fish overtaking the traditional cod markets also represent a real threat to the cod farming industry. The more recently observe d increase in wild stock catches will also result in reduced emphasis on the development of cod farming that is still far from being economical.

Main results – strategic actions to be taken

Two main actions were identified as a first step in improving the quality of intensively reared cod larvae, as a common initiative to be taken in collaboration of countries around the North Atlantic. Individual tasks were then identified for each action.

Action 1. Improved quality of larvae and juveniles

A comprehensive analysis of egg quality for determination of parameters to accurately assess the quality of cod eggs is needed. A comprehensive analysis and comparison of the various groups/types of live prey currently used for initial feeding of cod larvae is also needed, for determination of parameters to assess the quality of the live prey. Live prey to be included in such analysis include natural zooplankton and intensively produced copepods as well as rotifers and brine shrimp cultured at various locations within the Nordic countries. The comparison should also include previously published studies on copepods carried out by especially Norwegian scientists. Also needed is a nationwide analysis of cod larvae reared using various types of live prey, for determination of parameters for an assessment of the quality of cod larvae. Disease prophylaxis is furthermore urgently needed, with treatment of the live prey, larvae and juveniles using selected probiotic mixture.

Action 2. Broodstock management

Indicators for egg quality in the broodstock need to be defined. This should be carried out by analysing the concentration and ratio of selected ingredients in the blood, roes and milt of wild as compared with farmed cod and in relation to egg quality. Broodstock nutrition and fecundity are also issues of key importance and comprehensive feeding trials are needed, for investigating the effects of macro- and micro ingredients in broodstock diets on fecundity and egg/larval survival and overall quality.

The following conclusions can be drawn from the results:

An analysis of the current status stresses the importance of improved quality of cod larvae and juveniles as the basis for a successful and economical cod farming industry as a whole. The Strategic Action Plan suggested by the project consortium is based on an analysis of the current status, with common objectives and initiatives that may be addressed by the Nordic countries suggested. Cod larvae fed wild zooplankton have high quality and high growth performance, demonstrating that it is possible to achieve high performance of farmed cod larvae. This, however, requires that the enriched rotifers and *Artemia* are manipulated to make the diet nutritionally optimal for the cod larvae.

Actions to be taken include a comprehensive analysis of the effects of broodstock nutrition and describing important parameters that affect the quality of the live prey used during the first weeks of exogenous feeding of larvae. Overall, suboptimal nutritional quality of live prey represents a major obstacle that has to be overcome if cod farming is to become a major industry in the Nordic region.

Recommendations for continued studies:

Even though the best results for larval quality and growth are obtained in ponds, this production method is not optimal for industrial production of cod larvae, mainly due to the natural variability of the abundance and composition of copepods in natural pond systems. An optimal system therefore requires that the enriched live prey are manipulated in order to make the diet nutritionally and microbiologically optimal for the cod larvae, which in turns requires detailed information on the differences in composition of various live prey. The BASECOD collaboration therefore suggests a comprehensive comparative analysis of the effects of broodstock nutrition on the quality of eggs and larvae as well as of the quality of the various prey animals used in the production of cod juveniles. The semi-intensive production of cod larvae in the pond systems in the Faroe Islands is extremely important for this knowledge, but it has to be noted that the species abundance and composition may vary in the ponds. A repeated analysis and monitoring of the live prey and larvae from the ponds in the Faroe Islands may therefore be needed. The recommendations imply active collaboration with the involvement of Nordic funding bodies, with the common aim to promote the Nordic cod farming industry.

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Deliverables from the project

Three deliverables were defined within the project and all were fulfilled. The communication plan and identification of key participants in future work is delivered separately. The other two project deliverables are described in more details below, including an ***analysis of the current status*** in intensive production of cod larvae and juveniles, and the description of ***a strategic action plan*** for stable and safe production of cod larvae and juveniles, focusing of innovative approach.

1. An analysis of the current status

A SWOT analysis is a useful tool for incorporation into a strategic planning model. Such an analysis helps address individual items when defining a desired objective or end state (objective-driven strategic planning). The analysis describes:

- **S**trengths: attributes of the production that are helpful to achieving the objective.
- **W**eaknesses: attributes of the production that are harmful to achieving the objective.
- **O**pportunities: *external* conditions that are helpful to achieving the objective.
- **T**hreats: *external* conditions which could do damage to the objective

A draft of the SWOT analysis was prepared by Matis ohf., based mainly on the production of cod larvae and juveniles in Iceland. A further work on the draft was carried out at the first project meeting, held in Iceland Nov 12-13th 2009. A final version was then prepared at the meeting February 26th 2010, with adjustments made with respect to changes in the current status of cod farming in the Nordic countries since November 2009.

1.1 SWOT analysis of the production of Nordic cod larvae and juveniles

The main ***strengths*** identified are based on solutions to the question why cod should be produced in aquaculture.

a) *Already established markets* for products made from cod. Market for cod products also include different market niches for salted, dried and other products of cod.
b) *Processing knowledge and expertise* are existing within the Nordic countries.
c) *Favourable and steady environmental conditions* for juvenile cod farming which is a native species of the North Atlantic.
d) *Human resources and knowledge* of various fish species produced in aquaculture (cod, halibut, turbot, salmon etc).
e) *Similar equipment used for cod and salmon farming*, with already established technology, infrastructure, fish feeds, feeding equipments etc.
f) *Existing knowledge and technology for co-product utilization* (cod liver, milt, ingredients for salmon feeds etc.).
g) Farmed cod is mostly *free of parasites and is low in environmental contaminants*. This opens for the possibility of targeted marketing of farmed cod as superior to wild cod with respect to low content of environmental contaminants and parasites.
h) *High technology analytical tools* are available for analysis of cod products, cod eggs, zooplankton, enrichments media, live prey animals, cod larvae etc. High competence in genetic and analytical methods is highly useful for understanding the nutritional demands of cod larvae and juveniles.

Country based strengths:

Iceland: clean warm (and cold) water resources for control of environmental temperatures in cod juvenile production. Virus problems have not been encountered and the disease situation in general is less a problem compared with the other Nordic countries. Fisheries companies furthermore dominate the farming activities and the involvement of the whole value chain must be considered a strength. Iceland also has a world leading role in the development in fish processing and logistics.
Norway: extended knowledge and world leading role in the production of fish in aquaculture. Extensive coastline.
Faroes: knowledge in semi-intensive production of cod larvae and juveniles, with superior growth achieved as compared to intensively produced cod. Close to optimal temperatures for culturing of fish and especially the Atlantic cod (6-11°C).
Denmark: knowhow within processing and water recirculation techniques. Proximity to central Europe. Experience and tradition in culturing of different species.
Sweden: Experience and tradition in fish processing.
The Nordic countries: The Nordic countries have a common heritage in utilization of fish as food for human consumption and extended general knowledge within processing and technologies for production of high quality food products exists within the countries.

The main ***weaknesses*** are identified based on the present status in the production of cod larvae and juveniles in the Nordic countries.

a) *Lack of performance of cod juveniles*.
Intensive production of cod juveniles, the nutritional aspects of broodstock fish and cod farming in general are still in an early phase and a lack of performance may be expected during the early phase. The high production costs of cod juveniles is strongly related to the high mortalities experienced during the first production stages. Hence, improved survival of cod larvae and juveniles will improve the quantities achieved from each unit and thereby lower the production costs in all aspects.
The cod is a carnivore species and marine carnivores are known to have high demands for marine raw materials in the feed. Inadequate nutrition will therefore result in an unused potential for growth during early developmental stages. The semi-intensive approach gives better results but may also be inadequate for normal growth and development of larvae. High mortalities of cod juveniles are furthremore experienced at transfer to sea-cages, stressing the need for optimization of technology/protocols and nutrition/environment/ performance during early developmental stages. Overall, the nutritional requirements of larvae and the broodstock fish still need to be solved.
The quality of cod juveniles should be evaluated when the juveniles have reached a certain size, i.e. have passed the most important part of the metamorphosis (a body length of 12-15mm). Performance is the key with respect to such a terminology, measured by growth rate correlated with the temperature used. The quality of juveniles is, however, harder to apply, due to the lack of a definite definition of the term "quality". The main production bulk in Norway during the last years has been without *Artemia* and using only rotifers, resulting in high survival but a permanent growth suppression when compared to juveniles fed *Artemia* as well. The previous focusing in Norway to get rid of the *Artemia*

and early dry feed weaning of larvae is opposite to the approach in bass/bream farming. Furthermore, juveniles startfed with zooplankton clearly exhibit superior long-term growth rates over hatchery produced juveniles.

The components of zooplankton that need to be in the live prey offered to larvae have to be defined (fat acid content and composition, protein content, peptide and amino acid composition etc.). The nutritional composition of wild zooplankton may never be reached 100%, but the most important factors need to be provided. One problem to this is the knowledge of which nutrients/micronutrients/ingredients to look for. The common aim here should be to achieve the nutritional quality of wild zooplankton. Hence, it is important to withdraw knowledge from the semi-intensive approach in the Faroes and in Norway, as well from farming of other species (i.e. bass and bream).

b) *Lack of effective vaccines*.

Effective vaccines are not available against the most serious and most common diseases in cod. The cod furthermore seems to have poor immune memory and repeated immunization may therefore be needed.

c) Early maturation during the on-growing phase.

This has been addressed as one of the main problems in cod aquaculture. Results from the Codlight Tech project (EU FP6 CRAFT SME-2006-032859) and other national and international projects need to be collected and adapted for the benefits of the industry.

d) Fluctuations in supply and demand for cod juveniles.

Due to the fragile situation currently experienced in the Nordic cod farming industry, high fluctuations in supply and demand for cod juveniles are experienced.

Country based problems representing weaknesses.

- ✓ *Iceland*: financial government contribution in Iceland is scarce and only one hatchery and one transport vessel currently practised. Suboptimal and varying environmental temperatures during the grow-out phase furthermore represent weaknesses and so does the fact that the production of cod juveniles is in the hands of only one hatchery.
- ✓ *Faroe Islands*: a lack of interest (presently) in on-growing of cod, which is mainly regulated by product prices. High prices are currently experienced for salmon, and restricted sea-cage localities therefore available for cod. An overall lack of interest and/or facilities for on-growing of cod
- ✓ *Norway*: high environmental temperatures are common during the summer months in west Norway, giving sup optimal conditions for on-growing of cod. In November 2009, there were too many companies managing the production of cod juveniles, but these have been reduced to acceptable numbers in February 2010 (~4 producers).
- ✓ *Denmark and Sweden*: The lack of activity in culturing of cod that may be related to lack of facilities for on-growing in sea-cages. Current difficulties in financing cod research are furthermore experienced.

An increasing interest for cod farming has been experienced in Canada. The current status may, however, be described as waiting and seeing if the main problems will be solved and for the industry to become economical.

The ***opportunities*** are described based mainly on marketing of products of farmed cod.

a) *Numerous opportunities in the fresh-fish markets* within Europe. Currently there is increased access to both fresh and frozen fish in Europe. Cod farming invites great opportunities to deliver stable and good quality farmed cod.
b) *Cod farming can offer stable supplies* and is not subject to the fluctuations observed in the wild cod stocks availability to the market.
c) *More efficient processing* and longer shelf life compared to wild cod, leaving farmed cod more attractive. Products of uniform sizes also offer great possibilities on markets of farmed as compared to wild cod.
d) *Increased general health awareness*. A wide variety of protein sources may be used during the main grow-out phase, without being reflected in the muscle at slaughtering.
e) *Exploitation of new technologies* for help improving the quality and safety of the production and products of farmed cod.
f) Possibilities to *lower the production cost* of farmed cod that may be expected to follow similar trends as in the cost development in salmon farming.
g) *The use of co-products* in cosmetics, feeds for other species etc.

The main ***threats*** are identified based on the current status in the production and markets for farmed cod.

a) *Increased wild stock catches* are currently experienced but do not necessarily represent a realistic threat for the nearest future.
b) *Changed consumer behaviour***.** Markets for cod turn to other species that are cheaper and more accessible. This may result in the collapse of traditional markets for cod.
c) *High production costs***.** Lack of juvenile performance results in high production costs. As of today, production costs in cod farming are far too high and cod farming is not economical.
d) *Disease threats* related to high environmental temperatures during the summer months, especially in Norway, Denmark and Sweden.
e) *The economic situation* represents a real threat for the development of cod farming. The profits are currently too low with respect to production costs and hence, the driving force is missing.

1.2 Summary

The analysis stresses the importance of improved quality of cod larvae and juveniles as a basis for a successful and economical cod farming industry as a whole. The items identified were each discussed in details and the Strategic Action Plan then based on the current analysis and where common objectives and initiatives that may be addressed by the Nordic countries are suggested. Cod larvae fed wild zooplankton have high quality and high growth performance, demonstrating that it is possible to achieve high performance of farmed cod larvae. This, however, requires that the live prey are manipulated in order to make the diet nutritionally and microbiologically optimal for the cod larvae.

2. A strategic Nordic Action plan

2.1 Introduction

Cod farming is an upcoming industry in the Nordic countries, with goals set in 2007 assuming a steady increase to 10 million juveniles in Iceland and 90 million juveniles in Norway in 2013. Presently, however, the industry is facing numerous problems. Cod farming companies have been hit by the economical crisis in addition to increased quotas of wild cod that result in lower prices and dramatically reduced demand for farmed juveniles. The production costs are still too high and most of the companies are losing money on their farming operations. The production goals from 2007 have therefore not been achieved. Growth rates have in general been disappointing and diseases have caused problems for many companies. Nordic aquaculture of cod furthermore appears to suffer from the lack of quality in hatchery produced juveniles.

A stable production of high quality eggs and larvae is clearly fundamental for the success of cod farming. Fast growth and normal development of larvae is the basis for fast growing individuals that utilize their genetically determined growth potential to the maximum. Hence, acceptable growth rate in the grow-out phase of cod will only be obtained through high quality larvae and juveniles. A sustainable growth of the Nordic cod aquaculture industry therefore requires an improved knowledge base that allows a predictable supply of high quality juveniles for the grow-out phase.

The overall aims of the BASECOD project are to identify and describe suggested actions to be taken for stable and safe production of high quality cod larvae and juveniles. A strategic action plan was drafted at the first project meeting, held in Iceland Nov 12-13th 2009. A number of actions were identified, each addressing major problems encountered during intensive production of cod. At the second project meeting held in Oslo Norway 26th February 2010, two key actions were selected based on the project´s aims:

1) ***Improved quality of cod larvae and juveniles.***
2) ***Broodstock management. Parental effects on quality of eggs, larvae and juveniles.***

The two actions identified are described in more details in the following section and tasks for addressing the individual actions have been suggested and are briefly described. In addition to the BASECOD project participants, the following key actors have contributed to this work:

- Agnar Steinarsson from the Marine Research Institute in Iceland
- Dr. Ingrid Overrein from SINTEF fiskeri og havbruk in Norway
- Dr. Kurt Gamperl and his colleagues from the Memorial University of Newfoundland participating in the cod-farming-initiative in Canada.

Dr. Josianne Støttrup (Denmark Technical University) and Dr. Jana Pickova (Swedish Agricultural University) furthermore informed of the current status and the lack of ongoing farming of cod and research on cod in Denmark and Sweden.

2.2 Identified actions for production of high quality cod larvae and juveniles

2.2.1 Action 1 – Improved quality of larvae and juveniles

Farming of the Atlantic cod is an emerging commercial activity in regions of the north Atlantic, with production of juveniles primarily taking place in intensive hatcheries (McIntosh *et al*., 2008). The production of juveniles is still a bottleneck in cod farming. Intensively reared cod larvae grow slower (McQueen Leifsson, 2003) and develop more deformities (Imsland *et al*., 2006) than larvae reared semi-extensively on their natural prey, copepods, filtered from pond systems. The nutrient profile of both rotifers and copepods has recently been analysed (Srivastava *et al*., 2006, Hamre *et al*., 2008b; van der Meeren *et al*., 2008) and the data show that there are numerous differences in nutrient composition between the two food items. Copepods generally contain higher levels of various micronutrients compared to rotifers, and the levels are commonly considerably higher than the requirements reported for fish (Hamre *et al*., 2008ab). The larvae nutrition represents one of the main problems in large scale production of fish, with high incidence of larval vertebral deformities commonly observed (Kjorsvik *et al*., 2009; Puvanendran *et al*., 2009). Significant progress has been made towards optimizing the composition of feed and feeding regimes (Fletcher *et al*., 2007) but sub-optimal nutrition in larvae fed rotifers may partly explain the inferior performance of intensively, compared to semi extensively cultured larvae (Hamre *et al*., 2008a). Non-saturated and polyunsaturated fatty acids are the major nutrients that live feed contributes and recent findings show that nutritional deficiencies in the live feed can be complemented with fatty acid enrichment (Rivera and Botero, 2009).

Continuous access to high quality eggs, is a key issue in intensive production of marine larvae. A reliable method for assessing the viability of fertilized eggs during early development would be beneficial for the aquaculture industry, allowing low quality egg batches to be discarded before costly resources are devoted to their culture (Avery *et al*., 2009). A significant positive correlations has been observed between egg size and larval size during early production stages, with correlations weakening with time and no longer significant at an age of 9 months (Paulsen *et al*., 2009). Cod is a batch spawner and previous studies reveal highly variable quality of eggs from individual females as well as throughout the spawning period (Norberg *et al.*, 1991; Kjesbu *et al.*, 1996). Quality evaluations include fertility, measured for example as the numbers of viable larvae produced by each female, fertilization success and hatching rate of eggs in addition to survival and overall larval quality. The observation of cellular morphology during embryogenesis has furthermore been increasingly used for quality evaluation of eggs (Avery *et al*., 2009).

Various factors may affect the egg quality and large females will for example produce larger eggs of higher quality (Marteinsdottir *et al*., 2006). Previous studies furthermore indicate that first time spawners may produce eggs of reduced viability as compared to older females (Carr and Kaufman, 2009; Izquierdo *et al*., 2001). There are also indications that eggs from farmed fish are of poorer quality compared to wild fish, demonstrated for example by lower success of fertilization and hatch ratio (Rosenlund and Halldorsson, 2007). The eggs will furthermore be in

immediate contact with high variety of environmental bacteria already at hatching and high bacterial numbers may affect the success of fertilization and egg survival (Olafsen, 2001; Hansen and Olafsen, 1999).

A successful cod larviculture requires the provision of live feed (microalgae, rotifers and *Artemia*) during the early stages of larval development. This practice could potentially introduce pathogenic microbes into the production system (Makridis *et al*., 2000). The high organic load associated with intensive production of live food cultures selectively induces an increased proportion of opportunistic bacteria, and live feed-associated mortality has been considered to represent a major obstacle to the successful establishment of commercial marine finfish production (Hansen and Olafsen, 1999; Skjermo and Vadstein, 1999). However, the gastrointestinal tract is poorly developed during early larval stage of Atlantic cod (Olafsen 2001) and limited information exist concerning the nature and effects of the microflora associated with cod larvae and the live prey offered during early developmental stages (McIntosh *et al*., 2008; Korsnes *et al*., 2006). The possible benefits of probiotic treatment of cod eggs have been evaluated in Atlantic cod (Hansen and Olafsen 1989) and improved survival and growth has been achieved as a result of probiotic treatment of larvae (Lauzon *et al*., 2009). Increased survival has also been observed with inclusion of pre-digested dietary protein in formulated diets at weaning (Kvale *et al*., 2009). Optimization of hatchery production through improvements in dietary regimes, use of immunostimulants and a better control of the associated microflora has been found to result in an increased availability of cost-effective and high quality fry (McIntosh *et al*., 2008). The use of probiotics or efficient disinfection methods to control bacterial flora development in hatcheries is in general believed to lead to improvements in the growth and survival of marine finfish larvae (Verner-Jeffreys *et al*., 2003; Magnadottir *et al*., 2006; Schulze *et al*., 2006; Plante *et al*., 2007).

Overall, broodstock husbandry, genetics and nutrition may play a major role in causing vertebral deformities in Atlantic cod at hatch, while nutrition and prey type may play a major role during metamorphosis (Puvanendran *et al*., 2009). Major gaps in knowledge on fish larval nutritional requirements still remain, with high mortalities and quality problems commonly experienced in marine larviculture (da Conceicao *et al*., 2009). The aim of the tasks described below is to improve the knowledge of factors that may affect the organic and digestive development of cod larvae and the way these factors can affect their growth and survival.

Based on the current status, four main tasks were identified for this action:

Task 1-1: Egg quality

Analysis of eggs from broodfish held in captivity and wild caught fish at various locations (Norway, Iceland, Faroes) will give valuable information on the nutritional status of the parental fish. Furthermore, it is important to study the effects of age and size of parent fish within and

during the spawning seasons with respect to the quality of eggs and larvae. It must, however, be noted that observed differences between different spawning groups may be partly caused by different environmental temperatures at the various locations.

Suggested analytical approach for this task:

- Nutrient content (protein, amino acid profile, fat, water, ash). Nutrient content and the ratio of macronutrient components will give valuable information of the overall nutritional status.
- Fatty acid content and fat classes (GC and HPLC-ELSD). HPLC (monolithic silica column Chromolith®Performance-Si) with an evaporative light scattering detector (ELSD) allows separation and quantification of a broad range of lipid classes.
- Elementary analysis (Inductively coupled plasma mass spectrometry, ICP-MS). ICP-MS allows determination of elements with atomic mass ranging between 7–250, and a simultaneous quantification of all elements to nanograms. Separation is based on very high plasma temperature (10,000 K). Coupling to mass spectrometry involves extraction of ions from the plasma through a series of cones into a mass spectrometer, usually a quadropole.
- Proteomics for analysis of soluble peptides. Two-dimensional gel electrophoresis (2-D PAGE) and two-dimensional difference gel electrophoresis (2-D DIGE) for automated spot picking. The protein spots are then analyzed using MALDI-TOF and ESI-Q-TOF mass spectrometry.
- Metagenomics for detection of microorganisms in stomach content. Environmental shotgun sequencing with quantification and determination of algae, bacteria and virus groups recovered directly from the samples.

Task 1-2: Live prey analysis

A comprehensive comparison of the various groups of live prey currently used for initial feeding of cod larvae:

- Zooplankton in the Faroes. Note that an analysis of zooplankton in the Faroe basins is planned and these information should be used
- Intensively produced copepods (*Acartia*) from SINTEF in Norway
- *Artemia* used at Profunda in Norway, Fiskaaling in the Faroes, Fiskey in Iceland and the Marine Research Institute in Iceland
- Rotifers used at Profunda in Norway, Fiskaaling in the Faroes and the Marine Research Institute in Iceland

The comparison should also include previously published studies on copepods carried out by especially Norwegian scientists (see references above).

Suggested analytical approach as described for Task 1-1 in addition to detection of selected bacterial groups and/or other components, using specific antibodies in ELISA or through immune histology and image analysis.

Task 1-3: Larval quality

Growth performance, survival and general performances of zooplankton-fed larvae of bass, bream and halibut has been surpassed in intensive production using monocultures of live feed. As an example, intensively reared halibut larvae fed enriched *Artemia* at Fiskey Ltd. reach on average 27 mg ***dry weight*** after 47 days of feeding compared to 147 mg ***wet weight*** reported for halibut larvae fed zooplankton for the same number of days (McEvoy *et al*. 1998) which, according to the dry and wet weight relationship established by Olsen *et al*. (1999), corresponds to 25 mg ***dry weight***. Similar success has to be achieved for the cod. The semi-intensive production of cod larvae in the pond systems in the Faroes is important for setting the norms. It must, however, be noted that the species abundance and composition may vary in the ponds where temperatures and other environmental factors will affect copepod survival and growth.

This task may be further divided into two main sub-tasks:

- ✓ Analysis of larvae fed intensively produced copepods from SINTEF compared to rotifers/*Artemia* at Profunda in Norway. The comparison should also include zooplankton-fed larvae in Faeroes, halibut larvae produced at Fiskey Ltd. in Iceland and e.g. turbot larvae produced at the Marine Research Institute in Iceland.
- ✓ Expression studies (growth, disease resistance, stress parameters) carried out in two hatcheries using the same type of live prey (Profunda in Norway and the Marine Research Institute in Iceland)

Suggested analytical approach as described for Task 1-1 in addition to effects of live-prey enrichment using selected probiotics. The overall comparison should furthermore include analysis of juveniles at selected periods following weaning onto formulated feed.

Task 1-4: Disease prophylaxis

Results from intensive operations show that the quality of the live prey is strongly related to the microbiology of the prey. Various measures are applied for controlling microbial growth in live prey cultures. At Profunda in Norway and Fiskey Ltd. in Iceland, *Vibrio* spp. is used as an indicator for the quality of *Artemia* and various measures are applied for reducing bacterial numbers in the live prey. Various probiotic bacterial mixtures have furthermore been tested at Profunda in Norway and intensive operations commonly add probiotic mixtures to the Rotifer and/or *Artemia* cultures (Bactosafe, Remus etc.). Treatment using selected probiotic mixtures is therefore suggested as a disease prophylaxis approach through treatments of the live prey and larvae through the culture water and juveniles through the culture water or formulated feeds.

Suggested analytical approach as described for Task 1-1

2.2.2 Action 2 – Broodstock management

Studies indicate that the chemical composition of eggs may profoundly affect the egg quality of various fish species (Izquierdo *et al.*, 2001) and broodstock nutrition has been identified as one of the main causes of variable egg quality measured as success of fertilization and survival of successfully fertilized eggs (Sawanboonchun *et al*., 2008; Rosenlund & Halldorsson, 2007). Positive effects of astaxanthin supplementation to the broodstock diet on egg quality have been demonstrated (Sawanboonchun *et al*., 2008) and fecundity is affected by the content of arachidonic acid in the broodstock diet with indications of optimum requirements in the diet (Rosenlund *et al*., 2008). The protein content of the fish is reduced during the maturation process (Hamre *et al*., 2008c), indicating that it is important to ensure ample amounts of protein in broodstock diets. Also, certain nutritional components such as essential fatty acids and anti oxidative compounds, are of particular importance for parent fish as compared to growing fish, but too high concentrations may affect fecundity (Izquierdo *et al.*, 2001). Light and temperature used for manipulation of the timing of spawning may furthermore affect egg quality and there are indications of poorer quality of eggs from light manipulated fish (Rosenlund and Halldorsson 2007).

Overall, there are gaps in the knowledge of the relationship between defined components of the broodstock diet and the quality of the offspring. Defining such relationship is difficult as it needs a long term research. Analysing the concentration of selected ingredients in body tissues, including blood and roes/milt from wild as well as farmed cod should be carried out in order to reveal differences in the ratio of various components that could then be provided in the nutrition of the farmed broostock. This approach has worked for cows when there are problems with fecundity. Whether the same applies for the cod remains to be evaluated.

Based on gaps in the existing knowledge, two main tasks were identified for this action:

Task 2-1: Egg quality indicators in the broodstock fish

Analysing the concentration and ratio of selected ingredients in the blood, roes and milt. The analysis should include a comparison of wild and farmed cod in relation to egg quality (analytical approach as described in Task 1-1).

Task 2-2: Broodstock nutrition and fecundity.

Investigating the effects of macro- and micro ingredients in broodstock diets on fecundity and egg/larval survival and overall quality. Feeding trials of certain duration (minimum 6 months) are needed for such studies.

Fecundity has been assessed through the success of fertilization and egg survival among other criteria. A more satisfactory standard and definition of fecundity is, however, needed.

Macronutrient composition of diets may affect fecundity of the fish. High protein content in diet has for example been found to lead to reduced gonad size (Karlsen *et al*., 2006). Furthermore, tetradecylthioacetic acid (TTA) has been found to affect maturation in Atlantic salmon (Alne *et al*., 2009). The same may apply for the cod and testing the use of feed additives that have been found to affect maturation in other fish species may represent a novel approach for control of sexual maturation as well as quality of the off-springs in cod.

2.3 Conclusions and recommendations for next steps to be taken

Numerous actions were discussed through meetings held within the project, resulting in identification of the two major actions suggested for improved quality of intensively produced cod larvae and juveniles. Both actions describe initiatives that may be addressed in collaboration between the Nordic countries. Overall, suboptimal nutritional quality of the live prey animals used represents a major obstacle that has to be overcome if cod farming is to become a major industry in the Nordic region. Cod larvae fed wild zooplankton exhibit high quality and growth performance, setting the necessary quality standard for hatchery produced juveniles.

Even though the best results, regarding larval quality and growth, are obtained in ponds, this production method is not optimal for industrial production of cod larvae because of the natural variability of the abundance and composition of copepods in the ponds. An optimal system therefore requires that the enriched rotifers and *Artemia* are manipulated to make the diet nutritionally optimal for the cod larvae, which in turns requires detailed information on the differences in composition of various live prey. The BASECOD collaboration therefore suggests a comprehensive comparative analysis of the various prey animals used in the production of cod larvae. The semi-intensive production of cod larvae in the pond systems in the Faroes is extremely important for this knowledge, but it has to be noted that the species abundance and composition may vary in the ponds. A repeated analysis and monitoring of the live prey and larvae from the ponds in the Faroe Islands may therefore be needed.

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