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# FISH FARMING TECHNOLOGY

## ATLANTIC HALIBUT

**New-emerging candidate fish species**

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**Figure 1:** The Atlantic halibut (IMR, Norway)

# EXPERT TOPIC
# Atlantic Halibut

**Exploring the biological and socio-economic potential of new-emerging candidate fish species for the expansion of the European aquaculture industry – the DIVERSIFY project (EU FP7-GA603121)**

DIVERSIFY
New species for EU aquaculture

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One of the species included in the EU-funded DIVERSIFY project, which ran between 2013 and 2018 was the Atlantic halibut (*Hippoglossus hippoglossus*). The Atlantic halibut is the world's largest flatfish and can attain a weight of over 300kg. It is highly prized at markets worldwide, but availability of wild Atlantic halibut is decreasing.

Norwegian stocks are classified as viable, but fisheries are subject to strict regulation. This has led to a higher market demand for Atlantic halibut, which cannot be met by fisheries alone.

The Atlantic halibut (see figure 1) is a semi-fat fish, rich in omega-3 fatty acids, with a characteristic flaky white meat with few bones. Cultured Atlantic halibut has an excellent reputation and is traditionally marketed as large fish steaks or cutlets. It can be smoked or marinated in the typical Scandinavian style. These characteristics led to the inclusion of Atlantic halibut in DIVERSIFY, as a great candidate for fish species and product diversification in European aquaculture.

Research and cultivation efforts of Atlantic halibut started in the 1980's, and although the total annual production of cultured Atlantic halibut is increasing, it still only reached ~1600 tonnes in 2017 (Norwegian Directorate of Fisheries).

In Europe, Atlantic halibut farms exist in Norway and Scotland. The desired market size is 5-10 kg and production time is currently four-to-five years. Despite a significant research effort between 1985 and 2000, the complicated life cycle of Atlantic halibut made aquaculture progress slow, and very little research funding has been allocated thereafter.

However, during this time slow but steady progress has been made by the farmers in order to improve production stability, and interest in both cage and land-based culture is growing. The remaining bottlenecks for increased and stable production are related to a steady supply of fry and a need to decrease the production time.

The latter may be achieved with the recent establishment of "all female" juvenile production. This is expected to have a major impact on production time as females grow faster and mature later –80 percent of slaughtered fish <5 kg are mature males.

The project DIVERSIFY addressed these important bottlenecks with a coordinated research effort in reproduction, larval nutrition and husbandry and vaccine development. The combination of biological, technological and socioeconomic research activities developed in DIVERSIFY are expected

Figure 2- Atlantic halibut breeders

to support the diversification of the EU aquaculture industry and help in expanding production, increasing aquaculture products and development of new markets.

## Reproduction

Research in our project confirmed that wild-caught females spawned reliably and produced eggs consistently of very high quality (>85% fertilisation). Farmed females also produced eggs of high quality when their ovulatory cycles were identified, and stripping was carried out close to ovulation (see figure 2).

For commercial production, as well as breeding purposes, it is not practical to rely on wild-caught females. However, relatively few farmed females produced eggs consistently with fertilisation rates >80-85 percent. As a consequence, it may be necessary to include wild-caught broodstock also in future breeding groups in order to ensure a broad enough genetic material.

Plasma concentrations of sex steroids in farmed breeders were similar to what has been reported previously in Atlantic halibut, with annual profiles following ovarian growth and maturation. Highest 17β-estradiol (E2) levels were recorded just prior to spawning, in the beginning of February, while both E2 and testosterone (T) remained elevated through the spawning period.

No differences in average concentrations were seen between wild-caught and farmed females. Plasma concentrations of the gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were documented for the first time in Atlantic halibut.

Mean FSH concentrations were relatively stable during vitellogenesis, from October to early February, consistent with a constitutive release of FSH from the pituitary. Plasma FSH decreased to low levels during spawning but increased again after spawning was completed.

Plasma LH concentrations showed large individual variations through the reproductive cycle, but high levels were detected during spawning. This was consistent with previously reported results in other teleosts, including a number of flatfishes.

Implantation with gonadotropin releasing hormone agonist (GnRHa) did not advance

**Figure 3:** Oocytes examined under a stereoscope for developmental stage

**Figure 4:** First feeding stage larva

spawning time significantly in Atlantic halibut females, but an apparent synchronisation in spawning time between individuals was seen, as treated females had completed spawning one month before control fish were spent. In commercial production, synchronisation between individuals can be an advantage as staff efforts in egg collection can be concentrated to a relatively short period (see figure 3).

Atlantic halibut breeders need to be monitored for ovulation and stripped on a regular basis, and eggs are fertilised *in vitro*. Therefore, the use of GnRHa implantation offers a logistic advantage to the commercial broodstock management of the species, by reducing the spawning season.

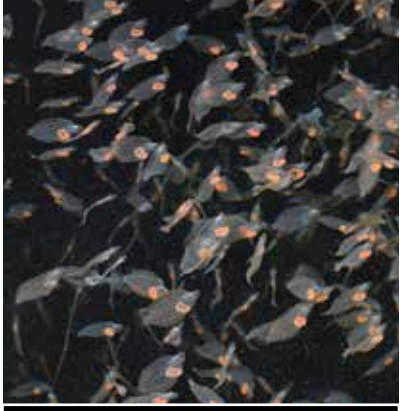

Figure 5: Atlantic halibut larvae at the IMR facilities (Norway)

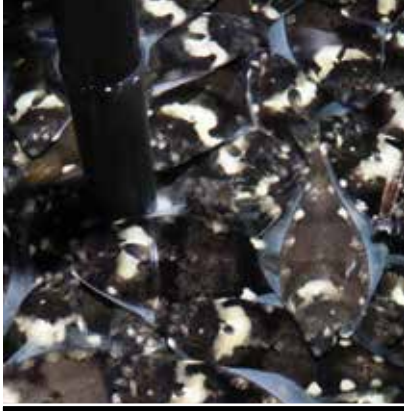

Figure 6- Atlantic halibut juveniles in a feeding experiment (IMR, Norway)

## Nutrition

For the development of a protocol for early weaning of Atlantic halibut larvae, we found a large difference regarding the larvae's feed intake on three different commercial diets at 28 days post first feeding (dpff) (see figure 4).

Larvae fed the commercial marine larval diet Otohime (Japan) had full guts after five days of feeding. This diet was used in an experiment aimed to find the earliest time of weaning at 15, 22 and 28 dpff. Weaning at 15 dpff resulted in almost 100 percent mortality, at 22 dpff approximately 30 percent mortality and at 28 dpff, almost zero percent mortality.

The conclusion was that diet characteristics are important to ensure feed intake in Atlantic halibut larvae and that the larvae are ready to feed on a formulated feed only at 28 dpff. Further experiments are needed to evaluate if the early larvae grow and develop well on these diets.

Also, a protocol for production of on-grown *Artemia* was developed and the nutrient composition was analyzed. *Artemia* grown for three days on the culture medium ORI-culture (Skretting, Spain) and then enriched with the medium LARVIVA Multigain (Biomar, Denmark) obtained an improved nutrient profile in many aspects.

The protein, free amino acid and taurine contents increased, lipid and glycogen decreased, while the ratio of phospholipids (PL) to total lipids (TL) increased. The fatty acid composition improved at one experiment, but not at the one carried out at the commercial partner. The micronutrient profiles were not negatively affected by culture of *Artemia* on the ORI-culture medium.

Since previous research had found that larvae fed on-grown *Artemia* developed into juveniles with better quality, larvae were fed on-grown *Artemia* compared to conventional *Artemia* nauplii in DIVERSIFY (see figure 5).

There were no differences in growth, pigmentation or eye migration between the two groups and the nutrient composition of the larvae after three weeks of feeding was very similar. The conclusion was that *Artemia* nauplii produced with modern methods have sufficient nutrient levels to cover the requirements of Atlantic halibut larvae.

Also, the hypothesis that larvae reared in recirculation aquaculture systems (RAS) would have another micro flora in the gut and, therefore, have different uptake of nutrients was examined. However, except for higher levels of the vitamin K derivative MK6, we found no differences in nutrient utilisation between larvae reared in RAS or flow through systems.

Finally, Atlantic halibut juveniles (one-gram body weight) were fed diets with five PL levels varying from 9 to 32 percent of TL. There were no effects of PL levels on growth or lipid composition in intestine, liver and muscle, 24 hours after feeding.

However, time after the meal affected the lipid composition of the intestinal tissue, with higher levels of neutral lipids one and four hours post-prandial, and higher levels of polar lipids, cholesterol esters and ceramide at 24 hours post-prandial, reflecting absorption of the lipids early after the meal.

It appears that Atlantic halibut juveniles regulate their lipid species composition to be independent of the diet when a range of PL/ Triacyl Glycerol is applied, as in the present study (see figure 6).

## Larval husbandry

A protocol for on growing of *Artemia* nauplii was developed and described. Use of on-grown *Artemia* during the critical period of metamorphosis in Atlantic halibut larva did not differ from use of *Artemia* nauplii with regard to growth, mortality and fry quality. In addition, the production of on-grown *Artemia* was labor-intensive, and high personnel costs may be prohibitive in implementation of this live feed source in commercial larviculture.

The commercial production of Atlantic halibut fry is currently carried out in flow through systems (FT), while there is a growing consensus that a RAS would offer more stable environmental and chemical water parameters that would lead to improved larval performance.

Production protocols for yolk sac and first feeding larvae in RAS were developed in DIVERSIFY. No differences in survival were detected between RAS and FT rearing during yolk sac incubation. When systems were primed for one-month, larval growth was significantly higher in the RAS group during first feeding. High mortality occurred in one of the FT tanks.

Taken together, results suggested that with adequate conditioning of the RAS, a stable system is established where growth and survival of larvae is as good as, or better than in FT systems with optimal conditions. The RAS was a more stable rearing system for Atlantic halibut larvae compared to the FT system.

Metagenomic characterisation of the bacterial communities in rearing water and larvae revealed that at least 300-400 different bacterial genera were present in the rearing systems. Significant differences were detected in the micro biota composition of the RAS and FT systems: both in silos and tanks, and in the water and the larvae.

No obvious correlation was seen between the micro biota in the water and the micro biota of the larvae. Characterisation of the micro biota composition provides important information for development of probiotic treatment of Atlantic halibut larvae.

## Fish health

In order to develop a vaccine against Viral Neural Necrosis for Atlantic halibut larvae, the capsid protein of Nodavirus was successfully expressed recombinantly in three different systems; *E. coli*, *Leishmania tarentolae* and in tobacco plant, and as expected there was variation in the amount of expression between the systems.

In addition, the recombinant capsid protein expressed in *Pichia* was provided from the EU project TARGETFISH. These four expression systems differ in the way the expressed proteins are post-translationally glycosylated. By constructing and using *E. coli* and *Leishmania tarentolea* expressing green fluorescent

Figure 7- Blood sample collected from a juvenile (IMR, Norway)

protein (GFP), it could be visualised by fluorescence microscopy that *Artemia* filtered efficiently and ingested these microbes, and thereby the harboring recombinant protein.

*Artemia* ingested recombinant Nodavirus capsid protein expressed by the various systems, which could be confirmed by immunoblotting.

The recombinant capsid protein expressed by the different system was then fed to *Artemia*, which were fed to Atlantic halibut larvae at 100 dph. Ten weeks later, the juveniles in all treatment groups were challenged by an i.p. injection (see figure 7) with Nodavirus to check for efficacy.

The challenged fish were terminated eight weeks post-challenge and tested for the presence of Nodavirus in the brain by real-time RT-PCR targeting the viral RNA2-segment. No significant difference could be seen between the different treatment groups, including the group with recombinant protein that has shown protection earlier.

This indicates that the size of the fish and the need to sort fish to minimise huge variation between individuals in different phases at the time of vaccination have its inherent limitations and should be carefully considered.

In conclusion, although it has been shown that *Artemia* will take up and accumulate the various forms of recombinant Nodavirus capsid proteins and act as a vector for oral delivery to larvae of Atlantic halibut, the challenge experiments indicate that this strategy of antigen delivery does not induce protection against Nodavirus infection, at least under the conditions used in this study.

A technical production manual has been produced for Atlantic halibut and can be downloaded from the project's website at www.diversifyfish.eu.

This 5-year-long project (2013-2018) has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration (KBBE-2013-07 single stage, GA 603121, DIVERSIFY).

The consortium includes 38 partners from 12 European countries –including nine SMEs, two Large Enterprises, five professional associations and one Consumer NGO- and was coordinated by the Hellenic Centre for Marine Research, Greece.

*www.diversifyfish.eu*

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