SOLVING BOTTLENECKS IN COMMERCIAL PRODUCTION OF ATLANTIC HALIBUT *HIPPOGLOSSUS HIPPOGLOSSUS* L.: THE DIVERSIFY PROJECT

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- So, for your Species presentation (at total of 30 min, but please limit the presentation to 20 min to have plenty of time for Q&As), I suggest you use your introductory presentation you gave at the Species Workshop, highlighting:
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- 1. the major challenges/bottlenecks of the project,
- 2. the important knowledge acquired and breakthroughs achieved,
- 3. the impact our work has already had or may have to the industry in the immediate future,
- 3. the need for further research or the new bottlenecks that are now obvious after 5 years of research in the species
-
- For this reason, avoid showing much experimental data and focus on the conclusions of the work from each WP or Task. My suggestion, as always, is limit your talk to 1 slide per minute (~20 slides) with interesting photos/videos, and use large font (24 preferably, 20 minimum) to ensure that you do not overcrowd the slide and that the information you present is visible/legible by the audience.

Major bottlenecks in halibut juvenile production

- Broodstock management:
- Larval husbandry- survival through early stages
- Nutrition
- Health – viral infections during early development

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Broodstock management

Bottleneck:
Unreliable supply of high-quality eggs

Hypothesis:
Farmed broodstock have inferior performance compared to wild

Approach:
- Documentation of performance
- Hormone therapy

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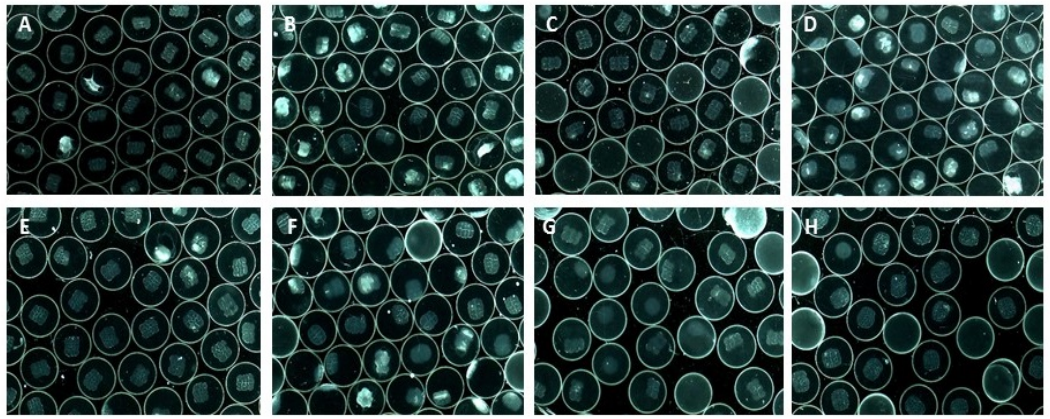
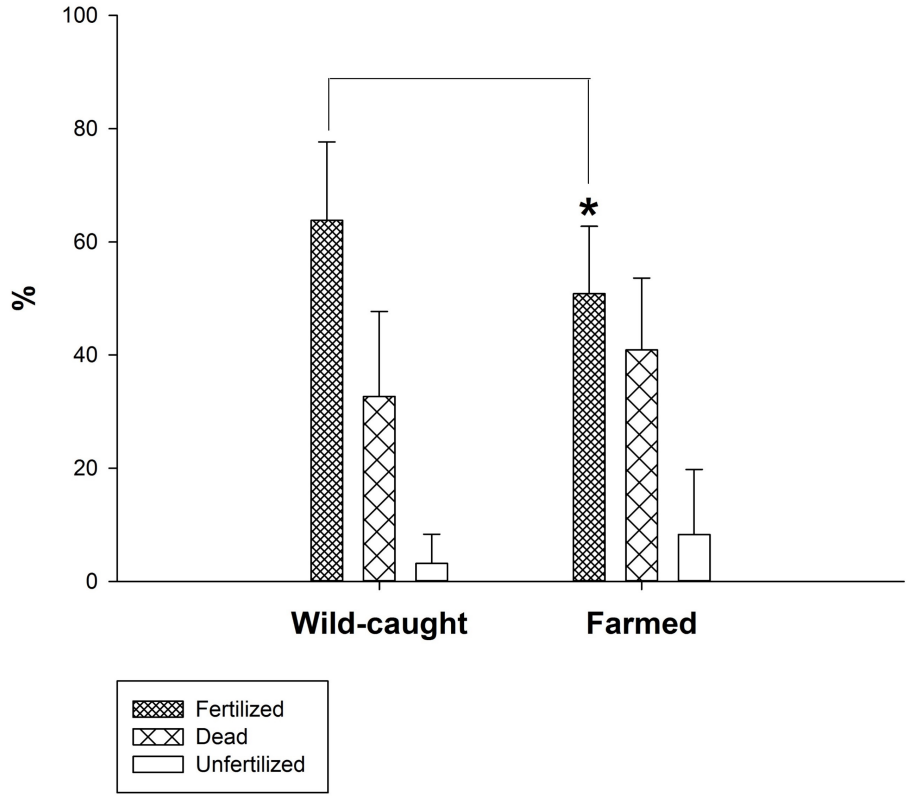
Egg viability in wild-caught and farmed females



In all females, egg batch no 3 was photographed and egg viability parameters analysed



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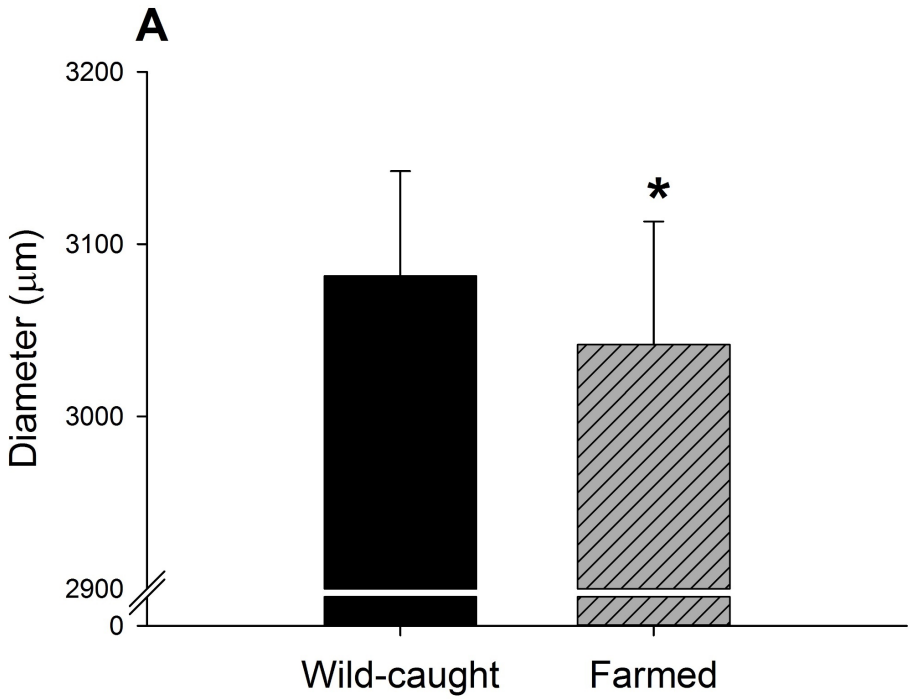
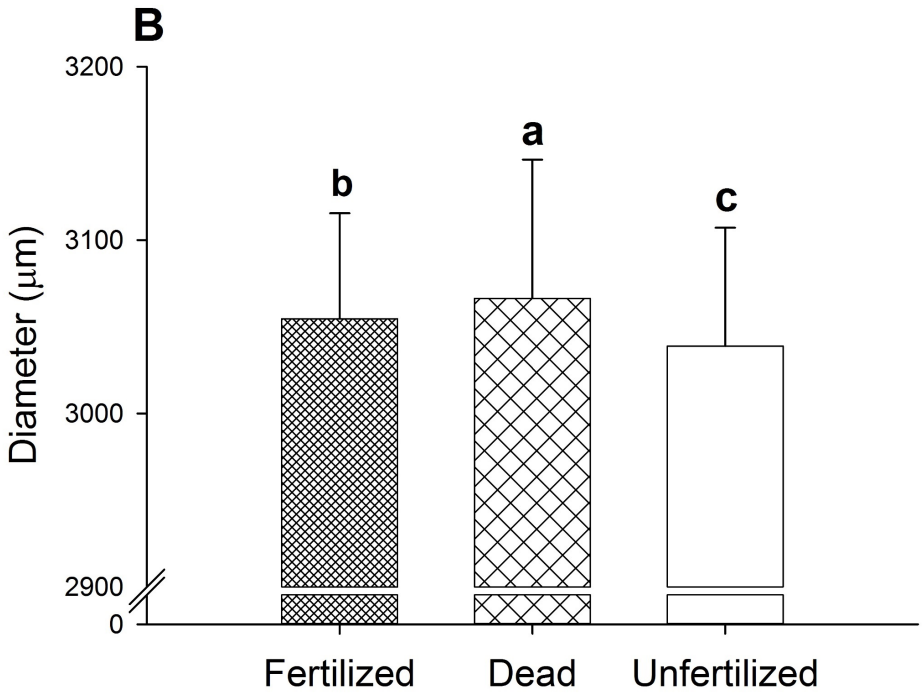
Egg diameter





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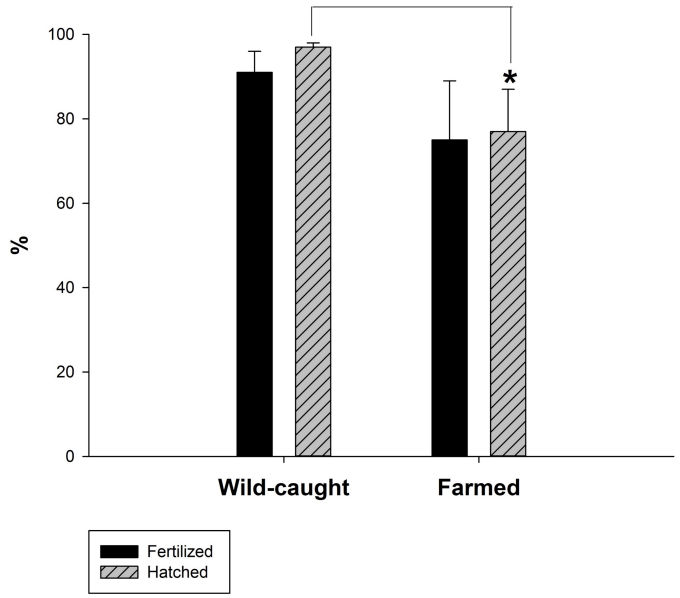
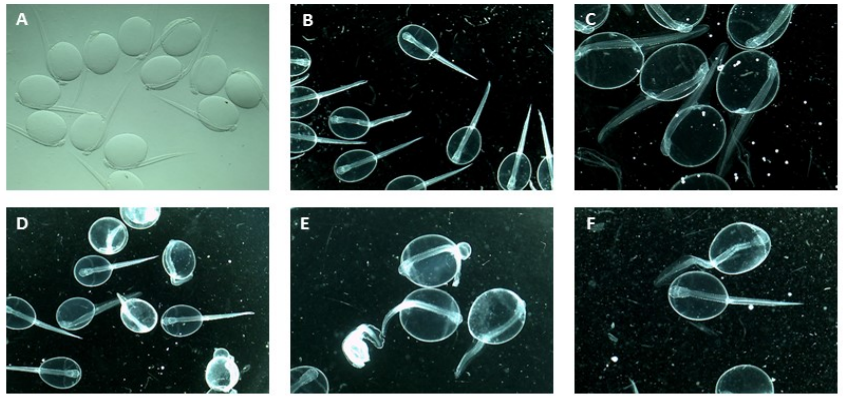
Fertilization, hatching and development in eggs from wild-caught and farmed females





- Eggs from the photographed groups were incubated in triplicate for calculation of hatching success.
- Newly hatched larvae were photographed

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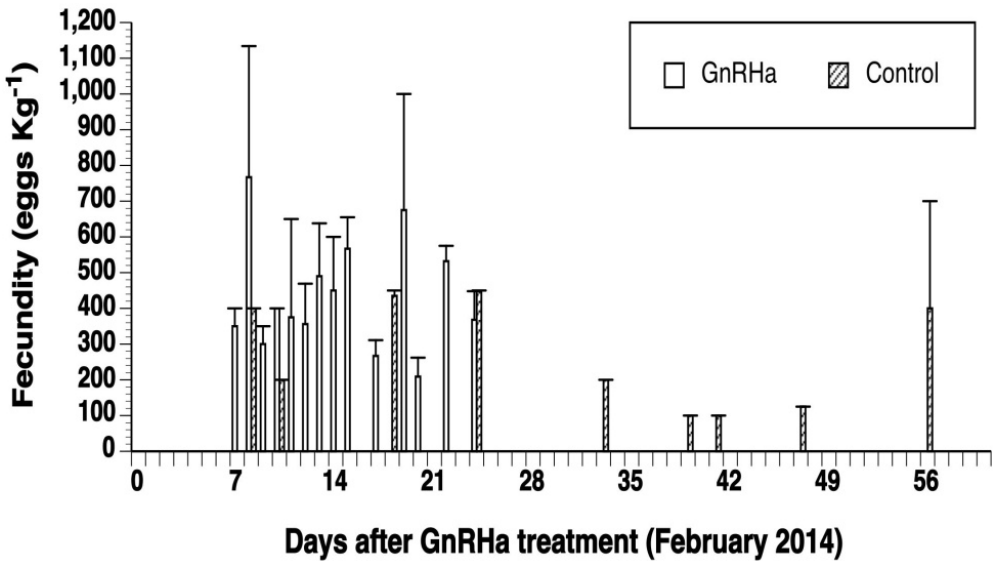
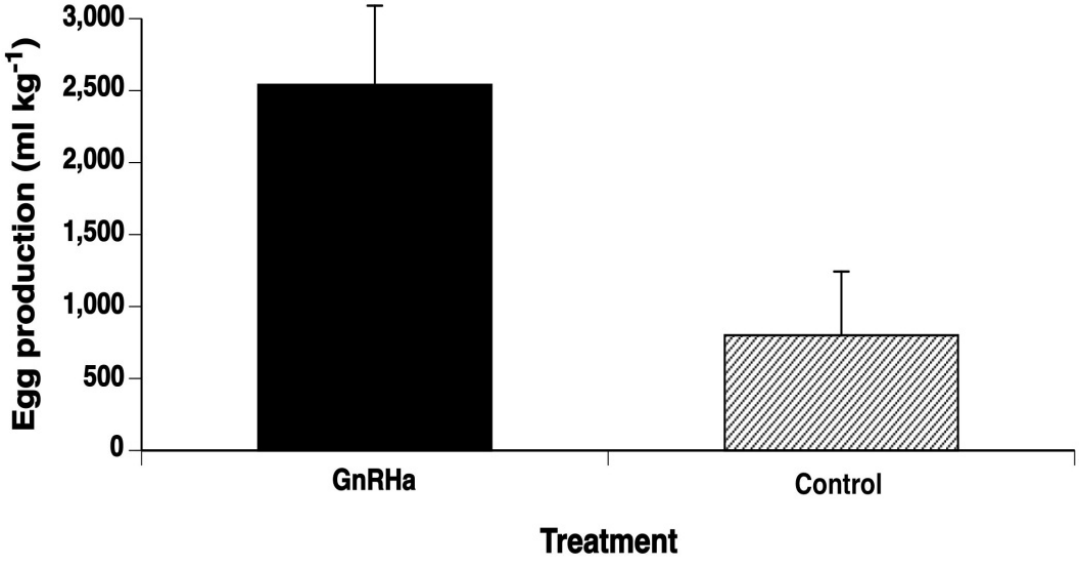
Effect of GnRH implantation on spawning time and fecundity





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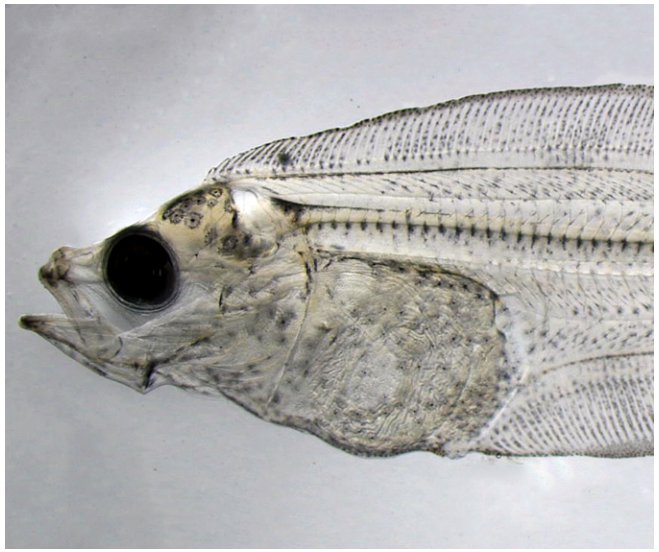
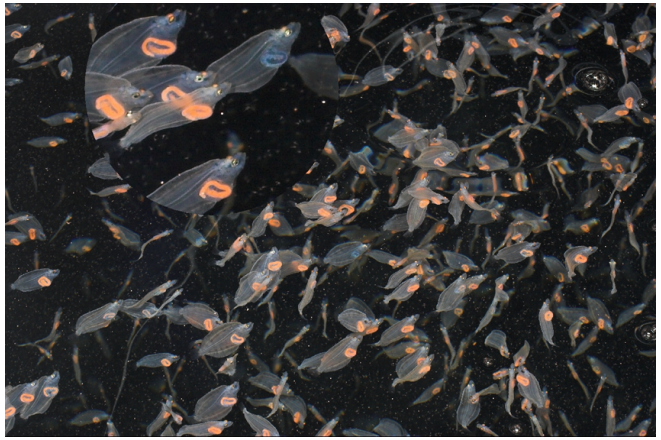
Larval Husbandry





Bottleneck:
Survival through early life stages

Hypothesis:
Recirculation (RAS) is a better rearing system than «traditional» flow-through tanks

Approach:
- Documentation of larval survival, development and growth
- Metagenomic analysis of bacterial composition

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First feeding:

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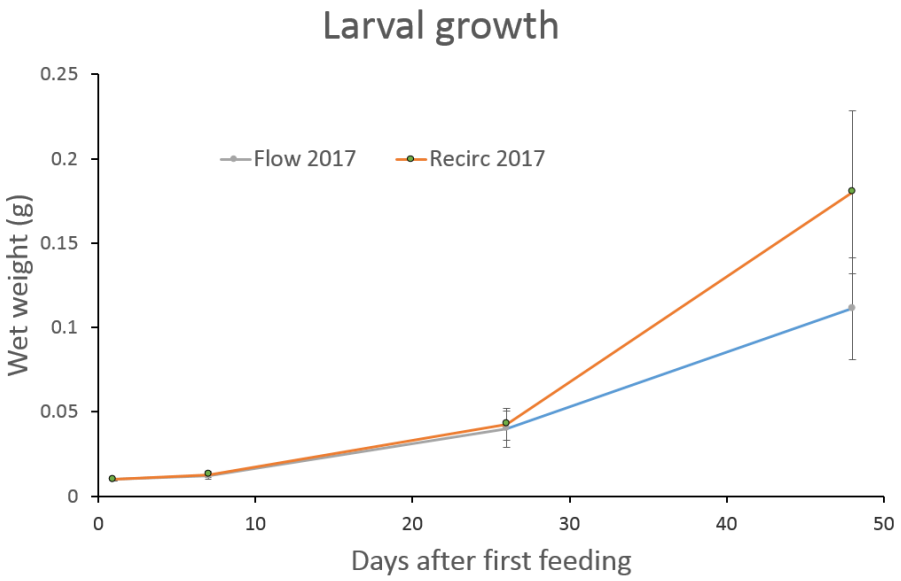
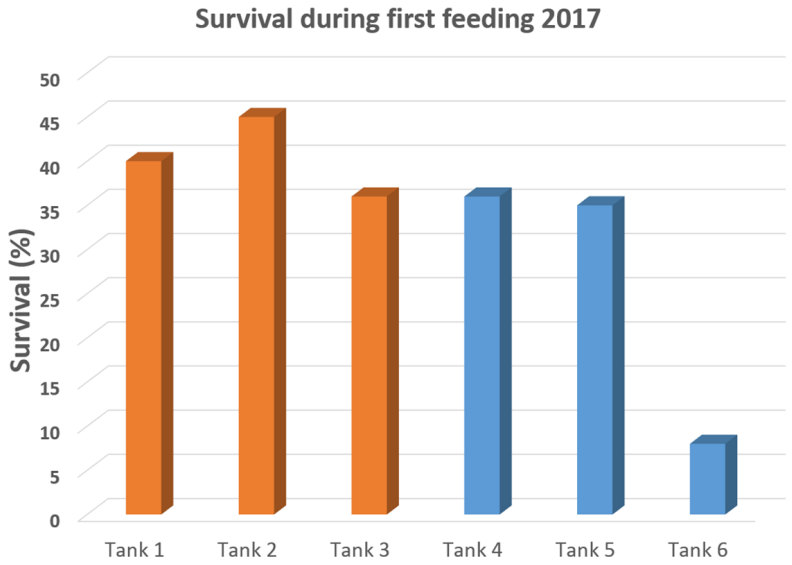
Larval growth and survival during first feeding in RAS and FT systems





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Larval Husbandry: metagenomic analysis of the bacterial composition in halibut larval rearing systems

- Water and larval samples were taken at various sampling points in both flow-through and RAS tanks during the yolk sac and first feeding stages.
- Using Illumina sequencing technique (MiSeq), the bacterial compositions of larvae and water were described.
- Significant differences were detected between the systems – important for development of functional probiotic treatment

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Photo: Øystein Sæle

Nutrition

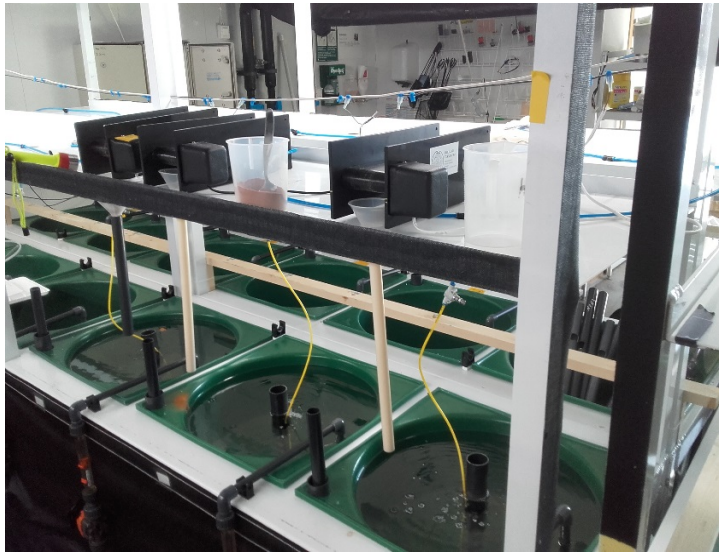
Bottleneck:
High dependence of live feed with suboptimal nutrient composition

Hypotheses:
- Larger Artemia are a better feed for halibut larvae
- Halibut larvae can be weaned to formulated feed earlier than today´s practice

Approach:
- Grow Artemia and feed them to halibut larvae
- Test different formulated feeds, 15, 21 and 28 days after start of first feeding

Early weaning of Atlantic halibut

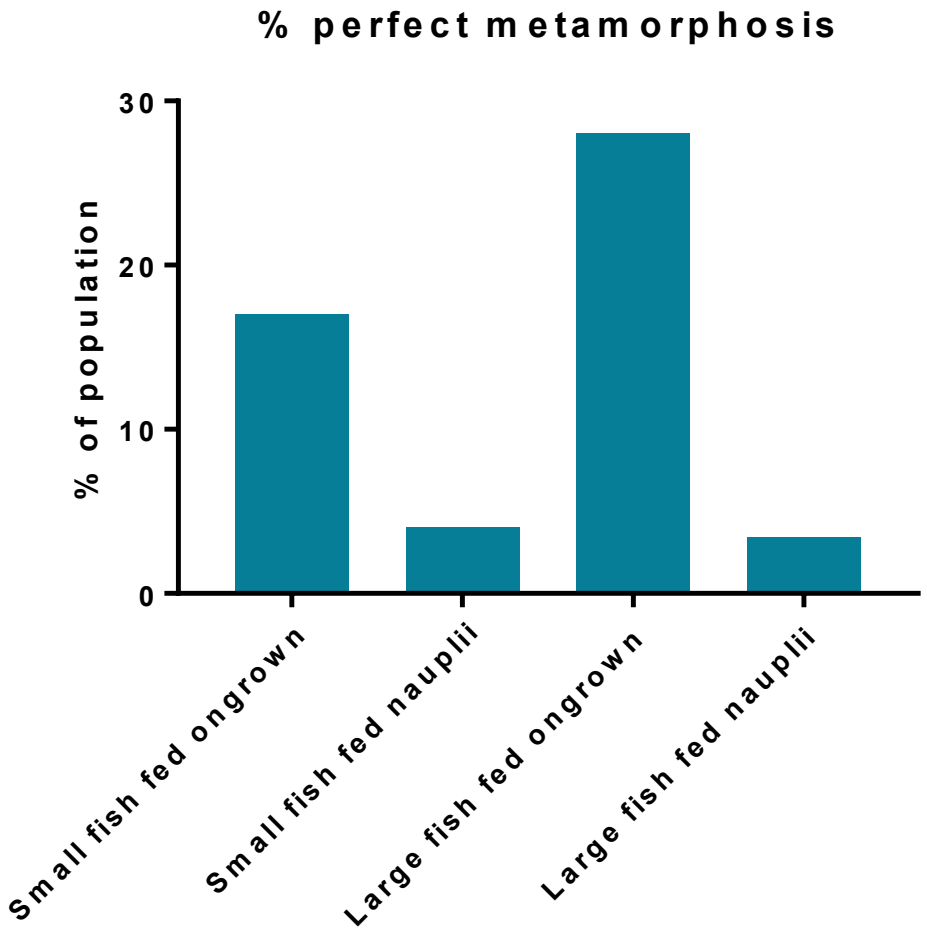
- Atlantic halibut are fed Artemia until 50-60 dpff, time for developed functional stomach.
- Formulated diets would ease larval rearing.
- The main problem has been to make the halibut larvae accept the feed



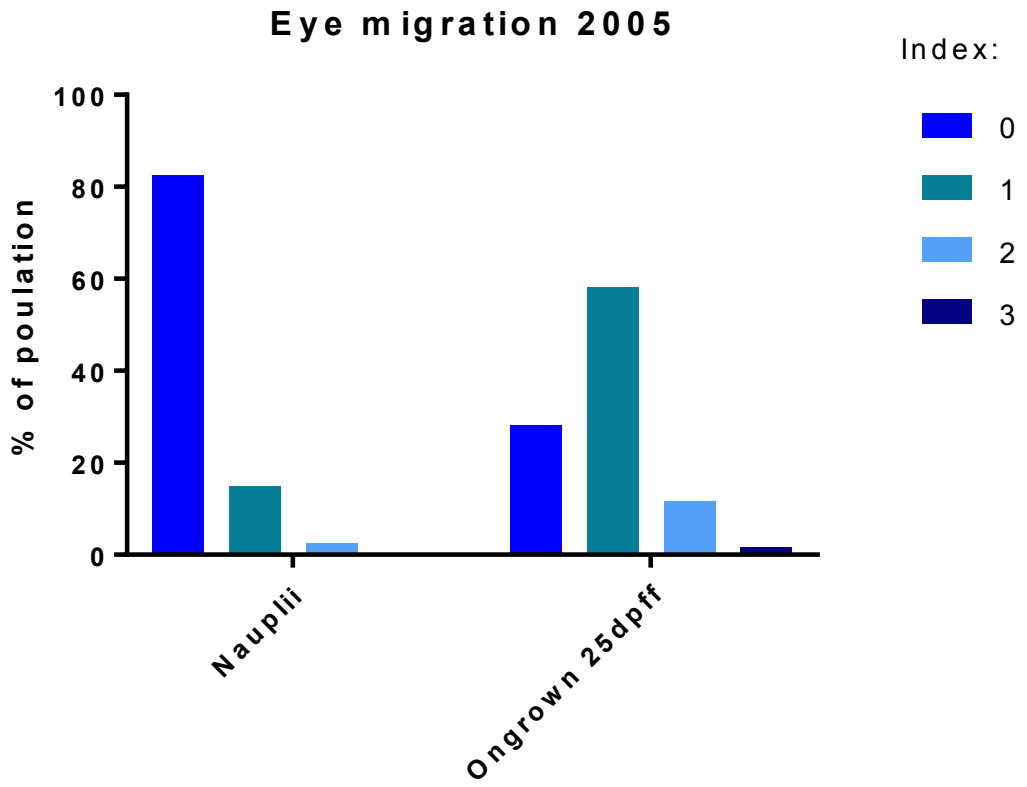
- Results:
  - Large difference in larval response to different feeds
  - Larvae would take the feed and survive from 28 days post firstfeeding (dpff) but not before
- Conclusions:
  - Choice of feed is important
  - Larvae can be fed dry feed from 28 dpff
  - Effect on growth, survival and juvinile quality must be tested in further experiments

Ongrown Artemia has been shown to improve juvenile quality in halibut

Olsen et al., 1999:

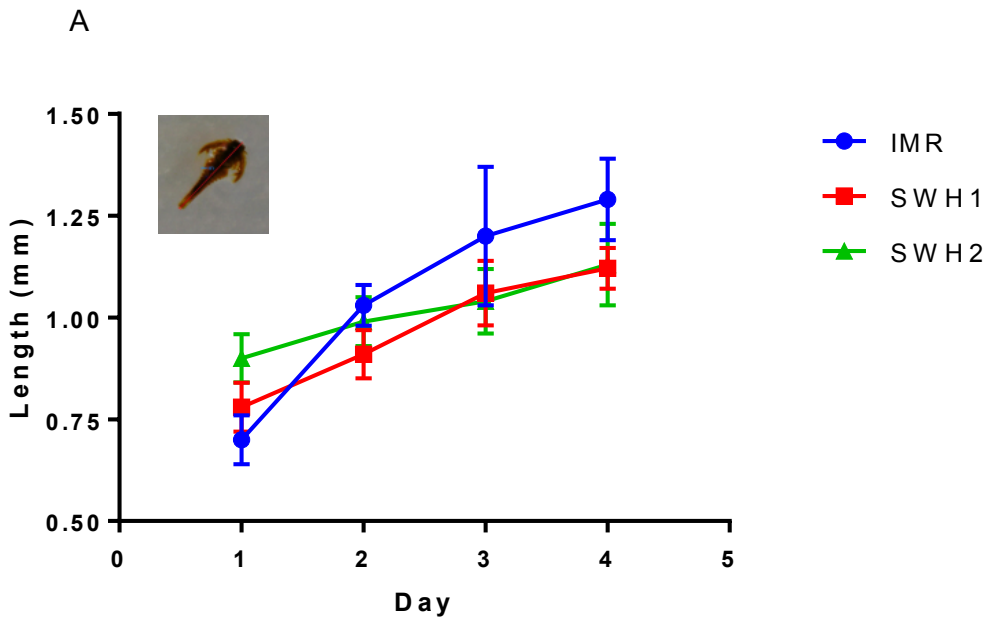


Harboe and Hamre unpublished:



Growth and change in nutrient composition of Artemia

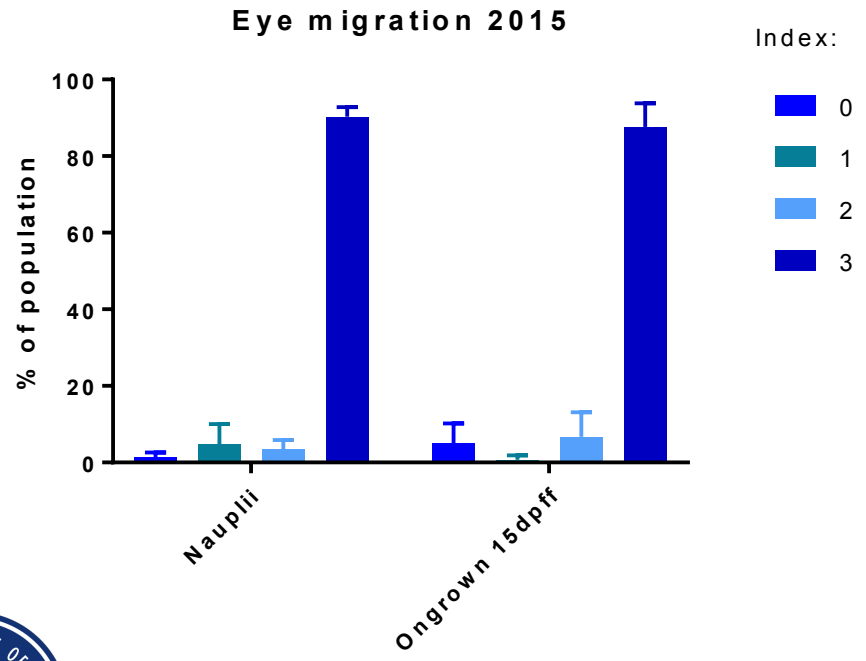
Lengthwise growth of Artemia



Feed: OriGreen or OriGo

- Improvements in nutrient composition:
  - More protein
  - Less lipid
  - Less glycogen
  - More free amino acids
  - More phospholipids
  - Minor changes in vitamins and minerals

Effect of ongrown Artemia on juvenile quality of Atlantic halibut



- Conclusion
  - Modern diets and rearing methods for Atlantic halibut make Artemia nauplii a nutritionally sufficiant larval diet

Fish health

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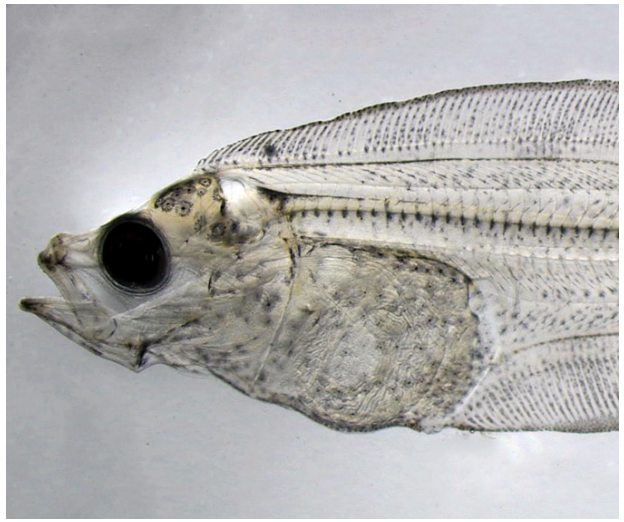
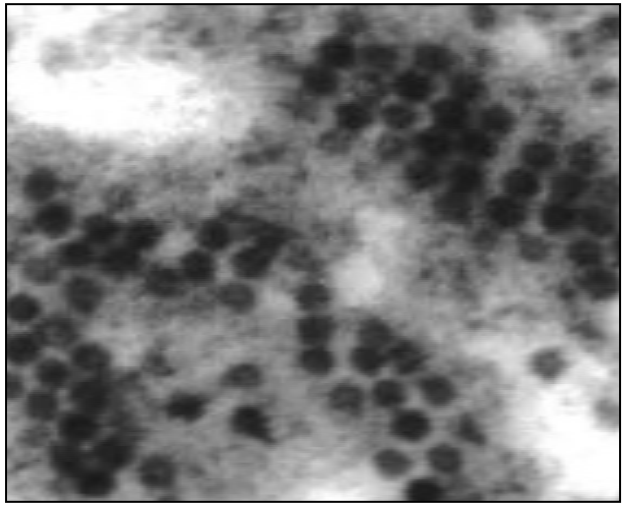




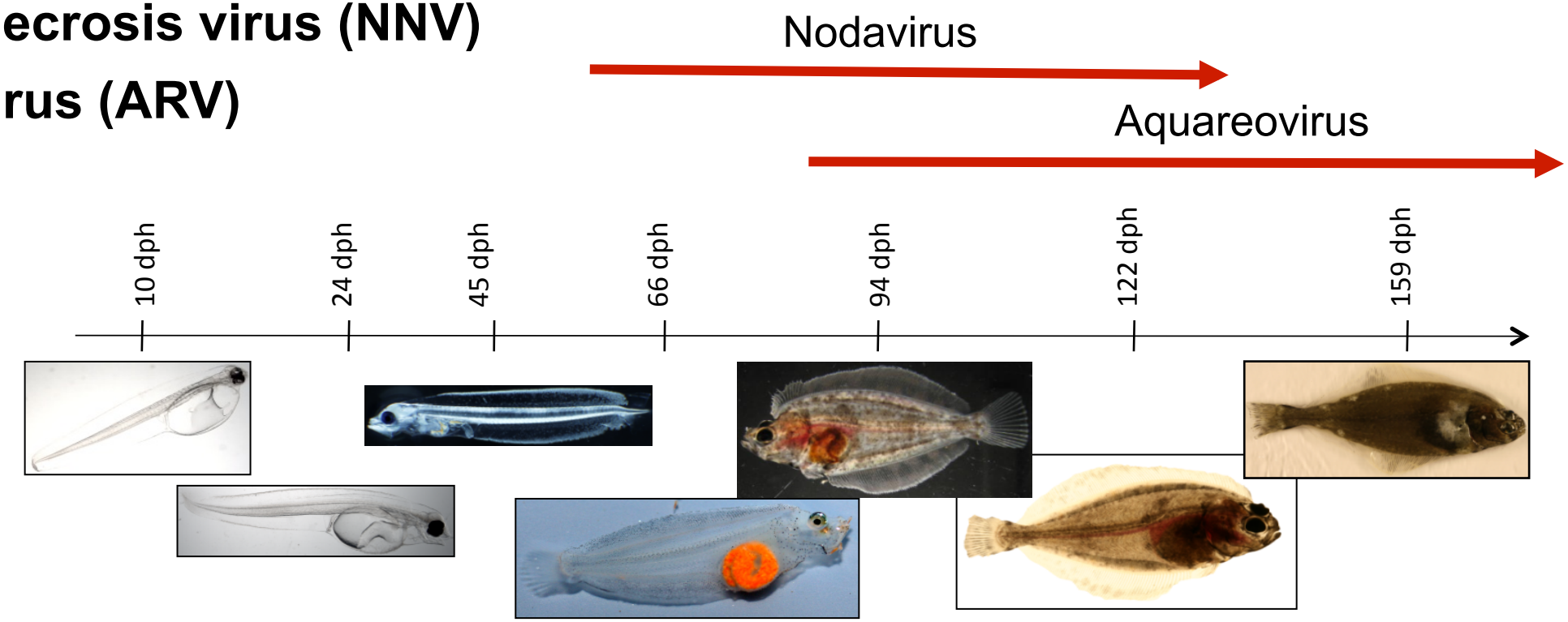
Bottleneck:
Mortality due to viral infections at early life stages

Hypothesis:
An oral vaccine can be cdeveloped and delivered to larvae through incorporation in live feed (Artemia)

Approach:
- Expression of capsid proteins in different systems
- Delivery to Artemia
- Feeding of antigen-containing Artemia to larvae
- Assessment of immune response

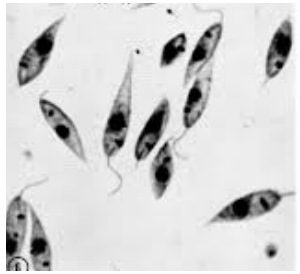
Viral diseases

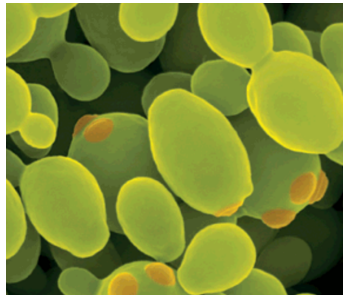
- Viral haemorrhagic septicaemia virus (VHSV)
- Infectious pancreatic necrosis virus (IPNV)
- **Nervous necrosis virus (NNV)**
- **Aquareovirus (ARV)**



Assessment of vaccine

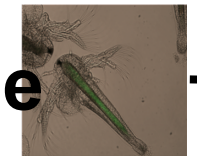
Production of antigen Protein expression in different systems

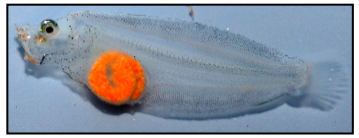


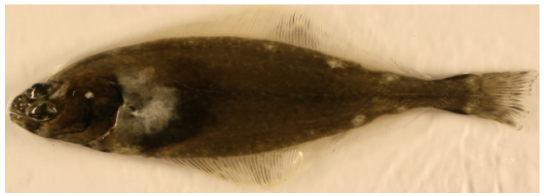


E.coli ***Leishmania tarentolae*** ***Pichia pastoris*** ***Tobacco leaves***

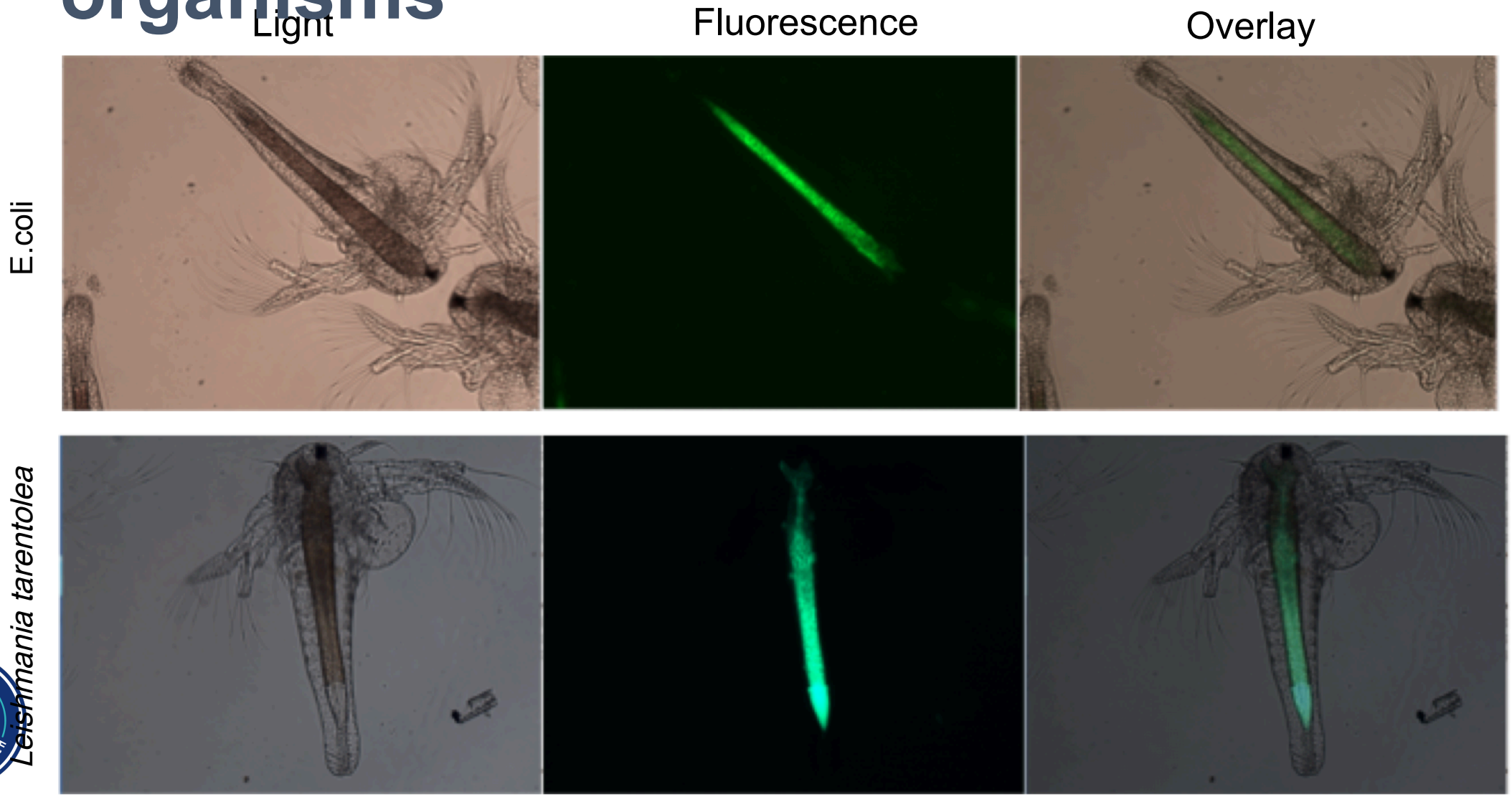
Delivery and challenge



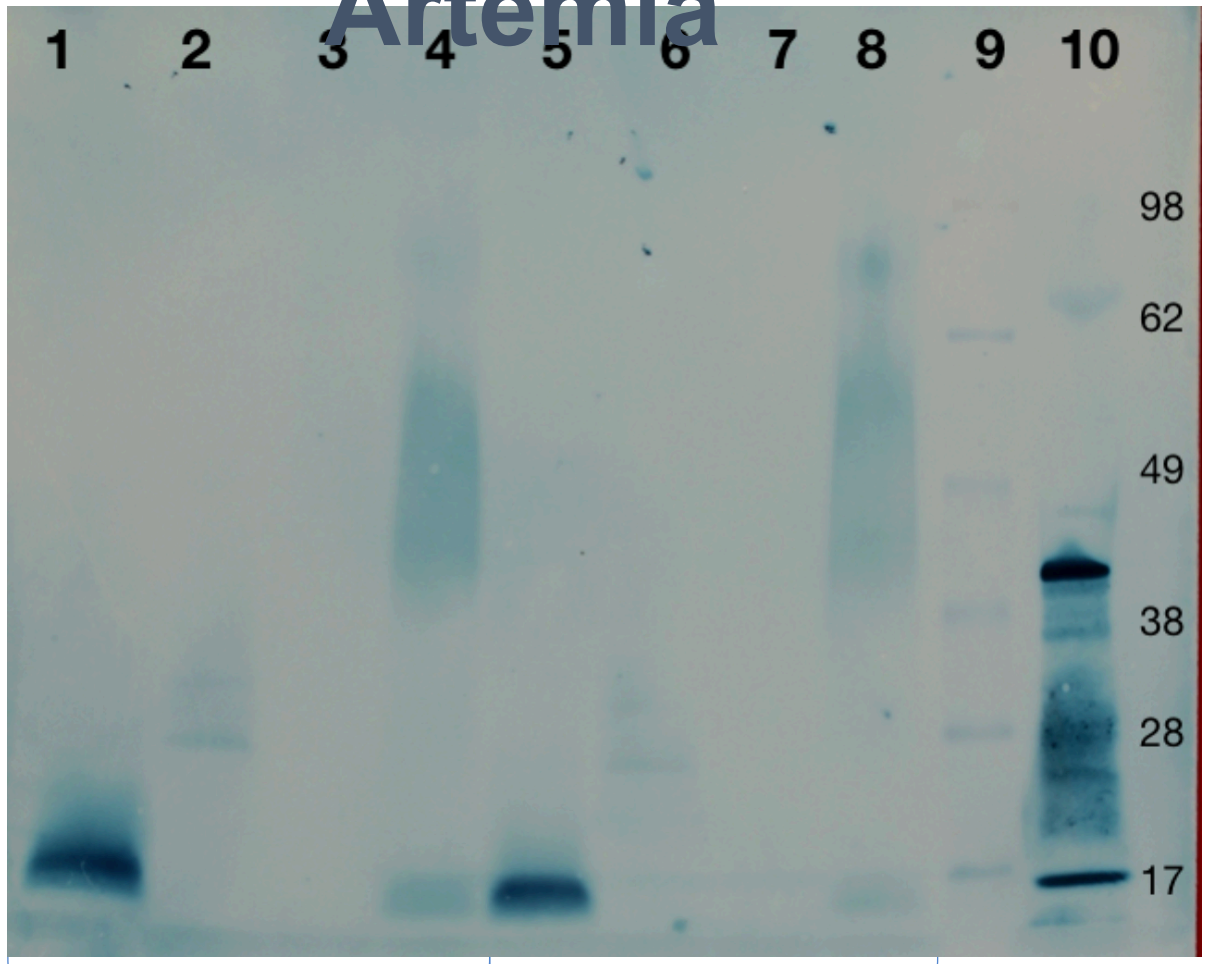




Test with GFP expressing organisms

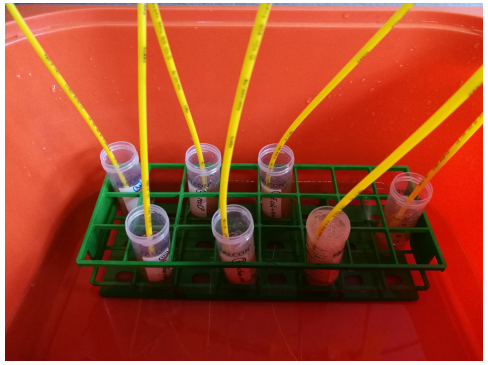


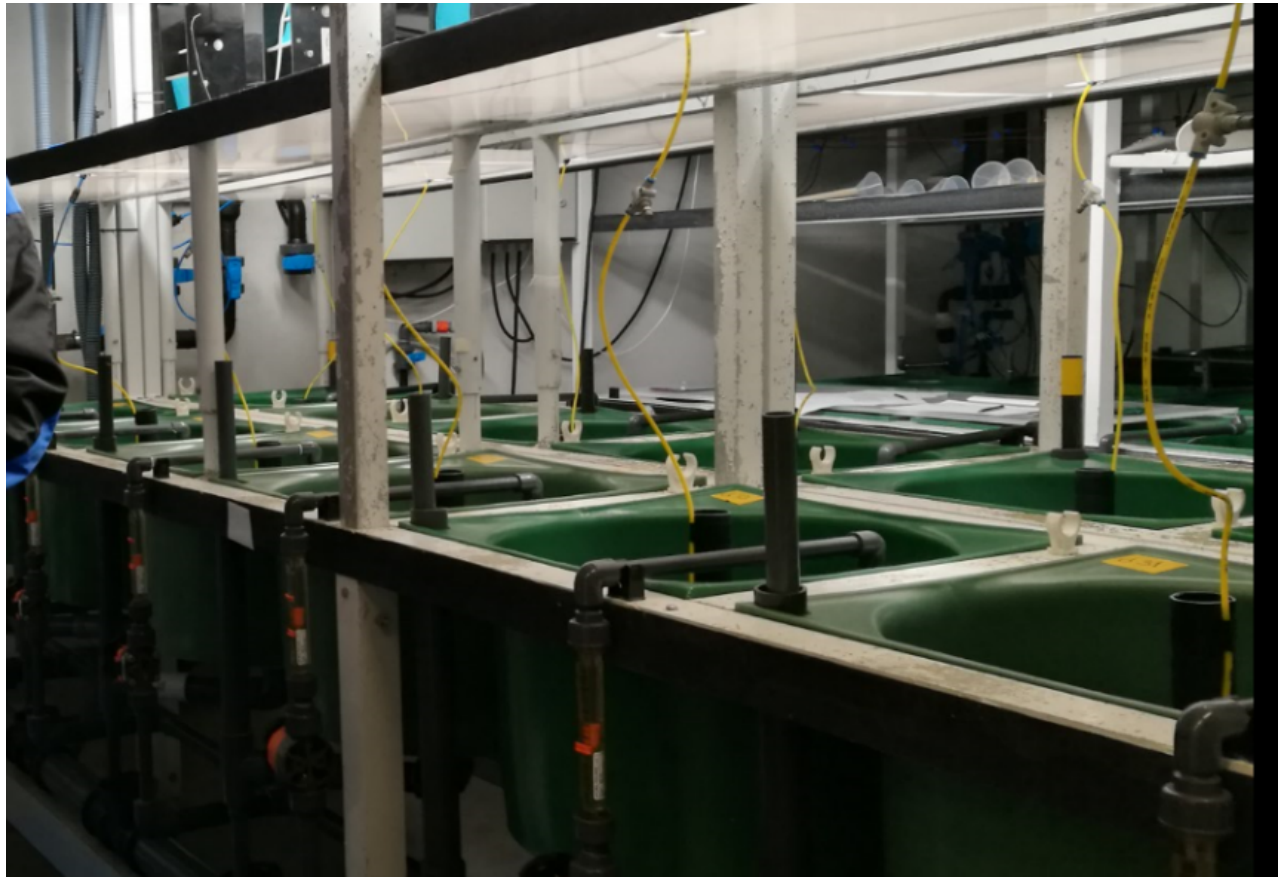
Uptake of protein by Artemia

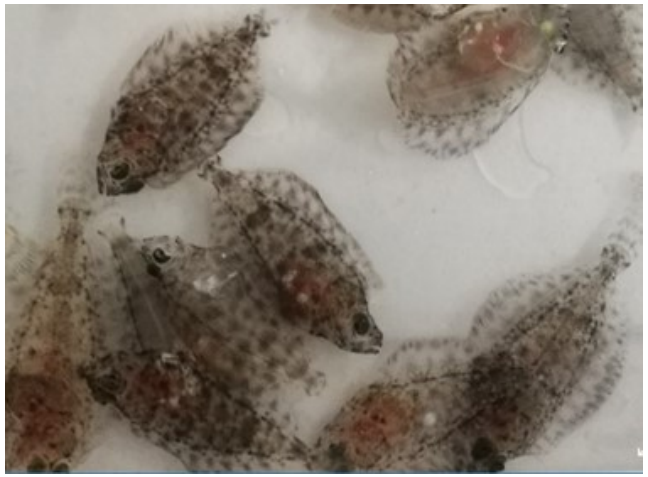


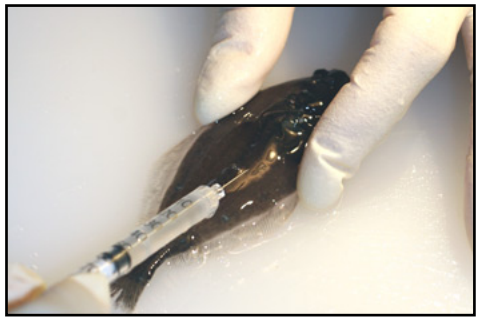
1. purified recCP expressed in *E. coli*
2. *E. coli* expressing capsid protein
3. *L. tarentolae* expressing capsid protein
4. *Pischia* expressing capsid protein.

Lab trial – oral and injection delivery

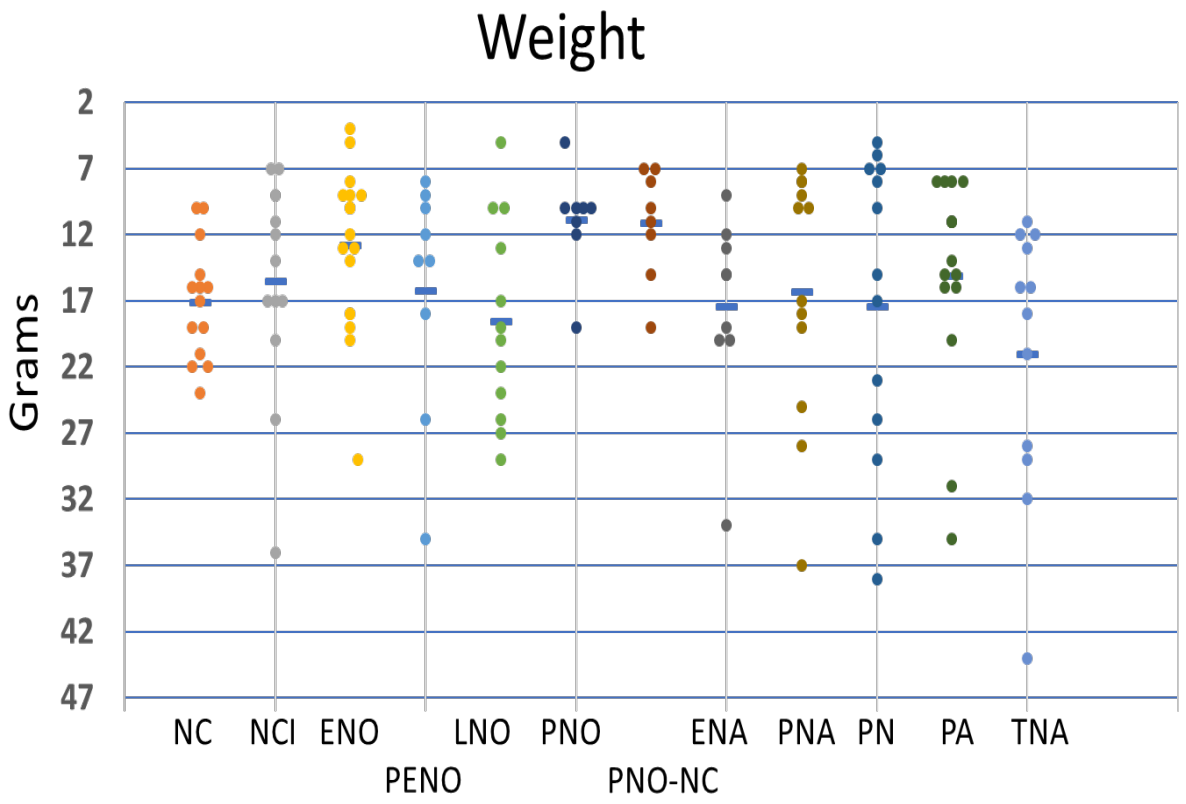








Protection?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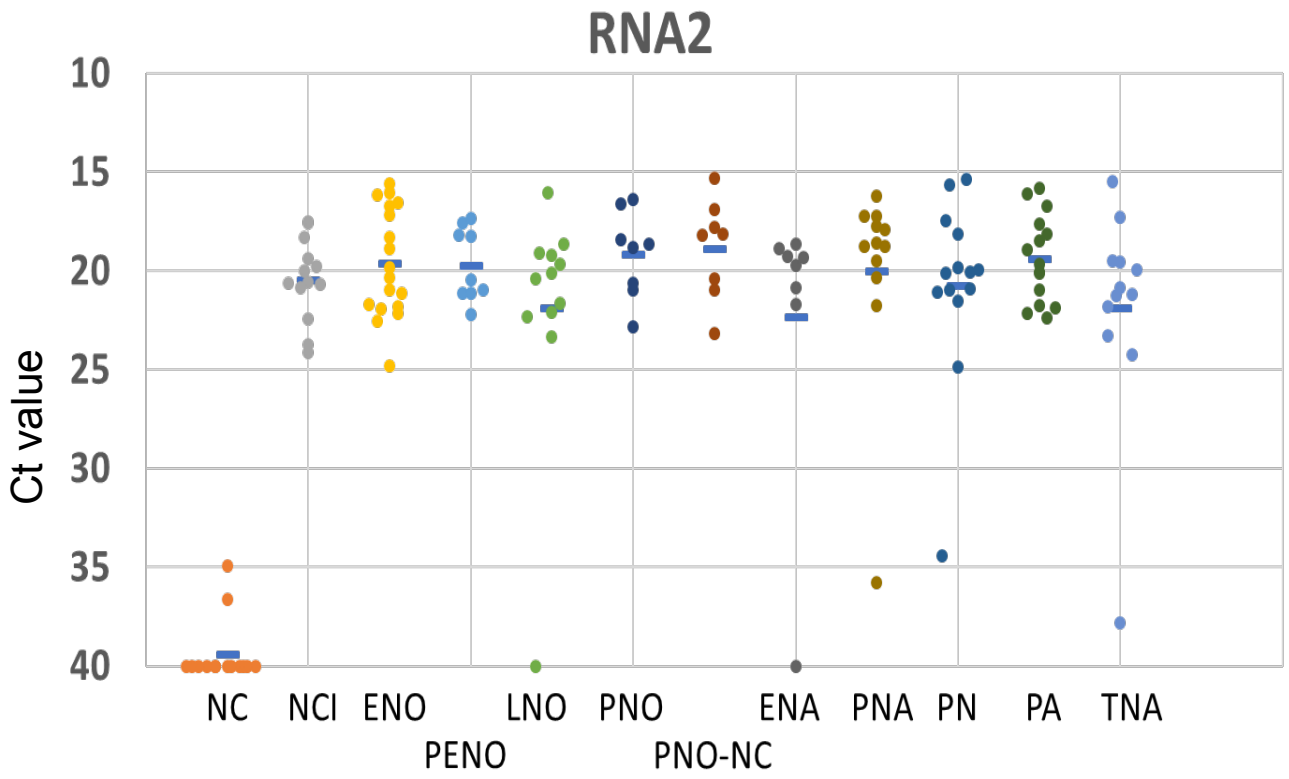
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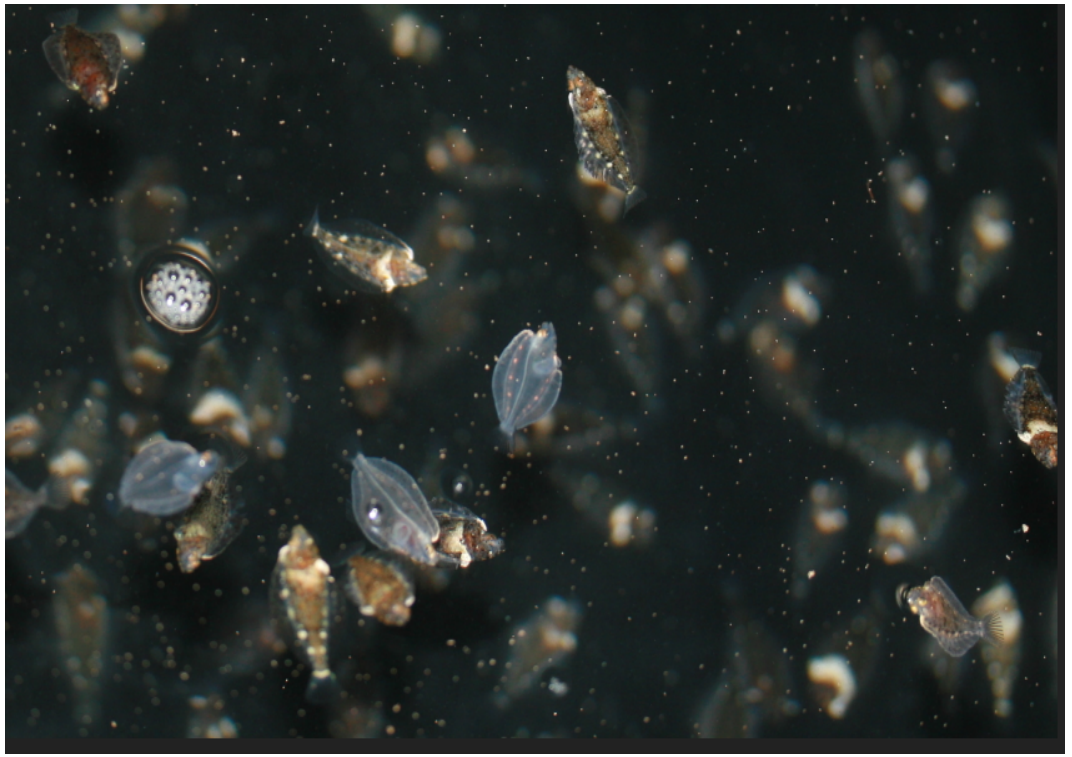
Notes from lab trials

- Vaccination trials should not be initiated before 100 dph
- A balanced diet during development could be important
- Best practice – hygiene during early phases

- Both *E.coli* and *L.tarantolae* are filtered from seawater and taken up by Artemia



What is the impact on the industry?

- Overall, production of Atlantic halibut is growing by 10-15% per year – industry has positive development.
- Stable and predictable availability of juveniles is required for continued growth:
  - Broodstock management – better knowledge improves output of gametes
  - RAS for larval husbandry systems
  - Ongrown Artemia will not improve larval quality
  - Vaccine development will continue

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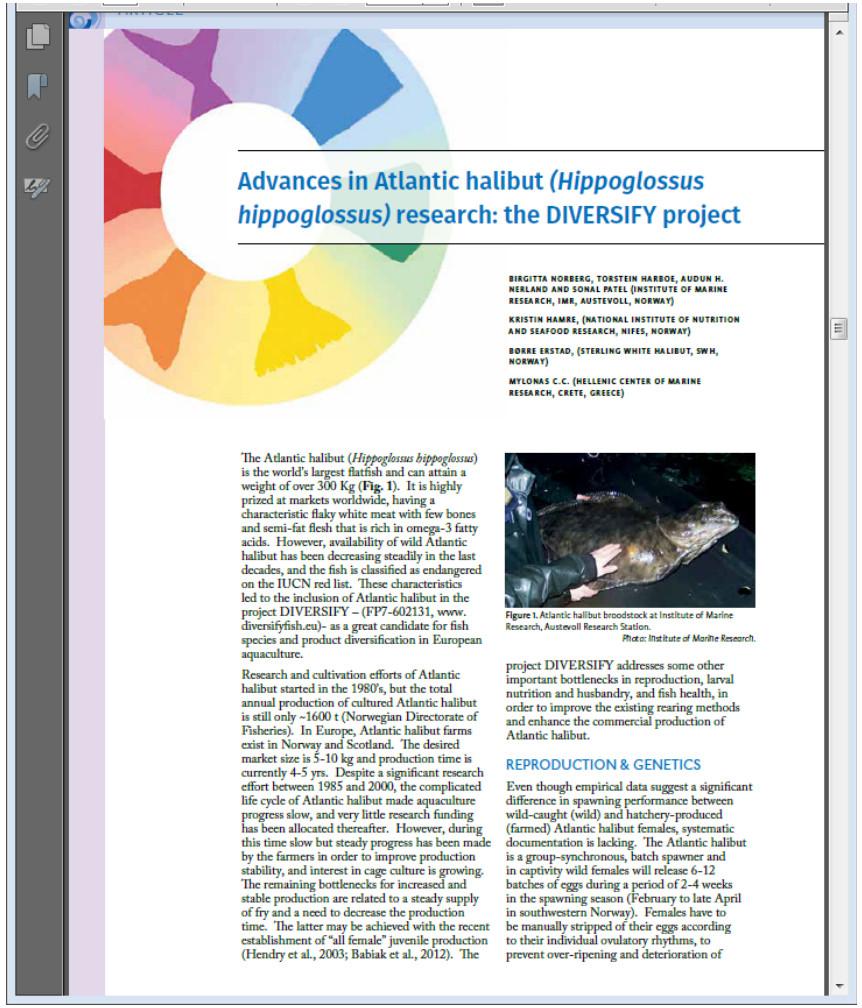
Further challenges and new bottlenecks

- Reproduction, broodstock management and breeding
  - Gamete quality – finding good, reliable and affordable markers
  - Improve stability and performance in out-of-season breeders
  - Use newly sequenced, assembled and annotated halibut genome to find good genetic markers for desirable traits

- Larval husbandry
  - Increase general survival and improve development from egg to juvenile:
    - Optimise RAS during early life stages (yolk sac and first feeding)
    - Improve stability in first feeding tanks
    - Improve eye migration and pigmentation

- Nutrition
  - Early weaning of larvae, to decrease dependence on live feed
  - Sustainable feed development for ongrowing
- Growth
  - Increase growth during first year of life
  - Avoid early maturation
- Health
  - Detection/screening of pathogens
  - Maternally transmitted viral diseases (ARV)
  - Parasite treatment
- Branding, Marketing & Sales

Thank you for your attention!



Advances in Atlantic halibut (*Hippoglossus hippoglossus*) research: the DIVERSIFY project

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The Atlantic halibut (*Hippoglossus hippoglossus*) is the world's largest flatfish and can attain a weight of over 300 Kg (**Fig. 1**). It is highly prized at markets worldwide, having a characteristic flaky white meat with few bones and semi-fat flesh that is rich in omega-3 fatty acids. However, availability of wild Atlantic halibut has been decreasing steadily in the last decades, and the fish is classified as endangered on the IUCN red list. These characteristics led to the inclusion of Atlantic halibut in the project DIVERSIFY – (FP7-602131, www.diversifyfish.eu)- as a great candidate for fish species and product diversification in European aquaculture.

Figure 1. Atlantic halibut broodstock at Institute of Marine Research, Austevoll Research Station.
Photo: Institute of Marine Research.

Research and cultivation efforts of Atlantic halibut started in the 1980's, but the total annual production of cultured Atlantic halibut is still only ~1600 t (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 4-5 yrs. 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 cage 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 (Hendry et al., 2003; Babiak et al., 2012). The project DIVERSIFY addresses some other important bottlenecks in reproduction, larval nutrition and husbandry, and fish health, in order to improve the existing rearing methods and enhance the commercial production of Atlantic halibut.

REPRODUCTION & GENETICS

Even though empirical data suggest a significant difference in spawning performance between wild-caught (wild) and hatchery-produced (farmed) Atlantic halibut females, systematic documentation is lacking. The Atlantic halibut is a group-synchronous, batch spawner and in captivity wild females will release 6-12 batches of eggs during a period of 2-4 weeks in the spawning season (February to late April in southwestern Norway). Females have to be manually stripped of their eggs according to their individual ovulatory rhythms, to prevent over-ripening and deterioration of

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