Final report

Workshop on bone disorders in intensive aquaculture of salmon and cod

NFR # 164773 "*Beinlidelser i intensivt oppdrett*"

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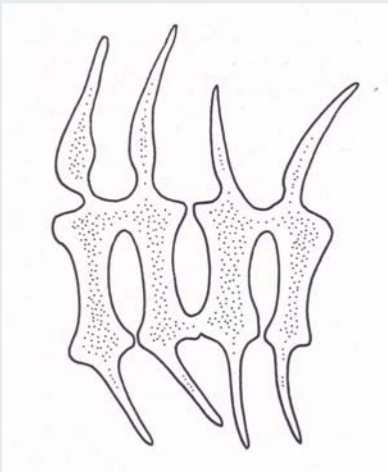

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Content

Final report from workshop on bone deformities in salmon and cod, Bergen May 10-11 2005

The National Institute of Nutrition and Seafood Research (NIFES) hosted a workshop on bone deformities in Atlantic salmon and cod in intensive aquaculture 10^{th}-11^{th} of May 2005. Present were international and domestic experts on bone biology and fish health, participants from the aquaculture industry, researchers and representatives from the Norwegian Food Safety Authority. Several research groups presented new results from ongoing projects showing that bone deformity is a topic that receives a great deal of research effort. More than 70 participants were present and 26 presentations were given.

Initially, an orientation on the history and the present situation of bone deformity in salmon aquaculture was given, also pointing out some risk factors related to fresh water and sea water conditions. Next, invited key speakers presented established facts and new knowledge on the basal biology of fish bone and cartilage. The major part of the presentations were dedicated to factors associated with bone deformity and field experiences from the industry before the Norwegian Food Authority concluded with some ethical considerations and an orientation on the recent modernisation of the legislation for aquaculture.

It became evident from both research and field experiences that bone deformities can appear in both early and late life stages and that this condition has a multi-factorial causality. In spite of lack of unambiguous evidence, some risk factors were identified:

- Unfavourable environmental conditions in juvenile rearing, e.g high water temperature and gas saturation (salmon and cod)
- Use of underyearling smolt with subsequent fast growth (salmon)
- Vaccination type and temperature at vaccination (salmon)
- Unbalanced nutrition with emphasis on phosphorous (salmon and cod)

The last session of the workshop was dedicated to group discussions and the outcome was presented in plenary. In short, standardisation of diagnosis should be a prioritised area along with intensified research on the identified risk factors. Furthermore, the aquaculture industry should contribute more in trying to reduce bone deformities, preferably in cooperation with research. Finally, all parties must be open and cooperate so that the whole industry can benefit from new knowledge.

The organising committee wishes to thank all the participants for their valuable contributions and their willingness to seek a solution to the bone deformity enigma.

Prof. Dr. Rune Waagbø, NIFES
Prof. Harald Kryvi, University of Bergen
Dr. med.vet Olav Breck, Marine Harvest Norway AS
Dr. Robin Ørnsrud, NIFES

Presentation summary

The presentations in the workshop were divided into four categories; I) Basic normal development of bone tissue, II) Factors associated with bone deformities, III) Field experiences from the industry and IV) Ethical aspects

I) Basic normal development of bone tissue

The purpose of the session was to give the listeners an introduction into the basic biology of bone in fish. The session was opened by Dr. Huysseune of Ghent University, Belgium, who gave an extensive presentation on the origin, growth and remodelling of bone tissue from an evolutionary point of view. An important aspect that separates fish bone from terrestrial animals is that the fish skeleton still displays the complex evolutionary history that has led to an astounding tissue diversity with many intermediate tissue types between bone, cartilage and connective tissues. Further, bone must be considered a living tissue with continuous remodeling, i.e. continuous resorption of old bone and formation of new bone. These processes are maintained by bone forming osteoblast cells and their counterpart, the bone resorbing osteoclast cells. The osteoclasts may be further subdivided into multi-nucleated giant cells, commonly found in the more primitive cellular boned teleosts while mononucleated osteoclasts are more common in the more advanced acellular-boned teleosts. These cells are thus involved in remodeling processes, which are neceessary to facilitate bone growth or as an adaptation to mechanical load. So, knowledge of the enormous diversity of skeletal tissues, with intermediate tissues types that are widespread under natural conditions and with several alternative pathways of skeletal tissue formation, is essential if these tissues are to be used in the diagnosis of skeletal disorders.

Dr. Eckhard Witten of the University of Hamburg gave a presentation enlightning differences between regular and pathological bone growth in Atlantic salmon. In normal bone growth, skeletal formation and growth in Atlantic salmon follows known pathways, such as cartilage formation (chondrogenesis), intramembranous bone formation, and perichondral/periosteal ossification eventually followed by endochondral ossification. Like many other teleosts, salmon can also develop skeletal tissues with characters intermediate between bone and cartilage such as chondroid bone, a mineralised tissue with cartilage-like cells embedded in a bone-like matrix. In many teleosts, chondroid bone is part of normal skeletal growth. It has been suggested that a switch from periosteal bone formation to chondroid bone formation facilitates fast growth. Formation of a particular type of skeletal tissue (cartilage, bone, and possibly intermediate skeletal tissues) can be triggered by altered mechanical load. Besides being part of regular skeletal development in salmon, a switch from periosteal bone formation towards the formation of chondroid bone, or even a whole continuum of skeletal tissues, also occurs under pathological conditions, in particular during the pathological compression of salmon vertebrae. Compressed vertebrae display shape alterations of their end-plates and changes in the composition of the intervertebral tissue. The spongiosa is unaffected, i.e. there are no fractures or enhanced bone remodeling nor are there differences in vertebral mineral content between compressed and normal vertebrae, but the growth zones of adjacent vertebrae blend towards the intervertebral space into different types of skeletogenic tissue that replace the notochord. Unaltered central parts of the vertebral bodies suggest that the compression of vertebrae is a *late event*, typically after sea water transfer that relates to a metaplastic shift of osteogenic tissue into chondrogenic tissue in the vertebral growth zone. It was proposed that an altered mechanical load could have caused this transformation.

Dr. Sindre Grotmol of the University of Bergen gave a detailed presentation of the osteological development of the early vertebral column in Atlantic salmon, including a detailed description of backbone structures facilitating differentiation between normal and pathological backbone development. The backbone of the Atlantic salmon consists of amphicoelous, “hour-glass” shaped, vertebrae and intervertebral ligaments, both of which include notochord-derived structures. As in other teleosts, the notochord develops into a prominent fiberwound hydroskeleton that sustains the locomotion of the larvae after hatching. It seems that the notochord plays an instructive role in the segmental patterning of the vertebral column by initiating a segmental differentiation of the notochordal sheath into chordacentra and intervertebral regions. The ring-shaped chordacentra form through mineralization of preformed matrix, within the outer half of the notochordal sheath. The mineralization process in each chordacentrum starts along the ventral midline and proceeds towards the dorsal side of the notochord in a bilateral, symmetrical manner, finally forming a complete mineralized ring. The mineralization process is preceded by segmental expression of alkaline phosphatase (ALP), which is an enzyme associated with bone mineralization, within morphologically distinct bands of chordoblasts. The expression of ALP within the notochord precedes ALP expression by presumed somite-derived cells external to the notochordal sheath. The results show that the vertebral bodies of salmon, unlike those, for example, of tetrapods, are ‘open’ structures with no outer delimiting bone elements. The compact bone may thus grow through extension at the rims of the amphicoel, while the cancellous bone may grow by trabecular elongation and branching. Furthermore, the utilization of preformed structures such as the notochordal sheath and the intervertebral ligament, through mineralization, may constitute an efficient and less metabolically demanding mode of growth.

II) Factors associated with bone deformities

This session dealt with research on factors or conditions that have been related to the development of bone disorders, from environmental conditions (temperature, light regimes, water quality), handling (vaccination), genetics and nutrition (phosphorous), including some studies on mechanisms. The author contributions refer to their respective abstracts in Appendix III.

Dr. Grethe Bæverfjord presented a review on her studies on bone deformities in Atlantic salmon, *Salmo salar*. Elevated egg incubation temperature regimes have earlier been identified as critical for development of organ and bone malformations. From further studies, she extended the risk period to include sensitive juvenile stages during the fresh water period. For water temperatures >12°C, increases in vertebral fusions were observed. The studies support the view that lesions in vertebrae induced in early life stages continue to develop with time. The research so far indicates variation in genetic disposition, but do not support genetics as a primary casual factor. Growth seems to be maintained at the expense of normal bone development, showing that sub-optimal mineral supply (phosphorous) cause severe jaw and vertebrae malformations. Further studies are in progress to examine if water quality parameters of intensive rearing (high levels of CO_2, low pH, and elevated oxygen levels) may have an impact on bone development and mineralization.

Comparatively, Lein et al. suggested that rearing conditions like water temperature, salinity, and water current may affect the normal development of organs in cod larvae. From their temperature studies, larvae raised with a gradient increase in temperature from 6 to 12°C during first feeding period was superior to larvae reared at 12°C with respect to frequencies of skeletal deformities. There was, however, no significant difference in growth between the temperature regimes up to 415 d° post first feeding. In halibut, frequencies of deformities like gaping jaws and yolk sac oedema were correlated with temperatures up to 12°C, and the critical period was identified as the first three weeks post-hatch. Obviously, there is a need for more knowledge on several environmental factors (temperature regimes, water flow, salinity, and tank material) during first feeding and early juvenile stages in halibut.

A case study of spine deformity occurring in cultured Atlantic cod, *Gadus morhua,* larvae and juveniles was presented by Totland *et al.* From the pathomorphological characteristics he suggested that a deformation of the notochord by pressure from the swim bladder or digestive tract may in turn cause malformation of the spine in the cod larvae. He indicated a critical time window with regard to development of the malformation from 18 to 36 days post hatching, when the initial formation of the vertebrae takes place. The overfilled swim bladder may have been caused by decrease in ambient pressure, super saturation of gas or abnormal gas regulation. Overfilling of the digestive tract may have been caused by overfeeding, large feed particles or accumulation of gas in the intestinal lumen.

Development and pathology of vertebral column deformations in Atlantic salmon was studied in individually tagged yearling Atlantic salmon smolts reared at a full cycle natural photoperiod regimes until slaughter (Fjelldal *et al.*). The study confirmed that severity of deformations detected by radiography in freshwater increases until slaughter, while this was not true for platyspondyly observed in the tail region at slaughter. This condition seemed to develop during smoltification or in sea water and a higher prevalence of platyspondyly was seen in small sized males at time of sea transfer. The vertebral column of the Atlantic salmon seems to display regional growth, with increased longitudinal growth in the caudal and truncal regions during and after smoltification, respectively, possibly related to photoperiod. Rapid growth affects the mineral rate and the mechanical properties of the vertebrae negatively, as illustrated by comparing under-yearling and yearling smolt. Thus, rapid growth seems to be a risk factor for developing vertebral column deformations.

Constant light is regularly used to increase growth of farmed salmon. Experiments have shown that reduced mineral rate in salmon can be found already after one week of continuous light regimes. This seems to be related to significant increase in the non-mineral part of extra cellular matrix (ECM) in trabecular bone and not to a decreased mineralization of the bone tissue (Wargelius *et al.*). Gene expression of molecular markers involved in proliferation (*sonic hedgehog)*, mineralisation (*alkaline phosphatase*), mineral binding (*matrix gla protein*) and connective tissue properties (*collagen I*) in bone did not reflect this finding. Further studies are being conducted with micro-array techniques to find molecular cues to the drop in mineral content caused by constant light.

Gil et al. studied the impact of high water carbon dioxide levels on Atlantic salmon smolts in the fresh water rearing phase. Although fish exposed to high CO_2 levels showed an increase in trabecular volume and a higher rate of bone remodelling at the end of the fresh water stage, no pathological bone alterations could be found. After transfer to sea water and a grow-out period to 3.1 kg (harvest size), no signs of spinal deformities could be seen.

Berg *et al.* presented an experiment showing how vaccination time and type may influence growth and development of spinal deformities. Generally, vertebra mineral rate and strength seem to increase after vaccination. A correlation between vaccine side effects like organ adherences, growth depression and development of vertebrate deformities was observed. The results could, however, not be verified in other experiments, illustrating that interacting factors take part in the pathogenesis.

Dietary phosphorus concentration has been identified as critical to mineralization and development of bone deformities (Bæverfjord). Koppe *et al.* studied effects of dietary P-concentrations (7 -20 g P/kg) on performance and bone mineralization in Atlantic salmon 1 g fry and 270 g post-smolts for 10 and 16 weeks, respectively. The dietary P-content had no effect on the growth performance, feed utilization, mortality and did not lead to bone malformations, while the tissue mineralization was strongly correlated with the dietary P-concentration. Albrektsen *et al.* expressed concern over the low level of bioavailable phosphorous in diets for Atlantic salmon under-yearling smolt based on fish meal from blue whiting. Despite high total content of P provided by the fish meal, levels were too low to support normal growth and development, and carcass P content was at levels previously associated with development of bone deformities in salmon. Thus, phosphorous supplementation has to be reconsidered with respect to P digestibility of the feed ingredients, growth rate and life stages, as well as overall feed compositions. Relevant strategies to improve the utilization of P in fish bones are under work. Diets with marginally low P and Zn contents induced deformities episodically observed in intensive farming of Atlantic salmon, e.g. jaw and vertebrae malformations, curled rib bones and soft bone in the whole skeleton (Helland *et al.*). The study included morphological description of the skeletal deformities resulting from marginally low dietary phosphorus (P) and zinc (Zn) from start feeding to juveniles. The experiment was divided into two periods, including a cross-over from low to high P diet. The combination of feeding low P diet in the first and high P diet in the second period, improved mineralization but deformities from the first period persisted.

Together with phosphorous calcium is a major element in bone minerals. Maintenance of blood and tissue phosphate and calcium ion levels between narrow limits is vital for normal function of physiological processes. A multitude of hormones take part in the integrated regulation of calcium, including vitamin D metabolites. Marine feed ingredients are among few that may contain this vitamin in excess. Lock presented the role and molecular action of vitamin D metabolites in bone mineral deposition, mediated through two vitamin D receptors (VDR). Tissues sensitive to changes in calcium availability, including gut, kidney and gills were identified by *in vitro* and *in vivo* techniques. The VDR in Atlantic salmon was cloned and the expression measured using RT-qPCR. Effects of the active vitamin D metabolite 1,25D_3 on bone development were demonstrated in developed primary osteoblast cultures based on fish scales.

Another lipid soluble vitamin often present in excess in fish feed through marine feed ingredients is vitamin A (retinol). In 2004, samples from Norwegian commercial feeds showed a range from 9 to 121 mg vitamin A kg^{-1}, while requirement for salmonid fishes is 0.75 mg VA kg^{-1}. Vitamin A is a potent teratogenic agent and may be related to development of bone deformities. Ørnsrud *et al.* presented a trial where elevated retinol levels were produced through micro-injection of retinol in salmon eggs. Elevated egg retinol levels did not influence vitamin A homeostasis or early skeletogenesis in the developing salmon embryo, as evaluated by gene expression of *cyp26* or *sonic hedgehog*, respectively, nor did morphological examinations of the developing spinal column reveal any signs of pathological development.

Larssen and Djupvik presented a statistical analysis from a large scale collection of data from ten slaughteries, representing a total of 268 randomly selected net pens of salmons from 36 localities along the coast from Hordaland to Nordland. One of eight main reasons for downgrading was "Spinal malformation". Data from the net pens included breed stock, hatchery, vaccination, and other early life conditions of salmon hatched in 2000 and put to sea in 2000 as under-yearling or in 2001 as yearling smolt. The final model explained about 50% of the total variation and indicated that a) hatching- and start feeding locality, b) smolt type, c) vaccine type and d) vaccination period were significantly associated with spinal malformation. Despite uncertainties, the associations clearly indicate that the risk of spinal malformation to a large extent is determined by conditions in early life.

The genetic variation for a vertebral deformity in Atlantic salmon was reviewed by Gjerde *et al.* The average incidence of 44684 offspring from 225 sires and 471 dams of Atlantic salmon from four different year-classes with vertebral deformations was 9.5, 7.6, 21.5 and 2.3 %. Water temperature during egg incubation could not explain these incidence differences among the year-classes. The heritability for deformity showed that spinal deformities were determined by a substantial additive genetic component. The genetic correlations of deformity with body weight and length were negative, indicating that high genetic growth potential was not the cause of the deformity. The correlation between the coefficient of inbreeding and the breeding values for deformity of full-sib families indicated further that inbreeding did not cause the deformity problems. In view of these results, it is recommended not to select breeders showing deformities themselves or from families with high incidence of deformed fish. These recommendations may not reduce the development of deformities itself, but will prevent a potential increase in the genetic susceptibility to vertebral deformities in the population.

Storset *et al* presented the Aqua Gen AS family breeding program of Atlantic salmon and rainbow trout, which is based on recordings of 11 traits, of which 9 are included in the breeding goal. Recording of the occurrence of bone deformities started with the year-class 1992. Routinely, deformity scores are assigned to test fish after 15 – 16 months in sea cages based on visual examination of live fish and include classes as humpbacks, shortened tail region and angular displacement of the lower jaw. The overall prevalence varied from 2.8 to 21.5 % between year-classes. A genetic study with two wild and four commercial strains of Atlantic salmon was presented. Twelve family groups from each strain were kept separately in the same hatchery from fertilization until tagging. After tagging (5-20 gram) the fish were mixed and the mixed population was divided and reared at three sea sites, in the south, north and middle part of Norway through the on-growing period. This study showed that bone deformity was observed in all strains, including the wild strains. In line with the recommendation by Gjerde *et al.,* Aqua Gen families with breeding values below 80 are excluded from further breeding. Since vertebral deformity is positively correlated with condition factor, Aqua Gen selects indirectly against spinal deformity by selecting against high condition factor.

Hyperthermia during egg incubation has been shown to induce malformations in Atlantic salmon as in other species. Molecular mechanisms involved in development of deformities during embryogenesis due to heat shock were investigated by Harald Takle. Hyperthermia induces apoptosis and up-regulates the gene expression of e.g. executioner caspases and atrial natriuretic peptide. Specific expression was found in embryonic tissue that can be related to development of deformities: neural tissue, pharyngeal arches, somites and heart. This correlates well with the deformities that can be found in fish that has been exposed to high rearing temperatures during embryogenesis.

III) Field experiences from the industry

The intention of this session was to obtain an overview of the present situation of bone deformity in aquaculture.

Djupvik et al. attempted to get an outline of the prevalence of bone deformities in the salmon industry and the economic impact this causes. Salmon from 36 sea sites from Hardanger to Helgeland were investigated at slaughter. Under-yearling and yearling salmon of the 2000 year class (00G) were compared with 93G. An overall prevalence of 7.5 % of skeletal deformities was found in the 00G, with the highest prevalence in under-yearling groups in southern part of Norway. Economic losses due to deformities are related to downgrading and rejection at slaughterhouse, the latter especially significant in under-yearling groups. In addition, reduced growth performance is seen. In total, the 00G experienced higher economic losses than the 93G. For 00G, losses due to downgrading alone are estimated to 0.30 NOK per kilo salmon slaughtered. Rejection and weight losses have to be added to this sum, and for the under-yearling 03G in southern Norway, total losses due to deformities are estimated to be between 2 and 3 NOK per kilo slaughtered. Skeletal deformities were regarded as probably being one of the most serious economic loss factors in the sea site production of farmed salmon, particularly for under-yearling smolt in Western Norway.

Carl-Erik Arnesen of Firda Seafood reported from his experiences as a producer “still confused”. Their enterprise includes rearing of salmon and rainbow trout in all life stages until slaughter and has implemented rearing advice from research to avoid bone deformities. A significant decrease in the amount of “short-tails” has been achieved the last years by:
1) Being more careful with temperature in freshwater, 2) having less biomass in the freshwater sites, 3) X-raying pre-smolts and 4) tougher grading of the fish. Nevertheless, spinal deformities still occur periodically in both rainbow trout and Atlantic salmon, typically after sea water transfer.

Similar results from Grieg Seafood were presented by Solveig Nygaard; bone deformities occur periodically and typically after sea water transfer. An outbreak of bone deformities in a generation put to sea in September-December 2003 was possibly linked to a poor vaccine. Three fish groups from different hatcheries, all vaccinated with the same product, displayed both vaccine side effects and bone deformities while a fourth group had received a different vaccine and displayed no vaccine side effects nor bone deformities. All four fish groups were of the same breed and were reared at the same sea location.

Hege Hovland of Cod Culture Norway presented what was the most commonly seen deformities in their facilities; head deformities e.g. in the jaw (gaping, shortened lower jaw), scull (flattened) and nose (shortened) were considered minor problems while deformities in the most cranial truncal vertebral bodies of the vertebral column, “neck bend”, were considered the most prominent deformity. This condition typically appears very early in production, at around 5 g, has negative implications for fish welfare and causes quality downgrading at slaughtering. Although in 2004/2005 considerably less individuals had neck deformities at Cod Culture Norway compared to earlier productions, the problem still persists.

Hovland advertised for better diagnostic tools that could make an early and more accurate diagnosis, preferably at low cost and with a swift reply. To date, the tools that seem most capable of meeting these demands are MR/CT techniques.

Generally, there seems to be a good correlation between the researchers' understanding of the bone deformity problem as perceived by the fish farmer and veterinarians. Similarly, the fish farmers seem to rapidly implement the advice coming from researchers on rearing conditions into their rearing practice. Although proper implementation of handling advice (rearing temperature, vaccination etc), deformities still occur and the unknown factor X remains to be found.

IV) Ethical aspects

Bente Bergersen from the Norwegian Food Safety Authority gave an extensive introduction into the Animal Welfare Act which, among other things, states that: "*Animals shall be treated well, and consideration shall be given to the instinctive behaviour and natural needs of animals, so that there is no risk of causing them unnecessary suffering…*". Furthermore, "*Animals have an inherent value. Care and consideration for their species specific characteristics shall be given when handling animals. This implies that animal's natural needs are to a great extent to be considered, and disease, injuries and pain are to be actively prevented(…) People who keep animals must be knowledgeable about the animal's behavioural needs, about its feed requirements and its social and environmental needs.*"
The implications of the animal welfare act for researchers is that more effort must be put into solving the bone deformity enigma to ensure that proper animal welfare is achieved.

Workshop summary

On day 2, the participants were divided into five groups for discussion on the themes given in *Appendix II*. The groups discussed the topics relative to salmon (3 groups) or cod culture (2 groups). Appointed group leaders and secretaries were informed that they may prioritise among the given topics, but should pay special attention to the second and the last topic. The outcomes of the discussions were presented in plenum. The following chapter represent abstracts of the outcome organised according to fish species and the given themes, even though some groups left the thematic organisation.

I. Salmon culture

1) "Top 10 farming conditions" that are related to development of bone disorders based on research or field experiences

Freshwater (FW) quality

- Hatchery and first feeding conditions
- Tank environment, temperature, feed composition etc in FW
- Supersaturation of oxygen and carbon dioxide levels and fluctuations

Vaccination

- Vaccination time vs temperature, fish size at vaccination, specific growth rate (SGR),etc
- Vaccination lesions (granuloma)
- Fish size at vaccination

Genetics

Conditions in seawater (SW)

- Temperature in SW
- Stressful conditions in SW

Nutrition

- Digestibility and availability of phosphorous in different marine feed raw materials – interactions with other nutrients
- Rapid growth and/or compensatory growth in SW means elevated mineral requirements
- Broodstock nutrition

Handling

- Light regime (under yearlings vs yearlings)
- Stress, feed composition etc at smoltification (especially for under yearlings)
- Handling in SW phase
- Broodstock rearing conditions

2) The following areas related to suggested causation were discussed:

Initiation - when and how?

There was a general agreement that deformities are a complex problem, with no single cause. Standardisation of diagnosis is needed, e.g. by placing deformities into categories such as:

1. platyspondylia (shortened vertebrae)
2. fused vertebrae (organised and unorganised)
3. deviations in single vertebrae (increased mineralization and X-ray density)
4. abnormal vertebral structure
5. stargazer
6. lateral caudal fold (Z-tail)
7. calcipenia

Severity varies. Cases observed in 1993-94 and 1998 were dominated by platyspondylia. This was also observed in other species (saithe) in same cages, which pointed to a nutritional origin. There is, however a more complex situation today. Initiation can occur at different life stages, and may have different causes. Critical phases are embryonal/yolk sac to first feeding stages, first feeding to smoltification and the SW phase.

Developmental disorder or physical compression?

There seems to be separate pathogenesis patterns, although the general opinion was that compression of vertebra can only occur following developmental aberration.

Growth relation - rapid imbalanced growth or erroneous growth?

Selection for rapid growth may affect nutritional requirements.

Relation of nutrition to bone and connective tissue development, growth, strength or regulation?

Several nutritional areas of concern were suggested:

1. Phosphorous (P) deficiency and incorrect Ca:P ratio in feed at different stages (especially underyearlings).
2. More knowledge needed on requirements of P and other minerals in relation to bone mineralization and growth rate in underyearling smolt.
3. Mineral digestibility and availability of different feed raw materials.
4. Interactions between marine and vegetable raw materials
5. Physiological and biochemical Ca:P regulation mechanisms

3) Diagnostic tools/new technologies in research

The participants suggested developing reliable models, classification criteria and use of alternative technologies in describing the deformities:

1. *Experimental models* for development of bone deformities needed in order to study causal effects
2. Nutritional causes
3. Vaccine associated causes
4. Teratogenetic effects

5. develop a standard for classification of deformities and registration of production data
6. 3-D image analysis techniques; X-ray microanalysis; Scanning electron microscopy; transmisssion electron microscopy; ultrasound analysis, mammography

4) Practical strategies to reduce the incidence and seriousness of bone disorders on short and long term

Please discuss their responsibilities and who should take the leading role.

Aquaculture related industry (farming, feed, equipment, pharmaceutical…) has prime responsibility and must contribute more than today. Co-operation with research institutions needed.

The Food Safety authorities ("Mattilsynet") must put more pressure on industry to enforce legislation.

Funding bodies – Research council/research fundation, Ministries…("Norges forskningsråd", "Fiskeri- og havbruksnæringens forskningsfond", "Departementene") responsible for assisting industry to find solution

5) Prioritised research strategies and research alliances

Basic research (long term) must run in parallel with applied research (problem solving on short term). More basic research is needed.

What is normal variation in bone development in fish? Experience clearly suggests that s*tandardisation* is needed.

Experimental models for development of bone deformities are needed in order to study causal effects (for example teratogenetic, nutritional, and vaccine associated causes).

It is important to seek and use *existing knowledge from other farmed animals,* as well as cooperate with European seabass and seabream industry.

Epidemiological studies and analysis could be performed on existing data, including comparable data from surveillance programmes.

Communication between industry and research
One group suggested establishing a task force involving research and farming sector for:

- Brainstorming
- Define priorities (test factor by factor – then interactions)
- Improve quality standards for diagnosis (X-ray etc.)
- Develop a "best guess" sampling protocol for early detection of deformities
- Need of a review, with recommendations to industry
- Annual workshops, with published precedings

Suggested scientific angles included

- Identify risk factors
- Well performed life cycle experiments
- Seek the mechanisms behind development of bone deformities
- Define growth and factors modulating growth
- Look at *development of bone* from a multiple approach (environmental, nutritional, handling stress, production parameters etc.)
- Study stage and bone specific nutrition

All must contribute and focus on removing barriers and improve cooperation

II. Cod culture

1) "Top 10 farming conditions" that are related to development of bone disorders based on research or field experiences

Gas saturation

- Total gas saturation important (O_2, CO_2, N_2), supersaturation of single gas species probably not problematic
- Variation in total gas saturation is a negative factor

Temperature

- Optimal egg and larval rearing temperatures are of utmost importance

Salinity

Hydrodynamics

- High swimming strain in larvae and juvenile stages may affect bone development

Nutrition

- Optimal nutrition important in both broodfish and its progeny but advances within cod nutrition are often considered industrial secrets and not readily shared with others

Microbiology

- Infections in gut and swim bladder may affect bone development

Surface skimming

- Removal of surface film important for proper swim bladder filling

Stocking density

- Proper density of both food and fish important

Genetics

Water quality

- Removal of excretion products such as ammonium and control of gas saturation important

Handling

- All kinds of stress imposed on the fish may have a negative impact on bone development

Growth rate

Photoperiod

Vaccination

2) The following areas related to suggested causation were discussed:

Initiation - when and how?

- Deformity may be caused by events early in development but expressed at a later stage, need for long term experiments since this issue is not solved

Developmental disorder or physical compression?

- Probably both

Growth relation - rapid imbalanced growth or erroneous growth?

- Certainly erroneous, probably both NB! Look at the whole fish

Relation of nutrition to bone and connective tissue development, growth, strength or regulation?

- Need to establish more knowledge on the normal biology of cod (E.g. cod has acellular bone, as opposed to salmon)

3) Diagnostic tools/new technologies in research

- Morphometry through use of x-ray, ultrasound microscopy or other image techniques is a valuable tool that quickly establishes pathologic conditions
- mRNA quantification techniques are valuable tools but involves high cost and requires expertise, results must also be interpreted with care since they do not directly relate to functional protein
- Cell cultures may be an interesting approach but requires highly skilled personell and is primarily useful when studying specific mechanisms
- Histology an important tool

4) Practical strategies to reduce the incidence and seriousness of bone disorders on short and long term

Please discuss their responsibilities and who should take the leading role.

- The Norwegian Research Council should take the leading role in developing a strategy to reduce bone disorders through organising a group that should coordinate the plans for further research
- The industry should invest more in research and development of proper farming conditions
- Innovation Norway helped build the cod industry and should also invest in establishing a strategy for disease prevention

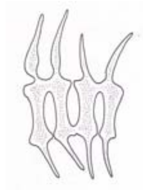
- Food authorities should be kept informed about new findings

5) Prioritised research strategies and research alliances

- Research funding should allow for more projects lasting 4-5 years rather than 2-3 years
- Research should be performed in cooperation with other research groups and industry to ensure that duplicate research is avoided and that new knowledge is shared
- Cooperation with groups doing research on other species than fish could be valuable

Appendix I – Abstracts

Bone disorder workshop May 10-11, NIFES Bergen



Title: Skeletal tissues in Teleost Fish: Origin, Growth and Remodeling

Author(s): Ann Huysseune[1] and P. Eckhard Witten[2]
Institution(s): [1]Ghent University (Bel), [2]University of Hamburg (Ger) & Dalhousie University (Can)

Abstract:
Bony fish possess an astounding variety of skeletal tissues, by far exceeding the diversity of tissue types that can be found in mammals. Some types of skeletal tissues are ancient, some evolved anew in the lineage of advanced teleosts. Many skeletal tissues (and their cells) don't fall into one of the classical textbook categories of cartilage, bone, enamel and dentine. From a developmental and structural viewpoint, several skeletal tissues display intermediate characters, for example intermediate between enamel and dentine, between dentine and bone, between bone and cartilage or between cartilage and connective tissue (1,2). Intermediate tissues and cell types are a naturally occurring feature in the skeleton of teleosts, but they can also occur under pathological conditions, thereby complicating the diagnosis of skeletal disorders.
Formation and growth of the teleost skeleton involves a variety of skeletal forming cells: chondroblasts, osteoblasts, ameloblasts and odontoblasts (1,3). Unlike in other vertebrate groups, especially mammals, bone and cartilage formation in teleosts commonly proceeds along alternative pathways, resulting in the formation of chondroid bone, the reduced role of endochondral bone formation, and the development of acellular bone (bone without osteocytes) in advanced teleosts (1,2).
Remodeling (resorption and new formation) of the teleost skeleton occurs lifelong, to facilitate growth, tooth replacement, mechanical adaptation, repair of the skeleton and to mobilise minerals (1,4). Depending on the animals' phylogenetic position and its bone type, resorption is predominantly conducted by mammalian-like multinucleated giant cells (basal teleosts with osteocyte-containing bone) or by inconspicuous, small mononucleated cells (advanced teleosts with acellular bone). The early appearance of multinucleated bone resorbing cells in basal teleosts suggests that their skeleton undergoes more remodeling than the skeleton of advanced teleosts (4).
Knowledge of the range of tissues that develop under natural conditions in the teleost skeleton, and of the way they grow and remodel, is essential if we want to use these tissues in the diagnosis of skeletal disorders.

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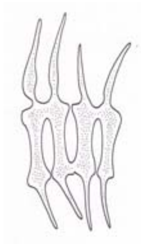

Title: Chondroid bone and metaplastic chondrogenesis: Elements of regular and pathological bone growth in Atlantic Salmon (*Salmo salar*)

Author(s): P. Eckhard Witten[1,2], Laura Gil-Martens[3], Brian K. Hall[2], Ann Huysseune[4], and Alex Obach
Institution(s): Univ. of Hamburg (Ger), [2]Dalhousie Univ. (Can), [3]Nutreco ARC (Nor), [4]Ghent Univ. (Bel)

Abstract:
Skeletal formation and growth in Atlantic salmon follows known pathways, such as cartilage formation (chondrogenesis), intramembranous bone formation, and perichondral/periosteal ossification eventually followed by endochondral ossification. Salmon bone contains osteocytes and, as part of the regular growth process, is remodelled by typical multinucleated bone resorbing cells (1). The latter features are shared with mammals and primitive bony fish but not with advanced teleosts such as tilapia or haddock (2, 3).
Like many other teleosts, salmon can also develop skeletal tissues with characters intermediate between bone and cartilage such as chondroid bone, a mineralised tissue with cartilage-like cells embedded in a bone-like matrix. In many teleosts, chondroid bone is part of normal skeletal growth. It has been suggested that a switch from periosteal bone formation to chondroid bone formation facilitates fast growth (1, 3). In sexually maturing male salmon, e.g., chondroid bone develops on the tip of the lower jaw and has been associated with fast growth of the jaw (ref). Formation of a particular type of skeletal tissue (cartilage, bone, and possibly intermediate skeletal tissues) can be triggered by altered mechanical load and is controlled by down-regulation of the CBFA-1/Runx2 gene and the up-regulation of the Sox9 gene (2).
Besides being part of regular skeletal development in salmon, a switch from periosteal ossification towards the formation of chondroid bone, or even a whole continuum of skeletal tissues, also occurs under pathological conditions, in particular during the pathological compression of salmon vertebrae (4). Compressed vertebrae display shape alterations of their end-plates and changes in the composition of the intervertebral tissue. The spongiosa is unaffected (histological structure and mineral composition are normal) but the growth zones of adjacent vertebrae blend towards the intervertebral space into different types of skeletogenic tissue that replace the notochord. Unaltered central parts of the vertebral bodies suggest that the compression of vertebrae is a late event that relates to a metaplastic shift of osteogenic tissue into chondrogenic tissue in the vertebral growth zone. We propose that an altered mechanical load could have caused this transformation.

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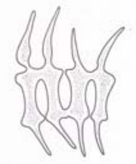
Title: From the notochord to the backbone……………………………

Author(s): Sindre Grotmol, Kari Nordvik, Harald Kryvi and Geir K. Totland
Institution(s): Institute of Biology, University of Bergen……………………..

Abstract:
The backbone of the Atlantic salmon consists of amphicoelous vertebrae and intervertebral ligaments, both of which include notochord-derived structures. As in other teleosts, the notochord develops into a prominent fiberwound hydroskeleton that sustains the locomotion of the larvae after hatching. The notochord comprises a core of epitheloid cells that is enclosed by an acellular fibrous sheath, which consists of a thin external elastic membrane, covering a thicker collagenous layer. The cellular core is composed of an inner tube of chordocytes, each with a large, fluid-filled vacuole. The chordocytes are surrounded by a monolayer of chordoblasts. The chordoblasts rest on a basal lamina, are germinal and produce the notochordal sheath. The first mineralized element of the vertebral body (chordacentrum) forms as an integrated notochordal structure, in which both notochordal cells and the sheath seem to be involved. The ring-shaped chordacentra form through mineralization of preformed matrix within the outer half of the notochordal sheath. The mineralization process in each chordacentrum starts along the ventral midline and proceeds towards the dorsal side of the notochord in a bilateral, symmetrical manner, finally forming a complete mineralized ring. The mineralization process is immediately preceded by segmental expression of alkaline phosphatase (ALP), which is an enzyme associated with bone mineralization, within morphologically distinct bands of chordoblasts. The expression of ALP within the notochord precedes ALP expression by presumed somite-derived cells external to the notochordal sheath. The early expression of ALP indicates that the notochord plays an instructive role in the segmental patterning of the vertebral column by initiating a segmental differentiation of the notochordal sheath into chordacentra and intervertebral regions. The vertebral body that develops around the chordacentra consists of four layers or compartments, two of which are formed through mineralization of preformed collagenous tissue (the notochordal sheath and the intervertebral ligament) and two of which are formed through ossification. The three inner layers have ordered lamellar collagen matrixes, which alternate perpendicularly from layer to layer, while the outer layer consists of cancellous bone with a woven matrix. The bone layers also differ in osteocyte content. Our results show that the vertebral bodies of salmon, unlike those, for example, of for tetrapods, are 'open' structures with no outer delimiting bone elements. The compact bone may thus grow through extension at the rims of the amphicoel, while the cancellous bone may grow by trabecular elongation and branching. Furthermore, the utilization of preformed structures such as the notochordal sheath and the intervertebral ligament, through mineralization, may constitute an efficient and less metabolically demanding mode of growth.

Title: **Rearing conditions and skeletal deformities in Atlantic salmon – What have we learned so far ?**



Author: Grete Baeverfjord
Institution: AKVAFORSK (Institute of Aquaculture Research), Sunndalsøra, Norway

Abstract:

Research on causal relations between rearing conditions and deformities have been a long term priority in AKVAFORSK. In this presentation, the major findings from several research projects will be summarized, with special attention to preventive measures based on present knowledge. The etiology of vertebral deformities is complex, and finding solutions to these problems requires sustained and long term efforts.

Temperature was identified as a highly potent causal factor early on, and egg incubation is a particularly temperature sensitive stage. Adjustments in egg temperatures have led to substantial reductions in some malformations, but skeletal deformities remains to be a problem. A recently completed study on the effect of temperatures in juvenile rearing have demonstrated that rearing temperatures have an impact throughout freshwater rearing, and that temperatures should be controlled, especially in the initial periods of juvenile rearing. The results show that for temperatures >12°C, there is a consistent increase in vertebral fusions. More fish are affected, and the severity of lesions increases. Also, these studies demonstrate that minor lesions in vertebrae induced in early life stages may continue to develop.

The importance of genetic variation has been demonstrated through several studies. No study so far, however, has indicated that genetics is a primary casual factor, but the results indicate that the variation in genetic disposition may modify adverse effects from environmental stressors.

Recent studies have confirmed the relation between dietary mineral supply and skeletal deformities. These results show that in a situation with restricted dietary mineral supply, growth is maintained at the expense of normal bone development, resulting in severe malformations of jaws and vertebrae (to be presented separately).

So far, no single factor related to water quality has been identified as critical to skeletal development. The typical water quality of intensive rearing, with high levels of dissolved CO_2 and low pH, may influence the mineralisation of skeletal structures, but only to a lesser degree. There are, however, indications in several experiments that water quality and rearing conditions typical for intensive freshwater production may still be a critical factor. Ongoing research indicate that excess oxygen supply, i.e. supersaturation of tank water, may impair normal development of vertebrae.

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Title: Effects of environmental factors on the development of deformities in Atlantic cod, Artic cod and Atlantic halibut

Author(s): Ingrid Lein and Lars Thomas Poppe
Institution(s): AKVAFORSK (Institute of Aquaculture Research)

Abstract:
Most of the information about environmental effects on the development of deformities in cold-water species is so far gained from Atlantic salmon and Atlantic halibut. Considering the very different environments of these two species, there are still many similarities between environmental effects in salmon and halibut. The knowledge from these species may be utilized in the work on cod. Factors like water temperature, salinity, and water current etc. are likely to affect the development of normal organs in cod larvae.

Several studies are initiated to gain more information about environmental effects on the development of cod. This presentation reviews results from ongoing experiments at AKVAFORSK on temperature effects on the development of cod and previous results on halibut.

Cod: One group of eggs from each of Artic and Atlantic cod were incubated at three different temperatures (4, 8 and 12°C, 4 replicates) until first feeding and thereafter first fed at 8°C. Another group of Atlantic cod eggs from the same batch as the first experiment were incubated at 4°C and first fed at three different temperature regimes (8°C, 12°C and gradual increase 6-12°C, 4 replicates). There was no significant effect of incubation temperature on survival during egg incubation or frequencies of deformed larvae at hatching and 415 d° post first feeding. When larvae were first fed at different temperature regimes, the highest frequencies of normal larvae was obtain when the temperature was gradually increased from 6 to 12°C compared to 8 and 12°C during the entire first feeding period. Larvae first fed at 12°C showed significantly higher frequencies of skeletal deformities than larvae held at the two other temperature regimes. Oedema was most frequently observed at 8°C and lowest at 12°C. This was the most frequent deformity observed in this experiment. There was no significant difference in growth between the three temperature regimes up to 415 d° post first feeding.

Halibut: Temperatures below 12°C did not affect the survival of halibut larvae during the yolk sac stage compared to 4, 6 or 8°C while the frequencies of deformities like gaping jaws and yolk sac oedema were strongly correlated with temperature. The highest frequency of normal larvae was obtained at the lowest temperature, and the larvae were most susceptible to higher temperatures during the first three weeks post hatching (p.h.). Similar results where found when water flow was introduced at different developmental stages post hatching. Survival rates and frequencies of normal larvae were also affected by the water salinity. Furthermore, it was demonstrated that different tank materials affect the survival and frequency of normal larvae negatively. Skeletal deformities have been demonstrated in metamorphosed halibut, but this type of deformities has yet not gained much attention. It is, however, to be expected that this problem will get more attention as the production of halibut increases. In particular, there is a need for more knowledge about the effect of environmental factors during first feeding and early juvenile stages in halibut.

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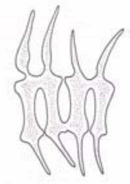
Title: Deformation of the notochord by pressure from the swim bladder may cause malformation of the spine in cultured Atlantic cod *Gadus morhua* larvae: a case study

Author(s): Geir Totland, Harald Kryvi & Sindre Grotmol………………
Institution(s): Institute of Biology, University of Bergen..

Abstract:
This study describes a malformation that frequently occurs in Atlantic cod in intensive culture systems. The malformation is characterised by a slight upward tilt of the head and an indented dorsal body contour at the transition between the head and the trunk, and is first evident to the fish farmer when the cod reach the juvenile stage. These abnormalities are associated with malformations of the neurocranium, the cranial region of the spine and the cranial part of the epaxial lateral musculature. The pathogenesis involves deformation of the notochord, which can be observed in larvae about seven days post hatching (dph) and onwards. The deformation consists of an increase in dorsal curvature of the notochord in the region above the swim bladder. In the same region, the notochord has an abnormal cross-sectional outline, characterised by a groove-shaped, longitudinal impression along the ventral surface of the sheath. In most cases, the swim bladder fills the impression, and in severely affected larvae, it nearly penetrates the notochord. The deformation of the notochord seems to be conveyed to the vertebral body anlage (chordacentra), which in teleosts are formed within the notochordal sheath. The vertebral bodies adopt an abnormal wedge shape with ventral concavity, and S-shaped neural arches are also frequently observed. A continuous range of degrees of severity of the malformation can be observed. All of these pathomorphological characteristics are compatible with the notion that the notochord has been subjected to an upward mechanical force, probably generated by a persistent increase in pressure between the swim bladder and the notochord during the period of development of the vertebral anlage. Chronic over-inflation of the swim bladder or pathological dilatation of the digestive tract may cause the lesions. Multiple factors related to the function of the swim bladder and/or digestive tract during the larval stage may thus induce this spinal malformation. Our results indicate that the critical time window with regard to development of the malformation is from 18 to 36 dph when the initial formation of the vertebrae takes place.



Title: Normal and pathological growth of the vertebral column in Atlantic salmon, *Salmo salar* L.

Author(s): Per Gunnar Fjelldal[1], Arne Berg[1], Sindre Grotmol[2], Geir Kåre Totland[2], Ulla Nordgarden[1], Wargelius[1] and Tom Hansen[1]
Institution(s): [1] Institute of Marine Research, [2] Institute of Biology, University of Bergen

Abstract:
In Atlantic salmon, vertebral column deformations may develop during different life stages, have multi-factorial origins, and are expressed in different regions of the vertebral column. To examine the development and pathology of vertebral column deformations in Atlantic salmon, we studied individually tagged yearling Atlantic salmon smolts reared at a natural photoperiod both in fresh and saltwater. All fish were radiographed in the winter before transfer to seawater, reared on until slaughter size when they were sacrificed and radiographed. Several of the vertebral column deformations found at slaughter were also detected on radiographs taken in freshwater, though the severity of the deformations had increased over time. However, deformations (platyspondyly) located in the tail region of the vertebral column were not seen on radiographs taken in freshwater, and may thus have developed during the smoltification or seawater stages. Platyspondyly in the tail region had a higher prevalence in males, which had a small size at transfer to seawater.
The normal growth of the vertebral column has been studied in several experiments, which have elucidated that the vertebral column of the Atlantic salmon display regional growth. During smoltification the vertebral column display increased longitudinal growth in the caudal part, whereas the longitudinal growth in the trunkal part is strongly influenced by photoperiod in seawater. These results show that in the vertebral column of Atlantic salmon, growth may be differentially regulated in various regions, and that photoperiod influence the pattern of growth.
In Atlantic salmon, certain aspects of the quality of the vertebral column such as the mineral rate and the mechanical properties of the vertebrae are affected by rapid growth. These differences are especially evident when comparing under-yearling and yearling smolt. This may imply that rapid growing fish are of a higher risk of developing vertebral column deformations.

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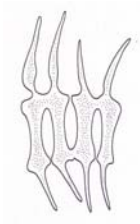

Title: Molecular dynamics of normal and light manipulated bone growth in under-yearling post-smolt Atlantic salmon (*Salmo salar* L.).

Author(s): Anna Wargelius, Per Gunnar Fjelldal, Ulla Nordgarden, Arne Berg and Tom Hansen
Institution(s): Institute of Marine Research

Abstract:
Fish in aquaculture often display skeletal deformities. Induction of fish bone deformities have been linked to development, inflammatory responses and conditions of fast growth. Fast growth in fish can be induced by several factors like light, temperature and feed manipulation. To induce differences in growth in Atlantic salmon, continuous light was given to under-yearling post-smolt Atlantic salmon during spring growth. In which one group of fish was given continuous light (LL) from late January until June while another group was given natural light (NL). To study the effect on bone, vertebrae were sampled once a month between January and June. To avoid regional growth differences, vertebrae were picked at the same location in every fish. Interestingly, the mineral rate dropped significantly after one week of continuous light. This effect seemed to be linked to a significant increase in non-mineral matrix content of extracellular matrix (ECM) and not to a decreased mineralisation of bone. Also both dorso-ventral diameter and lateral diameter increased and this is especially interesting since lateral diameter can be a measure of trabecular bone. This may imply that continuous light increase the incorporation of non-mineral matrix in the trabecular bone. At this stage we measured gene expression of molecular markers involved in proliferation (*sonic hedgehog*, *shh*), mineralisation (*alkaline phosphatase*, *alp*), mineral binding (*matrix gla protein*, *mgla*) and structural properties (*collagen I*, *col I*) in bone, but non of our markers displayed a response after one week of constant light. However after 5 weeks the NL group displayed a significantly higher proliferation detected by a peak in *shh* expression, which implies that cell proliferation is affected by light manipulation. Interestingly the growth of the NL group was characterized by two phases. In the NL group, the bone of the vertebrae displayed a phase of cell recruitment between February and March, and an increase in ECM mineralization between April and June.

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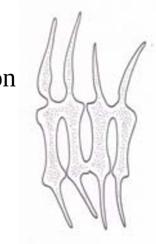

Title: Impact of high water carbon dioxide levels on Atlantic Salmon smolts (Salmo salar) : Effects on fish performance, vertebrae composition and structure

Author(s): Laura Gil Martens[1], P.Eckhard Witten[2,3], Sveinung Fivelstad[4], Ann Huysseune[5] and Alex Obach[1]

Institution(s): [1]Nutreco ARC (Nor), [2]Univ. of Hamburg (Ger), [3]Dalhousie Univ. (Can), [4]Bergen College of Engineering (Nor), [5]Ghent Univ. (Bel)

Abstract:

In recent years and due to the fast growth of the industry, the need for higher numbers of smolts has resulted in a significant increase in fish densities in the tanks during the freshwater period. This has been achieved by the addition of O_2 to the water and has resulted in a significant increase in the concentration of CO_2 reaching levels up to 40 mg/l CO_2. When fish are exposed to high levels of CO_2 for long periods, blood CO_2 will increase (hypercapnia) and blood pH will decrease resulting in respiratory acidosis (1,2). Fish compensate acidosis by increasing the plasma bicarbonate levels, excreting phosphate via the kidney (3) and mobilising ions from the bones (4).

The role of high CO_2 levels on fish performance, on bone structure and composition and as a potential cause of spinal deformities was studied. Two groups of fish were exposed to a low (control) and a high level of CO_2 for 134 days during the freshwater period. After smoltification, the fish were transferred to seawater and followed up for 517 days until they reached harvest weight (3.1 kg BW).

Differences in body weight between the control and the high CO_2 group were observed at the end of the freshwater period. Average weight in the group exposed to high CO_2 levels was 20.9% lower than in the control group. Specific growth rates (SGR) from the start of the experiment (10 g BW) to smolt stage were 1.63 and 1.36 for the control group and the high CO_2 group, respectively. Differences in body weight were kept during the initial stages of the seawater period, but were not observed at harvest weight. Nephrocalcinosis was not observed in any of the experimental groups at the end of the freshwater period. At smolt stage, X-rays revealed mild abnormalities in some vertebrae bodies, which could not be related to any experimental group. Despite the lack of signs of pathological bone alterations, the histological examination showed that the exposure to high CO_2 levels resulted in an increase in trabeculae volume and a higher rate of bone remodelling at the end of the freshwater period. This was reflected by a higher bone ash content in fish exposed to a high CO_2 level. These differences could not be observed at the end of the grow-out period. No external signs of spinal deformities were observed either at smolt stage or at harvest weight.

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Title: Vaccination strategy influence growth of the vertebral column
Author(s): Arne Berg, Per Gunnar Fjelldal and Tom Hansen
Institution(s): Institute of Marine Reseach

Abstract:
In two experiments we have shown that the vaccination strategy influences on the incidence of deformed vertebrae in Atlantic salmon. In the first experiment 1+ smolt vaccinated 8 months before transfer to seawater had more than 30% deformed vertebrae at slaughter. This group were vaccinated on a small fish size and on high temperature, and they developed most adhesions, and had the lowest growth rate. Fish vaccinated during spring, and the unvaccinated control, had less than 7% deformed vertebrae.

In the second experiment two vaccines were used. The vaccine group with most adherences, had the lowest growth rate and the highest incidence of “short tails”.

In other vaccination experiments where temperature, fish size, photoperiod or vaccine formulation has been used as experimental factor, the incidence of deformed vertebrae has not been influenced. However, mineral rate and vertebra strength increase after vaccination. Vertebrae morphology or intervertebrae space, examined on X-ray, has also been influenced in these experiments.

This show that vaccination strategy influence on the growth of the vertebral column, and that in some cases this may result in deformed vertebrae. So far the the mechanisms involved are unknown.

These experiments is part of the industrial project “Optimal vaccination strategy”, funded by Intervet Norbio AS.

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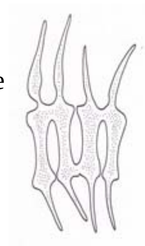

Title: Effects of dietary phosphorus concentrations on performance and mineralization in different life stages of atlantic salmon (*Salmo salar* L.)

Author(s): W. Koppe[1], M. Rodehutscord[2], P. E. Witten[3], Z. Gregus[1], and A. Obach[1]

Institution(s): [1] Nutreco Aquaculture Research Centre, [2] Institute of Nutritional Sciences, University of Halle-Wittenberg (Ger), [3]Univ. of Hamburg (Ger),/Dalhousie Univ. (Can)

Abstract:
This study was designed to evaluate the effects of varying dietary P-concentrations on performance, and bone mineralization in Atlantic salmon in freshwater and in seawater.
Two trials were conducted in freshwater (FW) and seawater (SW) with fish of 1 g (fry) and 270 g (post-smolts) initial body mass, respectively.
FW: Twelve diets were fed, ranging from 7g to 20g/kg in P-concentration. Diets were fed in excess of satiation to fry for 74 days. Finally the whole fish were analysed for their mineral content and bone microstructure.
SW: Twelve diets ranged from 7g to 16g/kg in P-concentration. Diets were fed to controlled satiation to post-smolts for 110 days and subsequently whole fish and skeletal tissues were analysed for their mineral content.
In both trials, the dietary P-content had no effect on the animals' growth performance and feed utilization and the mortality was low in all groups. No external malformations were observed, in agreement with histological analysis that revealed no negative effect on the animals' skeletal development. However, the mineralization in different body compartments was strongly correlated with the dietary P-concentration. Estimates of the requirement of dietary phosphorus for the different life stages will be presented.

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Title: Bioavailable P in fish meal formulated feed to Atlantic salmon smolt
Author(s): …Albrektsen S.; Hope B. and Aksnes A………………………
Institution(s): …Fiskeriforskning……………………….…………………..

Abstract:

Blue whiting (*Micromesistius poutassou*) is a small, lean cod fish of great importance to the Norwegian fish meal industry, accounting for 90 % of the raw material for commercial fish meal production in 2004. Fish meal produced from blue whiting generates lower growth and feed efficiency in salmon, halibut and mink, as compared to fish meal produced from other industrial fish species. The Norwegian fish meal industry has for several years supported research to improve the biological value of fish meals produced from blue whiting.

A regression model was applied to study effects of increased dietary P levels on growth and feed efficiency, and on the digestibility, retention values and deposition of P in the tissues of Atlantic salmon smolt (120 g). The fish were fed a practical fish meal formulated diet with graded dietary inclusion levels of inorganic P; 0, 0.3, 0.6, 0.9, 1.2 and 1.5 %. Within a feeding period of 12 weeks, classical signs of phosphorus deficiency developed in the fish. Deficiency signs included reduced growth and feed efficiency, lipid deposition in the body and reduced mineralization in carcass and bone. Supplementation of soluble P-salts in the diet (0.3 %) significantly improved the production results with respect to growth and feed efficiency, as well as the chemical composition of fish. A higher supplementation level of minimum 0.6 % inorganic P was required for optimal mineralization in carcass and bone. The highest supplementation levels (1.2 and 1.5 % inorganic P) seemed to have slightly negative impacts on growth and bone mineralization.
It was concluded that the digestibility of P from blue whiting meal as measured by stripping of faeces (24 %), was too low to support adequate amounts of available P for normal growth and development, despite high dietary content of P provided by the fish meal. Carcass P content was reduced to a level that has previously been associated with development of bone deformities in salmon. Further, fish fed the non-supplemented diet showed reduced body lengths and increased condition-factors, suggesting that the low availability of P from blue whiting meal might be limiting for normal skeletal development. Result suggests that the requirement for P supplementation in feed for Atlantic salmon has to be reconsidered with respect to variable P digestibility of marine feed ingredients, different growth rates and life stages as well as feed compositions.

Results from the trial with respect to growth and feed efficiency, P digestibility, as well as the retention values and deposition of P in the tissues of Atlantic salmon smolt will be presented. Practical implications to the feed producers and relevant strategies to improve the utilization of P in fish bones will be discussed.

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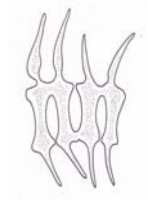

Title: Morphological description of skeletal deformities in Atlantic salmon subject to restricted mineral supply

Author(s): Synnøve Helland[1], Ståle Refstie[1,2], Kirsti Hjelde[1], Grete Baeverfjord[1]
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Abstract:
Intensive culture of Atlantic salmon (*Salmo salar*) has periodically resulted in high incidence of jaw, tail and backbone malformations. The present work gives the morphological description of the skeletal deformities resulting from marginally low dietary phosphorus (P) and zinc (Zn) from start feeding to juveniles. Salmon were fed three diet series, which had High P, Low P and Low P + Zn. The fish were fed these diets from start feeding to 20g, and at 20g the fish were individually tagged, mixed, and redistributed to tanks fed either High P or Low P. In order to study deformities in the fish we used photographs, X-ray images, and staining of bone and cartilage with Alizarin Red and Alcian Blue. We observed upper and lower jaw malformations, small, fused and malformed vertebra, curled rib bones, malformed tails, and generally soft bones in the whole skeleton. Salmon juveniles that were fed the High P diet in both periods showed few malformations. Fish that were fed the Low P diet in both periods showed a higher number of deformities and frequently multiple deformities were observed in the same fish. In fish that were fed the Low P diet in the first period and High P diet in the second period, the bones became mineralised, however, the malformations from the first period persisted. In the present study, diets with marginally low P and Zn contents induced deformities episodically observed in intensive farming of Atlantic salmon

Bone disorder workshop May 10-11, NIFES Bergen

Title: Vitamin D and calcium homeostasis

Author(s): E.J.R. Lock
Institution(s): Radboud University Nijmegen

Abstract:
Maintenance of phosphate and calcium ion levels between narrow limits is vital for normal function of the cardiovascular, neural and muscular systems and thus for larval and skeletal development, as well as for body growth, maturation and egg production. Endocrine systems of the brain, pituitary and gonads play key roles in these events and integrate with calcium-regulating hormones.

One of the major calcium (and phosphate) regulating hormones is 1,25-dihydroxyvitamin D_3 (1,25D_3). 1,25D_3 is not only involved in the uptake of calcium and phosphate, it also plays a role in bone development (deposition of calcium phosphates). The actions of 1,25D_3 are mediated by the nuclear Vitamin D receptor (VDR_{nuc}) that regulates gene transcription in over 30 target organs and a putative membrane receptor (VDR_{mem}) that mediates rapid (within seconds to minutes) biological responses.

To determine experimentally the roles of vitamin D in calcium homeostasis, tissues sensitive to changes in calcium availability, including gut, kidney and gills were identified by in vitro and in vivo techniques. The VDR in Atlantic salmon was cloned and the expression measured using RT-qPCR. To determine the effects of 1,25D_3 on bone development a primary osteoblast culture was developed using fish scales.

Bone disorder workshop May 10-11, NIFES Bergen

Title: Elevated retinol levels do not cause spinal deformities in the developing salmon embryo

Author(s): [1]Robin Ørnsrud, [2]Geir Totland & [3]Anna Wargelius
Institution(s): [1]National Institute of Nutrition and Seafood Research (NIFES),
[2] Institute of Biology, University of Bergen, [3] Institute of Marine Research

Abstract:
Hypervitaminosis A is a legitimate concern in salmon aquaculture due to the varying and sometimes high concentrations of vitamin A (VA) in fish meal and fish oil commonly included in formulated diets. In 2004, 21 control samples from different Norwegian feed manufacturers showed an average VA content of 31 ± 28 mg kg^{-1} (mean ± SD) and a maximum and minimum value of 121 and 9 mg kg^{-1}, respectively. Considering that the VA requirement for salmonid fishes has been set to 0.75 mg VA kg^{-1} (2500 IU kg^{-1}), it is conceivable that the varying and high levels of VA in salmon feed may present potentially harmful effects on salmon development and growth.

VA is a potent teratogenic agent, i.e. inducer of deformities during embryonic development. The effect of potentially toxic VA levels on development of salmon embryos and proper growth and maintenance of bone structures was investigated in the present study. The VA status of salmon eggs was elevated by injecting a VA containing oil directly into the developing egg at 42 day degrees after fertilisation using a micromanipulator with a nanolitre injector attached. Three groups of eggs were incubated at 6º until 810 day degrees post-fertilisation; one un-injected control group, one oil-injected control group witout VA and one VA in oil injected group. The retinol level in VA injected eggs was 785 ± 203 ng retinol egg^{-1} compared to 79 ± 5 and 109 ± 7 ng retinol egg^{-1} for the un-injected and oil-injected control groups, respectively. Eggs were sampled at 126, 294 and 810 day degrees. Gene expression of the retinoic acid specific catabolic enzyme CYP26 was used as a biomarker of VA homeostasis and the retinoic acid dependent morphogen sonic hedgehog (*shh*) was used as a biomarker of early skeletogenesis. Morphology of the prospective vertebral column was examined by staining mineralised tissue with alizarin red.
No differences in gene expression of CYP26 or *shh* were found in any groups at 126 or 294 day degrees indicating that elevated retinol levels do not influence VA homeostasis or early skeletogenesis. All three groups displayed normal development of chordacentra, neural and hemal arches at 810 day degrees indicating that elevated retinol levels do not cause malformation of the spine.
Elevated retinol levels in salmon eggs do not seem to cause spinal deformities in the developing salmon embryo

Bone disorder workshop May 10-11, NIFES Bergen

Title: Early life risk indicators of skeletal deformities in salmon at slaughter.

Author(s): [1]Larssen RB and [2]Djupvik HO
Institution(s): [1]Norwegian School of Veterinary Science and [2]Norwegian Food Safety Authority

Abstract:

Seven veterinarians associated with 10 slaughteries scattered along the coast from Hordaland to Nordland collected data from a total of 268 randomly selected net pens of salmons when these were delivered for slaughter. All salmons were graded according to standard procedures. A "random" sample from each pen of slaughtered salmon where subjected for additional classification, and for fish that were downgraded from Superior to Ordinary or Production, the main reason for downgrading was classified in one of eight alternative categories; one of these being "Spinal malformation". Data about breed stock, hatchery, vaccination, and other early life conditions were collected by the same veterinarians. The 268 net pens originated from a total of 36 localities. The study was restricted to salmons hatched in 2000 and put to see in 2000 (0 yearling) or in 2001 (1 yearling).

In the statistical analysis "Log prevalence of Downgrading due to spinal malformation" (LogMAL) was used as response variable, in both univariate and multivariate analysis. The final model(s) can explain about 50% of the total variation; and indicate that the following variables are associated with spinal malformation ("Prev"=Least square mean of LogMAL converted back to original scale): Hatching- and start feeding locality ($P<0.001$), Prev varying from 1 to 38%. Smolt type($P<0.001$), Prev in 0 yearling is 7% vs 4% in 1 yearlings. Vaccine type($P<0.001$), Prev varying from 1 to 33%. Vaccination period($P<0.02$), Prev of 13% in smolt period vs 4% in winter, but only within 0 yearlings.

Caution is important when analysing and interpreting data from observational studies. Other variables can be included to give other plausible models; and the model used does not account for potential problems due to multilevel structure and possible dependency in the data. However, the associations are so strong that they clearly indicate that the risk of spinal malformation to a large extent is determined by conditions in early life i.e. before the sea phase.

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Title: Genetic variation for a vertebral deformity in Atlantic salmon
Author(s): Bjarne Gjerde , Ma. Josefa R. Pante and Grete Baeverfjord
Institution(s): AKVAFORSK.

Abstract:
The incidence of vertebral deformities was observed on a total of 44684 offspring of 225 sires and 471 dams from four different year-classes (hatched in 1992, 1993, 1994 and 1995) of farmed Atlantic salmon. The deformities, observed on ungutted fish at different farms, were classified as humpback anterior or posterior the dorsal fin or as shortened tail. The average incidence of fish with vertebral deformations was 9.5, 7.6, 21.5 and 2.3 percentages in the four year-classes. The different water temparature during egg incubation could not be explained these incidence differences among the year-classes. No significant difference in percentage of deformed fish between immature fish and sexually maturing males and females was observed in two of the year-classes, while in the two other year-classes these differences varied both in sign and magnitude. Deformed fish were in general significantly lighter, shorter and had a higher and more variable condition factor than non-deformed fish within each sex-maturity category. The heritability for deformity (on the underlying liability scale) was 0.36±0.14, 0.22±0.09, 0.25±0.12 and 0.00±0.00 in the four year-classes showing that spinal deformities were determined by a substantial additive genetic component. The genetic correlations of deformity with body weight and length were negative indicating that high genetic growth potential was not the cause of the deformity. The correlation between the coefficient of inbreeding and the breeding values for deformity of full-sib families was -0.22 (P=0.017), -0.10 (P=0.26), 0.16 (P=0.08) and -0.04 (P=0.63) in the four year-classes, indicating that inbreeding did not cause the deformity problems. In view of these results, it is recommended not to select breeders from families with high incidence of deformed fish and, of course, not at all the breeders showing deformities themselves. Such a procedure is not likely to reduce the deformities significantly, but will prevent the increase in the genetic susceptibility to vertebral deformities in the population.

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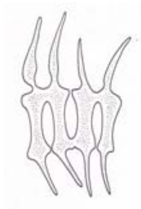

Title: Prevalence of bone disorders in breeding populations and wild strains of Atlantic salmon

Author(s): Arne Storset, Torunn Aasmundstad and Sven Arild Korsvoll
Institution(s): Aqua Gen AS

Abstract: Aqua Gen AS is running a family breeding program for Atlantic salmon and rainbow trout where selection of individual broodfish is based on the performance of full-sibs and half-sibs that have been tested and scored for specific traits. Today calculation of the breeding values is based on recordings of 11 traits, of which 9 are included in the breeding goal. With a generation interval of four years, Aqua Gen's Atlantic salmon breeding operation consists of four subpopulations, all originating from broodfish caught in Norwegian rivers in the early 1970s.

Recording of the occurrence of bone deformities started with the year-class 1992 (denoting first feeding year). Routinely, deformity scores are assigned to test fish after 15 – 16 months in sea cages based on visual examination of live fish. The deformities are classified as humpbacks, shortened tail region and angular displacement of the lower jaw. Some year-classes have also been classified based on number of deformed vertebrae in spinal columns from filleted fish. The first four year-classes have been thoroughly analyzed by Bjarne Gjerde, AKVAFORSK, and this work has been published and will be presented during the Workshop. The subsequent year-classes have been analysed by Aqua Gen's geneticists. Phenotypic variation within and between year-classes will be presented. The overall prevalence varies from 2.8 to 21.5 % between year-classes. The phenotypic variation between families can be large, but only a small proportion of this variation is due additive genetic variance (heritability, h^2). Heritability estimates vary, and ranges between 0.05 - 0.2 (on the observed scale). Due to the fact that deformity score assigned to whole fish is a binary trait, using linear models gives imprecise heritability estimates, especially when the prevalence is low.

Unpublished data from a study of 6 strains of Atlantic salmon, two wild and four commercial will be presented. The latter will be presented as anonymous data. Twelve family groups from each strain were kept separately in the same hatchery from fertilization until tagging. After tagging (5-20 gram) the fish were mixed and reared together. The mixed population was divided and kept on three sea sites in the south, north and middle part of Norway during the ongrowing period. This study showed that bone deformity was observed in all strains including the wild strains, reproduced directly from the rivers Alta and Namsen.

Aqua Gen does not use the estimated breeding values for bone deformity as a selection criterion if it is >80. Below that value families are excluded from further breeding. This is due to the general low heritability estimates and the supposition that the main causative factors are environmental in origin. Individual breeders with external signs of deformities are not reproduced, neither in the breeding nucleus nor in commercial egg production.. Since vertebral deformity is positively correlated with condition factor, and because we select against high condition factor (shape), we are indirectly also selecting against spinal deformity. This scheme is not likely to reduce bone deformities significantly, but the genetic development is under control and the measures taken will at least prevent a deterioration of the genetic makeup of our breeding population as far as skeletal deformities are concerned.

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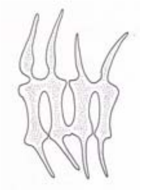

Title: Molecular mechanisms involved in development of deformities in early life stages

Author(s): Harald Takle
Institution(s): AKVAFORSK

Abstract:
Hyperthermia during the embryogenesis has been shown to induce malformations in Atlantic salmon (*Salmo salar*). To examine the involvement of executioner caspases in hyperthermia-induced cell-death in a poikilotherm vertebrate species, five genes encoding *caspase-3A*, *-3B*, *-6A*, *-6B* and *–7* were cloned from Atlantic salmon (*Salmo salar*) and the temporal and spatial expression was studied in thermal stressed salmon embryos. Increased apoptotic activity as evidenced by cleavage of nuclear DNA was shown in salmon embryos incubated at 18-20°C for 84 hrs after acclimatization at 8°C. Hyperthermia-induced activation of the executioner caspases was demonstrated by the increased mRNA levels of *caspase-3B* and *caspase-7* after 12 hr and 54 hr heat exposure as quantified by real-time PCR. Profound up-regulation of *caspase-6A/B* mRNA was first shown at 54 hr and onwards, indicating that this enzyme functions downstream of the other examined caspases. The 2-2.5 fold increase in the mRNA expression of the heat shock protein *Hsp70* gene coincided with the peak mRNA values of the executioner caspases. Whole mount *in situ* hybridization of the salmon embryo identified *caspase-7* mRNA in the lens exclusively, while the expression of *caspase-3B* and *caspase-6A/B* was much more widespread. Of special interest was the hyperthermia-induced *caspase-6A/B* expression in the heart, while the exposed and control embryos showed similar *caspase-6A/B* expression patterns in multiple tissues, including the brain, somites, pharyngeal arches and eye at the completion of somitogenesis. *Caspase-3B* showed a particular high mRNA positive signal in the intestine, while no mRNA was identified in the heart or somites. Altogether, these results shed light on the teratogenic effects of hyperthermia during early embryonic development.

Appendix II - Discussion themes for workshop groups

Bone disorders in intensive aquaculture of salmon and cod – Workshop

Discussion themes for the workshop groups

On day 2, the participants will be divided into four groups for 2 h discussion on the themes given below. The groups will discuss the topics relative to salmon or cod culture. They may prioritise among the given topics, however, the second and final topics are of special interest. The outcome of the discussions will be put down on overheads or PCs for plenum presentations, and included in the report for the Research council.

1. *Please list, and if possible discuss "Top 10 farming conditions" that are related to development of bone disorders based on research or field experiences*

2. *The following areas related to suggested causation are discussed:*
    - Initiation - when and how?
    - Developmental disorder or physical compression?
    - Growth relation - rapid imbalanced growth or erroneous growth?
    - Relation of nutrition to bone and connective tissue development, growth, strength or regulation?

   Please discuss the relevance of these areas and suggest scientific angles to how we should increase our knowledge.

3. *A lot of diagnostic tools/new technologies in research are now available.* Please discuss briefly these tools and how they should be used in future research
    - 3-D image analysis techniques; X-ray microanalysis; Scanning electron microscopy; transmisssion electron microscopy; ultrasound analysis
    - mRNA expression studies
    - cell cultures and cellular/biochemical markers of bone and connective tissues
    - use of early markers in sequential studies
    - new markers/tools to be developed

4. *The industry is dependent on practical strategies to reduce the incidence and seriousness of bone disorders on short and long term.* These are related to the roles and responsibility of
    - Industry (farming, feed, equipment, pharmaceutical…)
    - The Food and feed authorities ("Mattilsynet")
    - Funding bodies – Research council/research fundation, Ministries…(”Norges forskningsråd”, ”Fiskeri- og havbruksnæringens forskningsfond”, ”Departementene”)

   Please discuss their responsibilities and who should take the leading role.

5. *Overall prioritised research strategies and if possible suggest research alliances.* Based on the state of the art, please suggest the way forward to learn more about causation and risk factors. Please list prioritised areas and which institutions that may contribute alone or through concerted actions. This may also involve institutions that are not yet participating in fish bone health research.