FINEFISH Collective Research Project
(*Contract Nº012451*)

Malformations in farmed fish
Guidelines for classification

(*WP2: Standardisation of Environmental Fish Monitoring*)

IV. Atlantic salmon (*Salmo salar*)

Contribution of Partner No12
(AKVAFORSK)
Grete Baeverfjord
Kirsti Hjelde
Synnøve Helland
Ingrid Lein

Atlantic salmon (*Salmo salar*)

The aquaculture production of Atlantic salmon has increased rapidly, from next to nothing in the 1970ies to a big industry of great economic importance. The existence of malformation as a factor causing economic losses was recognized in the mid-1990ies, and since then, this topic has been the subject of a number of studies seeking to find the causes.

In Atlantic salmon, causative factors for spinal deformities have been identified in most life stages from embryonic to harvest size at about 4-5 kg. Also, for several of these causative factors, deformities at harvest can be linked to environmental factors in freshwater, without any visible morphological alterations in the period between. Thus, an examination performed at an early stage is not a guarantee for the final outcome of the fish group.

Diagnostic procedures

X-ray is the most reliable of the diagnostic methods available. External examination is of value only with the most prominent deviations, or else as performed by persons with particular training. Even in these cases, palpation will detect only half of the deviations visible on X-ray. Higher precision can be obtained by filleting and inspection/palpation of the exposed spine. In small fish, whole mount staining with alizarin red and/or alcian blue is an alternative.

The standard diagnostic tool for fish skeletal malformation is a lateral view X-ray, preferably with the right side of the fish down, and the head pointing left.

For fish < 100g, mammography equipment is generally preferable. In small fish, the contrast in images taken with standard equipment will be too low. Access to mammography can normally be obtained in hospitals or specialized clinics for human mammography screening. In fish < 100g, acceptable images can be obtained with standard X-ray setups, like those found in animal clinics. For both options, a skilled radiography technician will be able to improve the output considerably.

Fish radiography

The fish to be radiographed must be properly frozen, fixated or radiographed fresh. This is to avoid the fish going into rigor while lying bent, if this happens it is difficult to straighten it out to make good pictures. Please refer to sampling protocol at the end of this document.

A skilled radiography technician should assist you, and depending on the equipment you might have to try and fail a little to make good pictures. The main issue is to find the radiography dosage that allows you to see all the vertebrae in the cranial part of the back, while you still can see the tail vertebrae. Radiography technicians are trained to lower the dose of radiation to decrease risk for the patient. Since our patients are dead that is not necessary, and you can focus totally on picture quality. You can put many fish in one picture, but it is best to avoid the outer inch of the film frame, as the darkening can differ a little from the rest of the picture.

The dosage is preset in the radiography source, and is decided by mAs and kV. Generally increased mAs give a darker picture while increased kV lower the contrast. If you reduce the kV, you usually have to increase the mAs to get pictures with the same degree of darkening. Good quality pictures of fish skeleton therefore have a relatively low kV and high mAs.

Fish < 100g

To make good quality pictures of small fish, you should preferably use mammography equipment, which can be rented at hospitals or special mammography clinics. This gives pictures with high resolution and good quality.

Dosage might vary a little with the equipment, but the given doses are among those we have used in different equipment:

- Ca 1g: 47 kV, 10 mAs
- Ca 7g: 23 kV, 4 mAs
- Ca 50g: 55 kV, 10 mAs
- Digital system: 22 kV, 50-100 mAs

Film-focus distance is usually fixed in mammography equipment.

Fish > 100g

In fish bigger than 100g, regular radiography equipment found in hospitals and vet clinics usually give satisfying picture quality.

Again, dosage might vary with the equipment, but suggested doses are:

- 200g: 53 kV, 8 mAs
- 2kg: 63 kV, 16 mAs
- Digital system: 32kV, 50 mAs

Film-focus distance is variable, but about 70-80 cm is usually ok.

Whole mount staining with Alizarin red

In fish < 1g, whole mount staining with Alizarin red is the preferred procedure for malformation diagnostics. Samples must be fixed and later processed by a laboratory. For sampling and fixation procedures, please refer to the standardized procedures included at the end of this document.

When to examine

The following stages are recommended in a standardized screening program:

20 g size	Will detect the malformations induced during embryonic development, and those induced during first feeding
Smolt transfer	Status at seawater transfer
1 kg +/-	Time/size is relative, and should be combined with check for vaccination side effects or similar. Will be valid as prediction of results at harvest, even though further development of malformations can be expected as fish continue to grow.

Malformations induced during embryogenesis can probably be detected at any time after first feeding, when examined by appropriate methods, and should be considered if such an aetiology is suspected.

In general, skeletal deformities in Atlantic salmon continue to develop as the fish grows. Thus, the full expression of the deformity problems in a group of fish may only be fully expressed as fish reach harvest size. Early screening of fish groups (pre-smolt screening) may therefore serve as an indicator of early-onset problems, but may still fail to detect problems that are expressed later in the life cycle.

Normal

The Atlantic salmon spine normally consists of 57-60 vertebrae, and there is a certain regional variation in vertebral morphology along the spine. Also, the morphology may be variable in relation to developmental stage and age.

The cranial vertebrae are narrower than those further caudally, that is, the length:width proportion as seen on lateral X-ray is less than 1:1. From the area under the dorsal fin and backwards, this proportion length:width relation should be closer to 1:1. The tail vertebrae are narrower, and complete or partial fusions are not uncommon in this region, also in fish in which the spinal column is otherwise normal. In parr, the vertebrae are in general narrower and more delicate than in smolt and subsequent seawater adapted life stages.

Figure 1. Atlantic salmon with normal spinal columns. 80g salmon parr.

Malformations of the Atlantic salmon skeleton

In the following, the most typical malformations of the spine, head and ribs are illustrated, mainly by means of X-ray images. Wherever available, whole-mount stains and photos of fish are included.

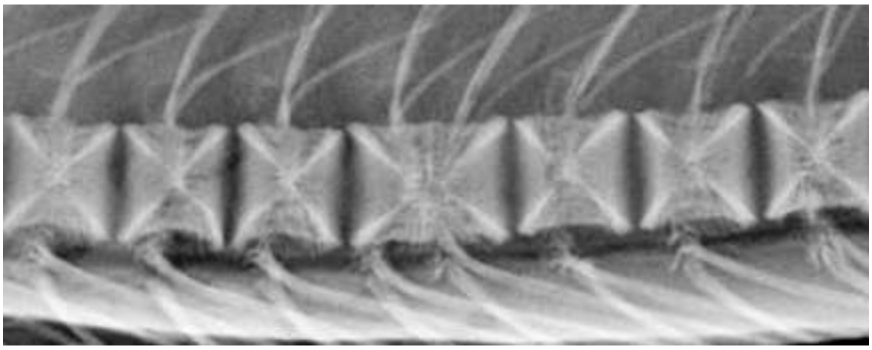
I. Fusions and fusion-associated changes

This section covers pathological changes where two or more vertebrae are more or less completely amalgamated. This is one of the most common types of vertebral pathology in the salmon.

Fusions may develop as vertebral bodies are first formed during embryogenesis. Some of these fusions will organize and appear as a slightly prolonged but otherwise normal vertebral body, identified only by the presence of two spinal arches. These vertebrae may be denoted *block vertebrae*, a common term in mammalian pathology.

Fusions may be induced also in post-embryonic life stages. They exist as simple fusions, involving two vertebral bodies, and complex fusions, involving more than two vertebrae. They can also be classified as complete fusions, in which the vertebral centra are fused, or incomplete, in which the vertebral bodies are still separate, but the endplates are more or less flattened and the intervertebral space may be reduced. The following terminology is suggested:

Simple fusion:	Fusion of two vertebrae, with vertebral centra coalesced
Complex fusion:	Fusion process involving more than two vertebral bodies
Multiple fusions:	More than one fusion centra separated by normal vertebrae
Complete fusion:	Fusion of two or more vertebral bodies, in which vertebral centra are amalgamated
Incomplete fusion:	A fusion process involving two or more vertebrae, in which vertebral bodies are more or less separate, but vertebral endplates of adjoining vertebrae are flattened. Intervertebral space may be reduced or absent, indicating that the process is moving towards a complete fusion.



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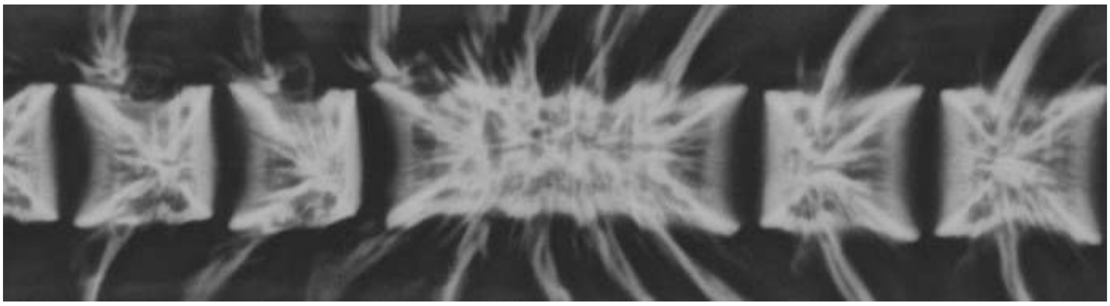

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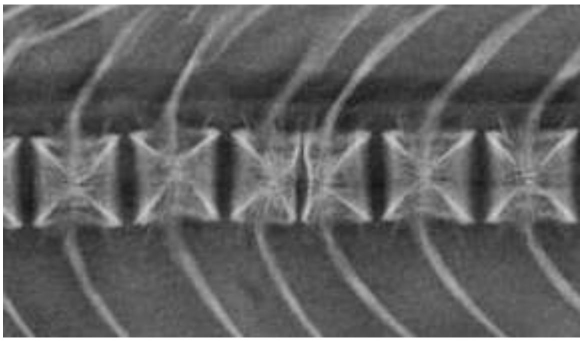

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Figure 2 . Common types of fusions in the spine of Atlantic salmon. a) simple, complete fusion, b) complex complete fusion, c) incomplete fusion

In a typical lesion, you will find a complete fusion at the centre, with one or more vertebrae on each side with distorted symmetry and flattened endplates. This can be taken as indicators that the process is active and that a further involvement of the adjoining vertebrae is in progress. Thus, a process like that should be counted as one lesion.

The number of vertebrae involved in a fusion process is normally a practical indicator of the severity of the lesion. Thus, in diagnostics, it is suggested to report the number of affected vertebrae per individual in addition to reporting presence of fusions. When recording number of affected vertebrae, do include both completely and incompletely fused vertebrae.

Time of onset

Fusions can be induced at any time during freshwater rearing, from egg incubation and until seawater transfer. Experimental results indicate that the juveniles may be most susceptible in early life stages, i.e. from first feeding and onwards, but there is no point at which the susceptibility comes to an end. Likewise, it is assumed that new fusions can be induced also during seawater rearing, although the evidence for this is insufficient at this stage.

Development of lesions with time

Fusion processes can be contained and result in a reorganized block vertebra with no indication of further development.

The most common course is, however, that the process continues to develop with time.

Some intermediate stages are illustrated by this figure, from Witten et al 2006:

Fish #1 Fish #2 Fish #3

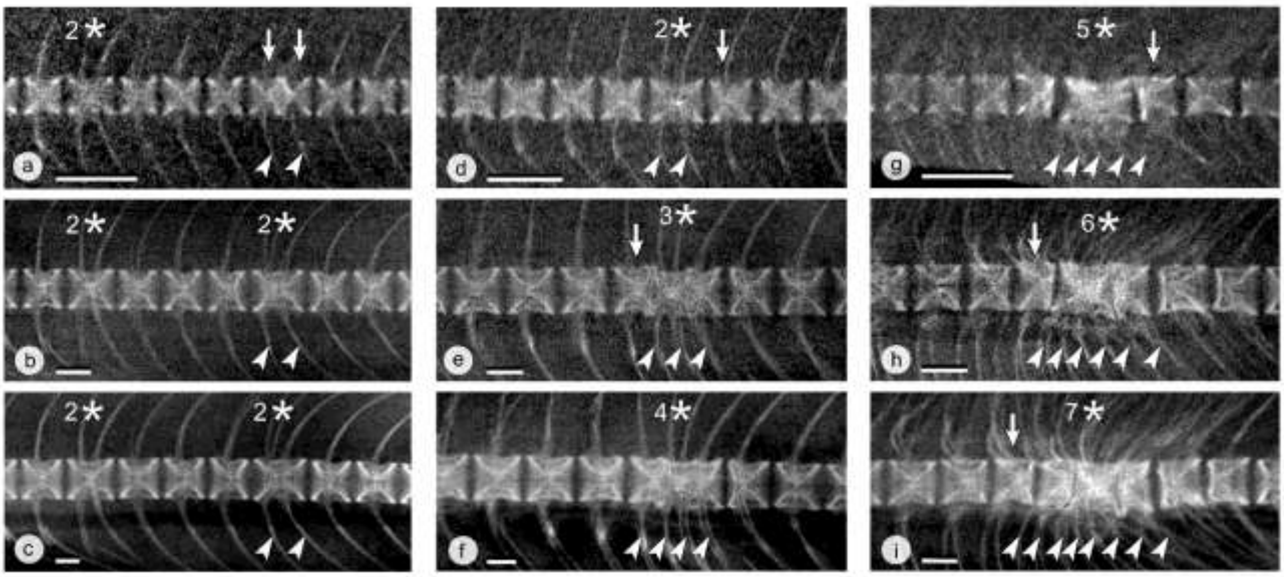


Figure 3. Development of vertebrae fusion in three different animals (animal one: a –c, animal two: d – f, animal three: g – i). Vertebral columns from each animal are shown in the same size at different scales to visualise shape alterations, scale bars equal 500µm. White stars indicate fused vertebrae, the number in front of the star refers to the number of fused vertebrae. White arrows label vertebrae in the process of fusion. White arrowheads label haemal arches. The X-rays represent the animals at pre-smolt stage (a, d, g), six months after seawater transfer (b, e, h), and 12 months after seawater transfer (c, f, i). X-rays of pre-smolts (a, d, g) show that animals of the same age group display different degrees of vertebral body fusion ranging from the starting fusion (a), fully fused and largely remodelled vertebrae (d), to multiple vertebrae fusion with amalgamation of adjacent vertebral bodies (g). Seawater stages show fused and completely remodelled vertebrae that became a regular part of the

spine (b - c left), the almost complete reshaping of fused vertebrae (b - c right), and the failure of reshaping with continuing incorporation of adjacent vertebral bodies (e, f and h, i).

From: Witten, P.E., Obach, A., Gil-Martens, L. and Baeverfjord, G. , 2006. Vertebrae fusion in Atlantic salmon (Salmo salar): Development, aggravation and pathways of containment. Aquaculture, 258, 164-172

Gross pathology

The gross morphology of salmon with fusions in the spinal column depends on the severity of the lesion and the location of the fusion. Some examples are shown below.

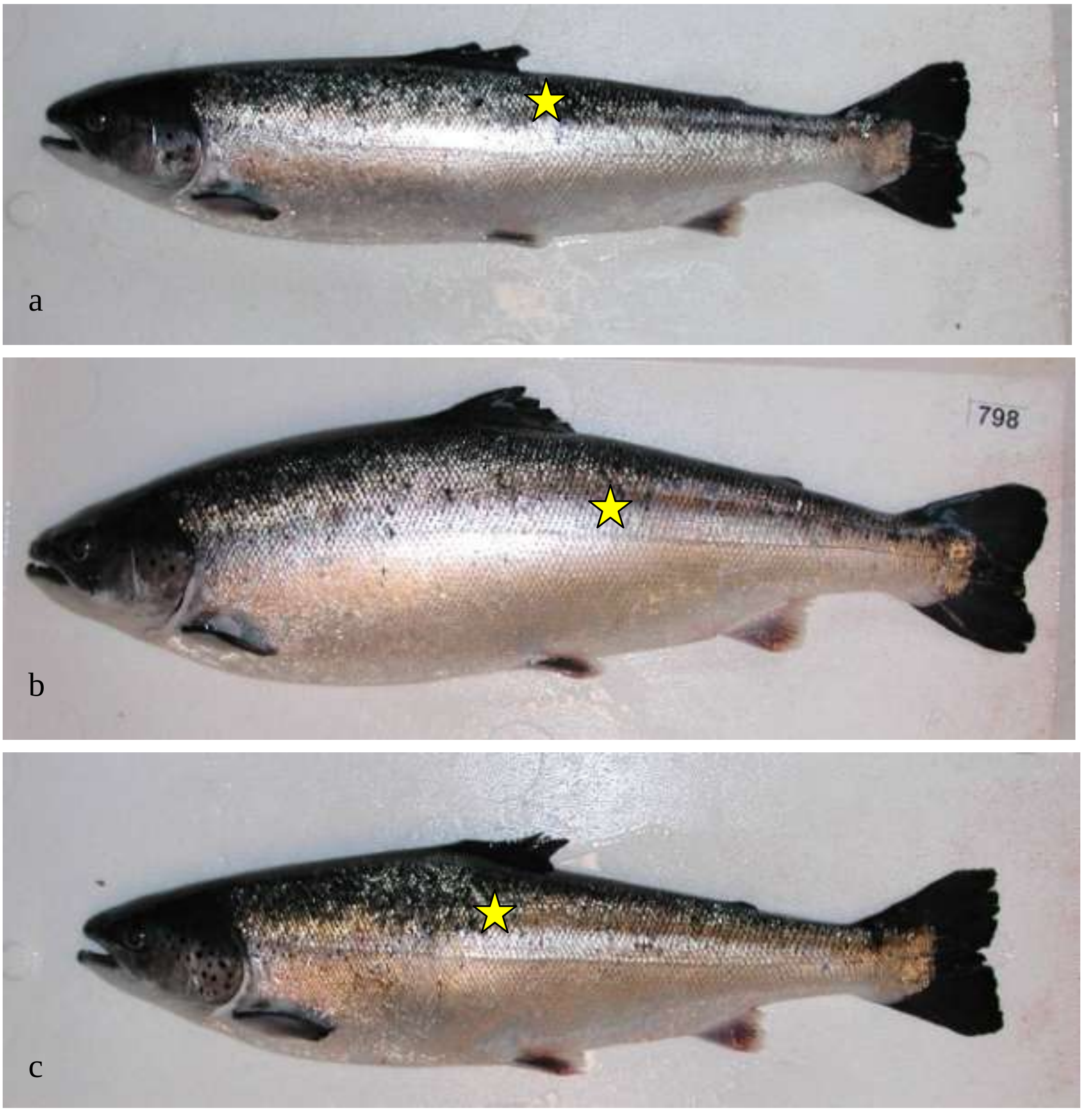


Figure 4. Salmon (approx 2,5 kg) with fusions in the spinal column. These are the same three fish as in figure 3. In a) a fish with a simple, well organized fusions shows no external sign of shape alteration. In b) a lump may be identified at the site of the fusion, but not with certainty.

This fusion consists of four vertebrae. In c) the shape of the fish is clearly deviant, and the site of the fusion is easily identified. Yellow asterisk indicates site of fusion.

Figure 5. Shape alterations of Atlantic salmon parr (approx. 60 g) due to fusions in the spinal column. a) Fish with fusions with a lumpy appearance, b) normal fish of same size

Fusions and body stricture

Some cases have been observed in which there is as a stricture of the trunk of the body. In the least severe cases, this deviation is observed as a malpigmentation in the area around the dorsal fin. One side is regularly more affected than the other side. With increasing severity the stricture affects the muscle and skin. The abdominal fin may be dislocated towards the side of the fish. In light cases, there may be no skeletal pathology observed, but in severely affected fish, skeletal malformations are commonly seen, typically as complex fusions both in the stricture area and at other locations on the spine.

Figure 6. Atlantic salmon with body stricture.

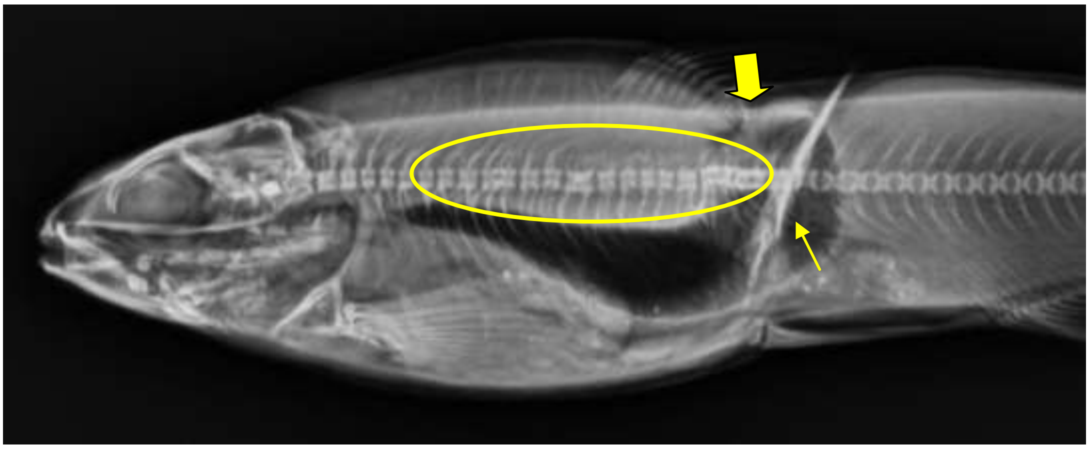
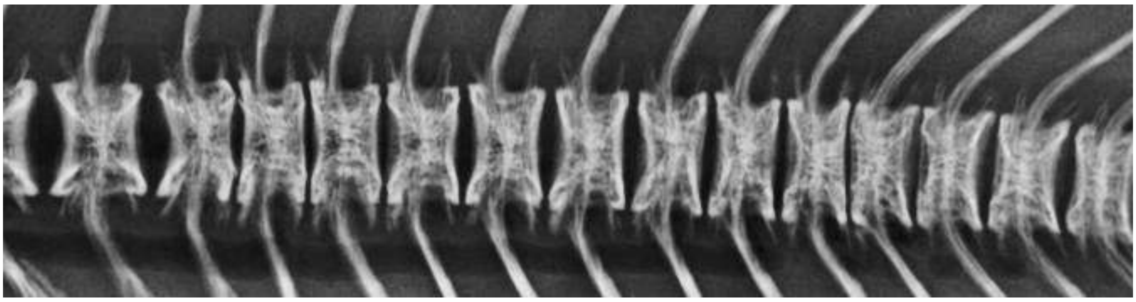


Figure 7: Radiograph of the same stricture fish shown in figure 6. Multiple, complex fusions in large parts of the spine (ring), and an abnormal image of swim bladder and abdominal fin (small arrow), as well as of the dorsal musculature (large arrow).

II. Platyspondylia

Platyspondylia was introduced as a term to describe vertebral changes in which vertebral bodies are flattened (length: width > 1) or compressed. Early stages are observed already at 20 grams, but at this stage the diagnostic is insecure. Also, at this point in time, the term platyspondylia is used to describe a range of morphologically variable conditions, which probably will be split into separate diagnoses at a later date. Platyspondylia is usually not observed until some time after seawater transfer (body weight >1kg), although there are some links indicating that causative factors may still be found in early freshwater stages. In a feed trial, early platyspondylia was observed at 20 g. Similar vertebral morphology has also been seen in commercially farmed fish.



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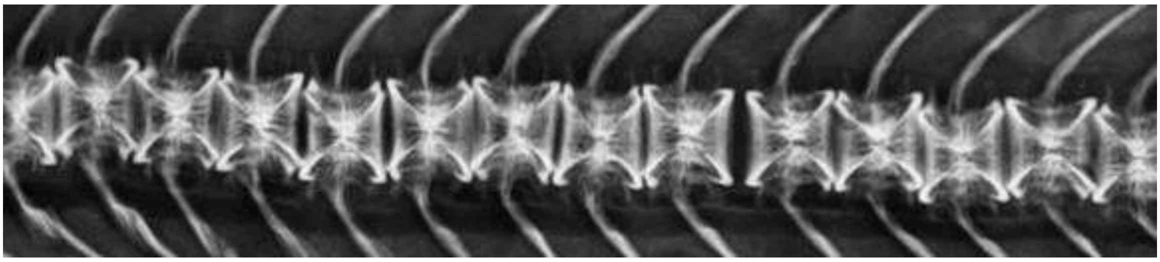

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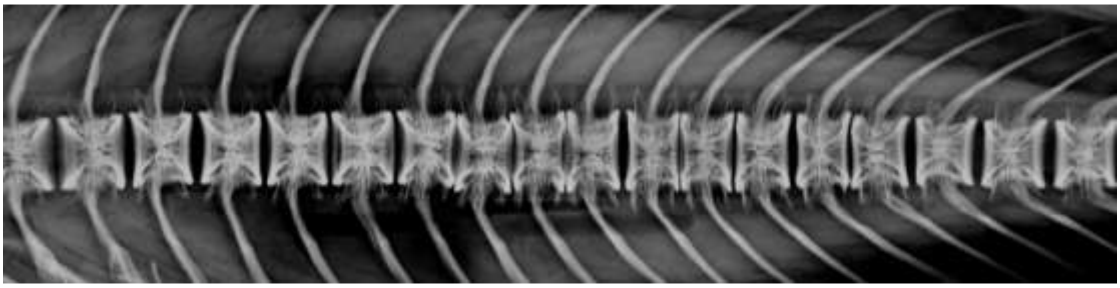

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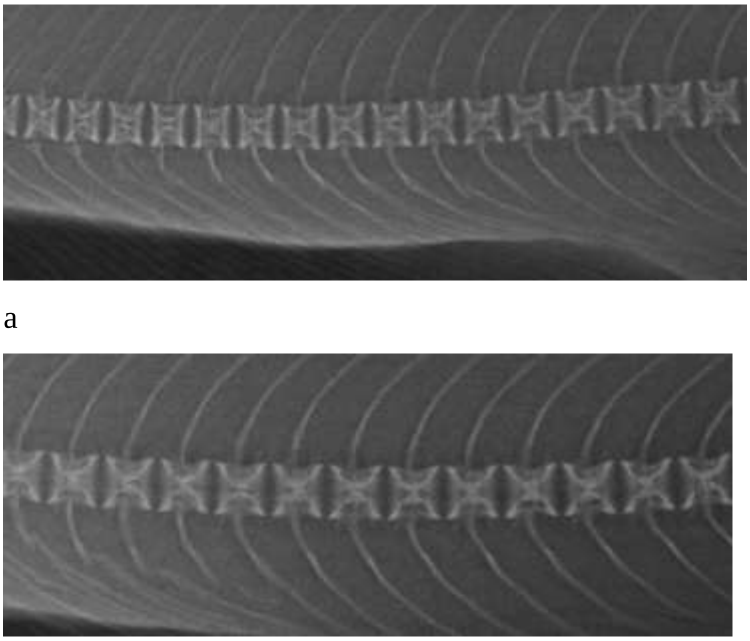
Figure 8. Platyspondylia in large salmon (size range 1,5-4 kg). a-c) different varieties which all would be classified as platyspondylia at present.

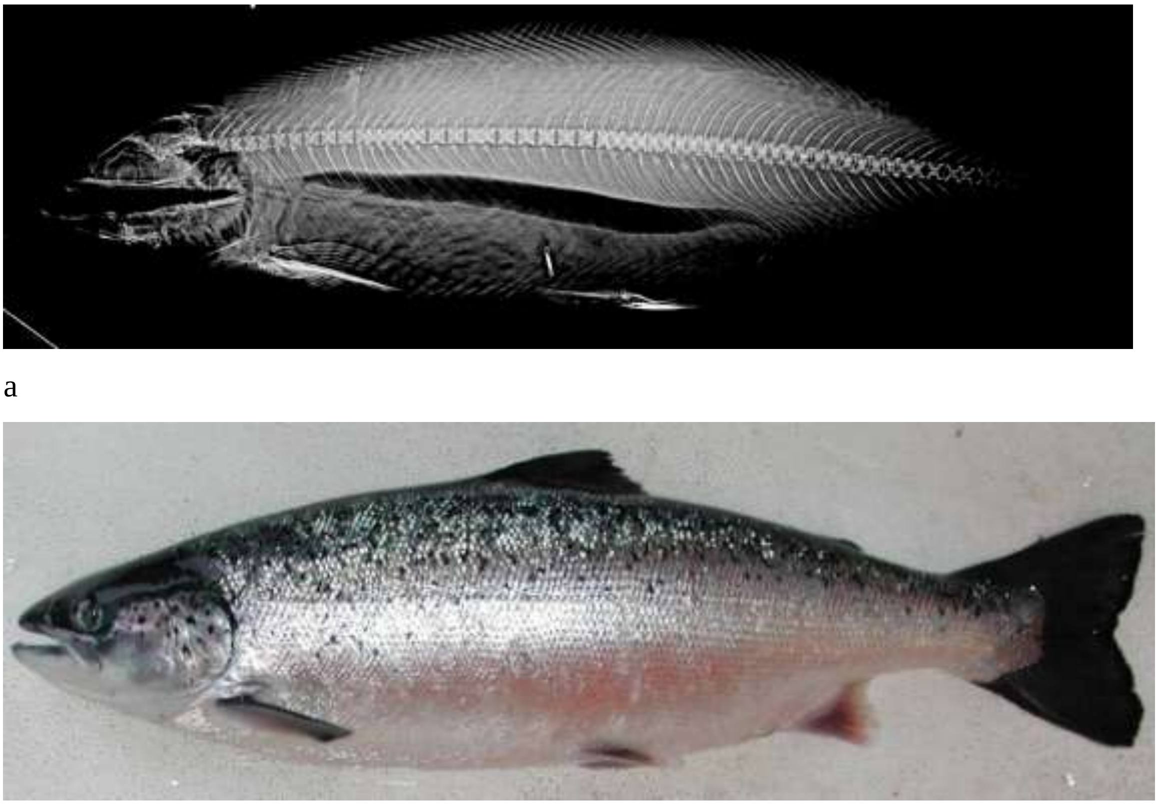


a

b

Figure 9.

Suspected early signs of platyspondylia. a) Vertebrae are too narrow, and intervertebral spaces too wide. b) Normal vertebrae in fish from same group.



a

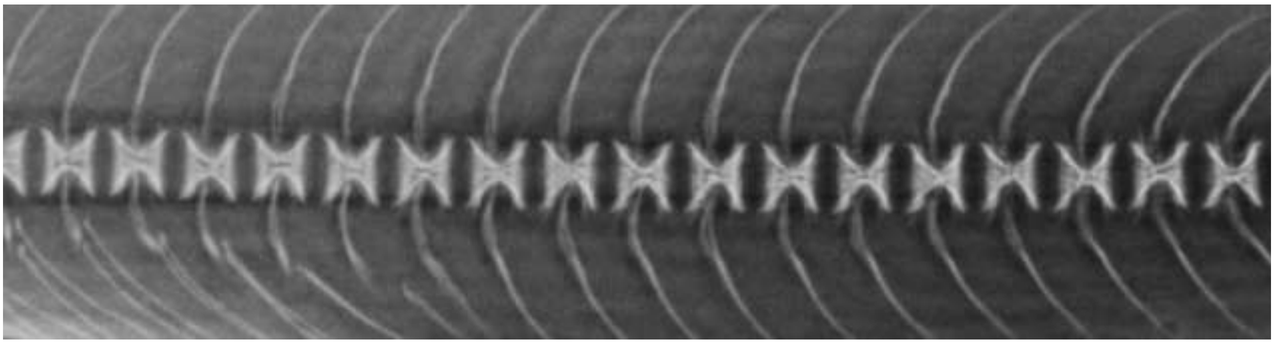
b

Figure 10. Harvest size Atlantic salmon with severe platyspondylia. a) Radiography. b) Photo of same fish.

III. Osteopenia

This term is used to describe the situation where mineralization of vertebrae is incomplete. The causes may be diverse, but the assumption is that most cases result from an imbalance in dietary mineral supply. This is most likely to occur in periods of rapid growth, e.g. in the period post first feeding and in the period of rapid growth in autumn following seawater transfer.

In the acute stage, vertebrae appear as “ghost-like” in X-ray.



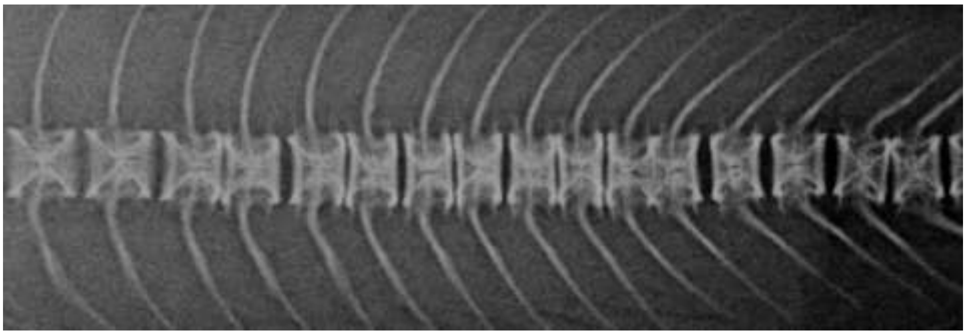
a

b

Figure 11. Acute stages of osteopenia caused by inadequate mineral supply. a) 100g smolt fed on a commercial diet. b) 2kg salmon, seawater-adapted, fed a suboptimal diet from seawater transfer and on. Contrast is enhanced in images, uncorrected the contrast is lower than normal.

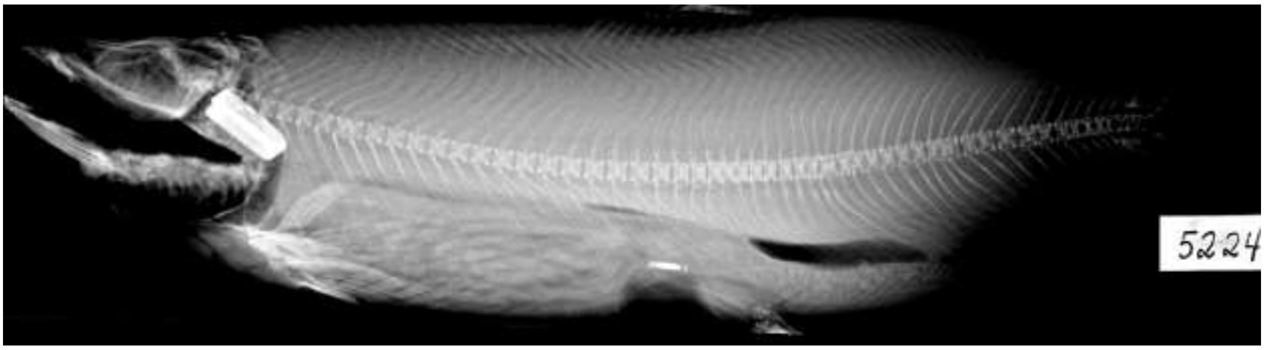
In subsequent periods of moderate growth rates, or if dietary mineral supply is increased, the structures may be remineralized, but the shape of the vertebrae may be permanently distorted. In severe cases, the osteopenia may develop into “true” platyspondylia (Fig 12 b).

a



b

Figure 12. Remineralized stages of osteopenia. In a) vertebrae shape is distorted, but outline of each vertebra is approaching normal. Thus, fish will appear relatively normal. In b) osteopenia has developed into severe platyspondylia.



a

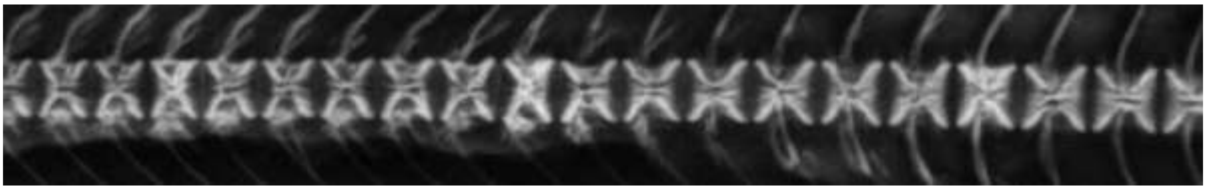
b

Figure 13. Platyspondylia in harvest size Atlantic salmon. In this individual, the platyspondylia developed from preceding osteopenia. a) Radiography. b) Photo of same fish.

IV. Hyper dense vertebrae

Single hyperdense vertebrae are sometimes observed in fish in the freshwater stages. The hyperdense vertebrae are identified on X-ray as single vertebrae with an increased radiodensity compared to its neighbours. The size of the affected vertebra can be normal or smaller than normal. In an individual fish, it is common to observe a few (1-5) hyperdense vertebrae regularly spaced, separated by 4-5 seemingly normal vertebrae. The phenomenon has been causally linked to inadequate mineralization (Helland et al., 2006).

a



b

Figure 14. Parr with hyperdense vertebrae. a) Radiography of whole fish (flesh caudal to vertebra 4-5 removed), showing presence of 3 hyperdense vertebrae under the dorsal fin. b) Detail of the affected spinal region

On whole mount staining, it was detected that the trabecular network of the hyperdense vertebrae appears denser than normal. Histological analyses demonstrated that the internal structure of hyperdense vertebrae is partially collapsed and that the internal spaces are filled with ectopic cartilage.

Hyper dense vertebrae are early observed as axial deviations caused by one small vertebra. This single small vertebra later develops into a hyper dense vertebra observed. The further development of this kind of vertebra is shown to be approximately half normalising and half developing into fusions.

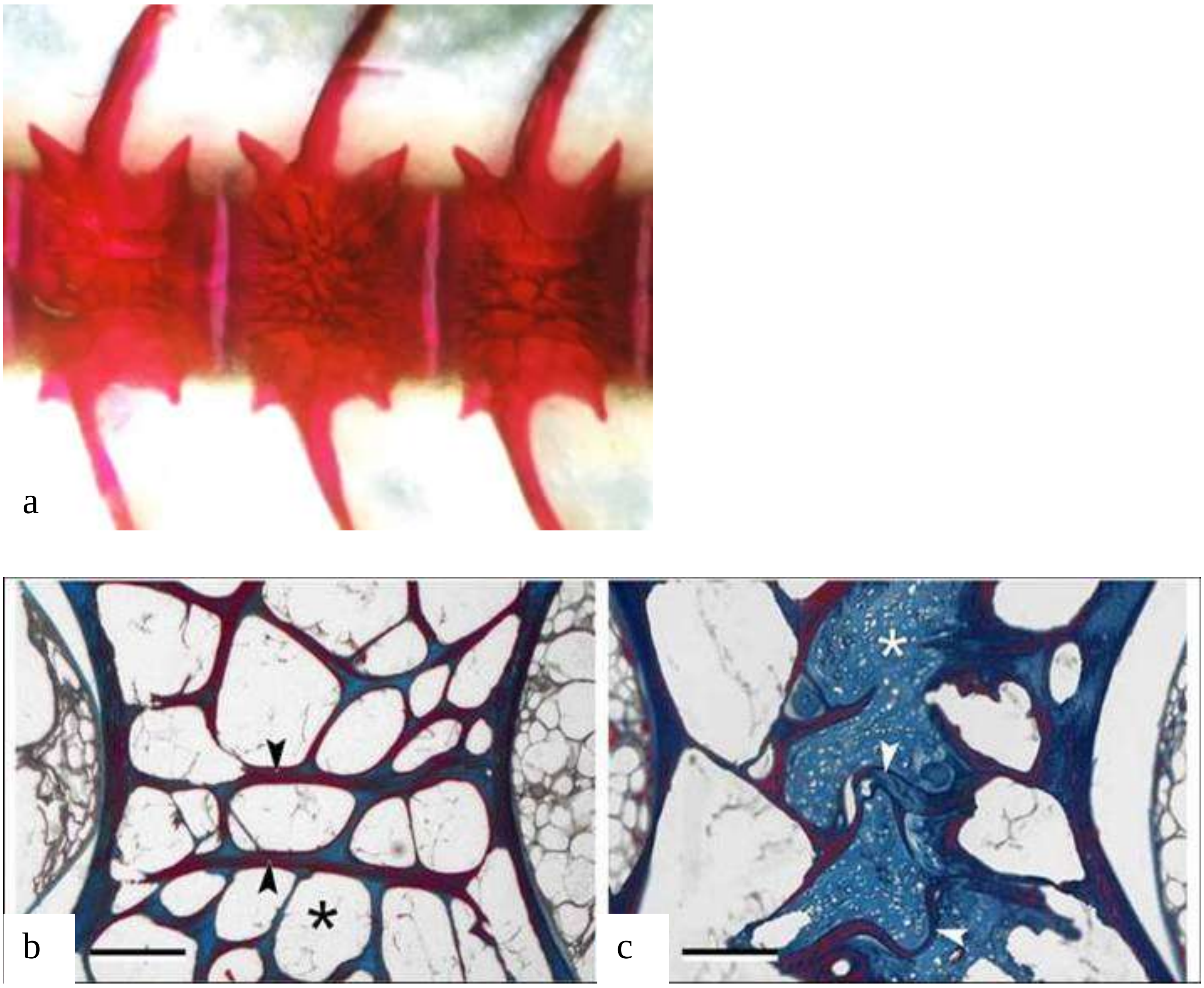


Figure 15. Hyper dense vertebra, details. a) Whole mount staining with Alizarin red, showing increased density of trabecular network in a hyper dense vertebra (center) b) Histological structure of normal vertebra. c) Histology of hyper dense vertebra, showing collapse of structural elements and presence of cartilage.

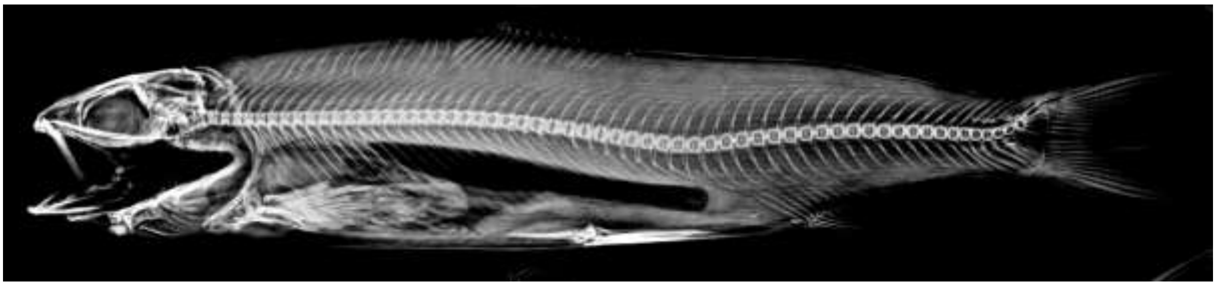
From: Helland, S., Denstadli, V., Witten, P.E., Hjelde, K., Storebakken , T., Skrede, A., Åsgård, T., Baeverfjord, G. 2006. Hyper dense vertebrae and mineral content in Atlantic salmon (Salmo salar L.) fed diets with graded levels of phytic acid. Aquaculture 261, 603-614

V. Axis deviations

Axis deviations can be observed both in the vertical and the horizontal plane.

Scoliosis is the well-known condition where the spine curves in the vertical plane, i.e. sideways. This condition was traditionally related to vitamin C-deficiency. In our times, this aetiology is less probable, as the commercial feed production has improved in this field. The condition may still be observed occasionally. If suspected, the fish should be observed from above, or supplemental X-rays should be taken from above. Some cases of severe scoliosis of the tail are occasionally observed. These fish will probably be identified and removed at vaccination.

a



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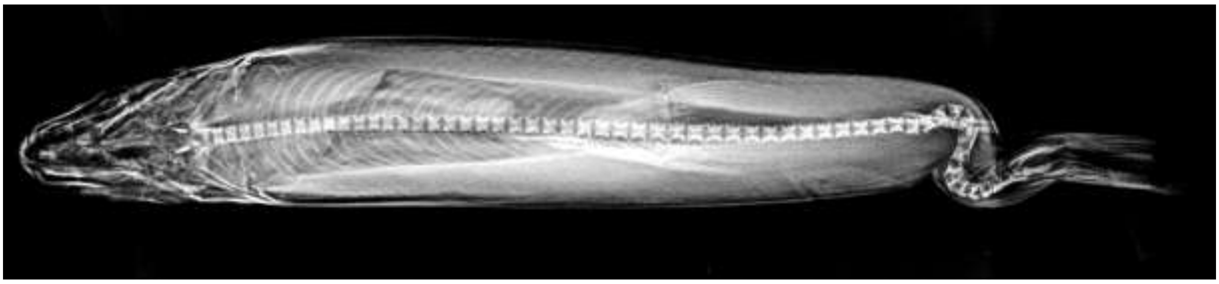


c

Figure 16. Salmon parr with scoliosis. a) Dorsal view. b) Lateral view of same fish as a). c) Severe scoliosis of tail.

Lordosis and kyphosis. Dorso-ventral axis deviations (lordosis, kyphosis) are not common in Atlantic salmon. Pathology of these types may be seen in individual fish. The most common observation of dorso-ventral axis deviations is that related to single vertebrae being small or structurally deviant. The axis deviation seen as a result of this is generally minor, and the use of the terms lordosis alternatively kyphosis may seem exaggerated.

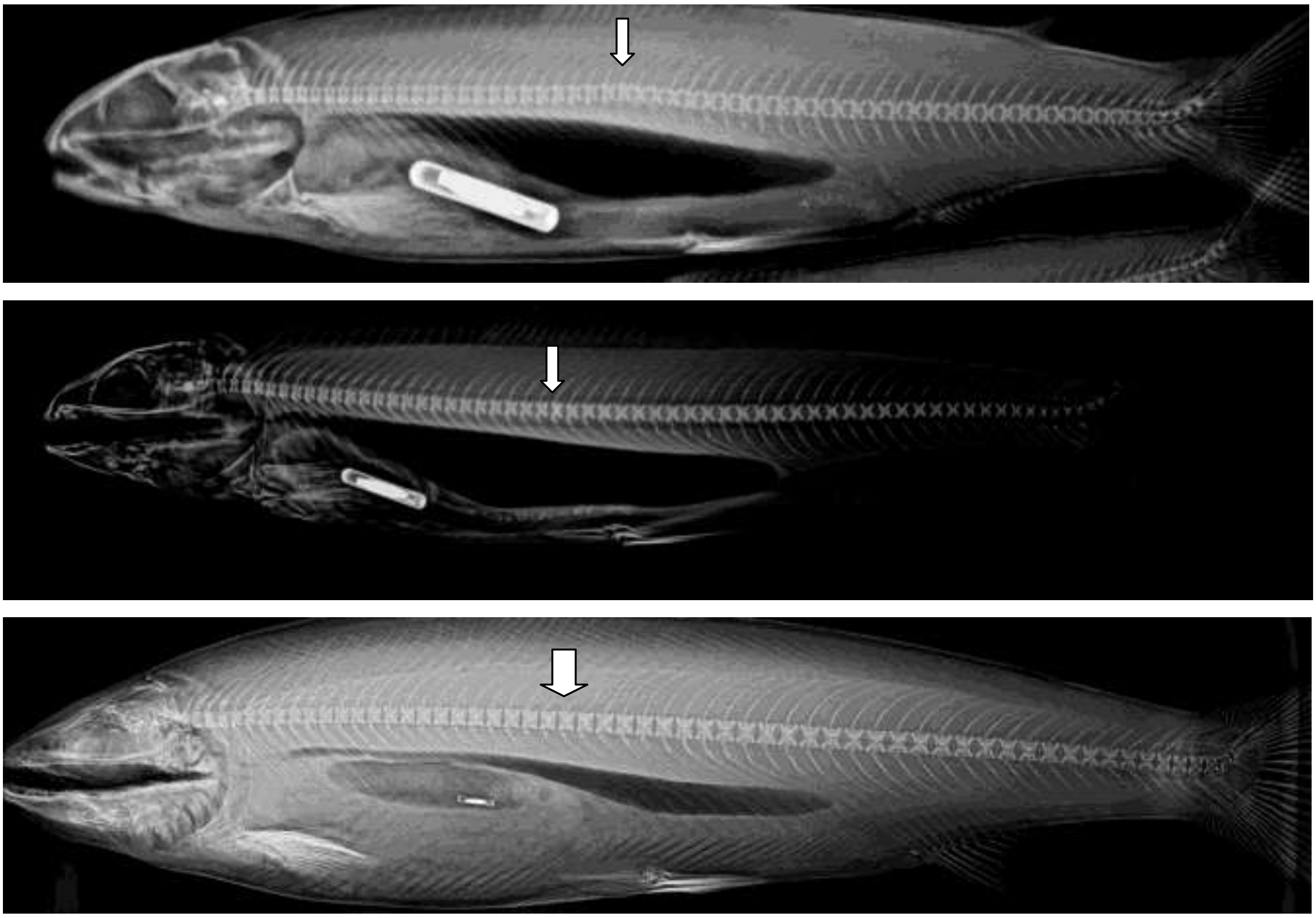


Figure 17. Slight axis deviation in Atlantic salmon parr, caused by deviation in single vertebra (marked by arrow). The same fish is shown in two later stages, where the small vertebra in the upper image have been replaced by first a high density vertebra and later a normal vertebra.

Proper lordosis and kyphosis appears rarely, and then often in combination with other deformities.

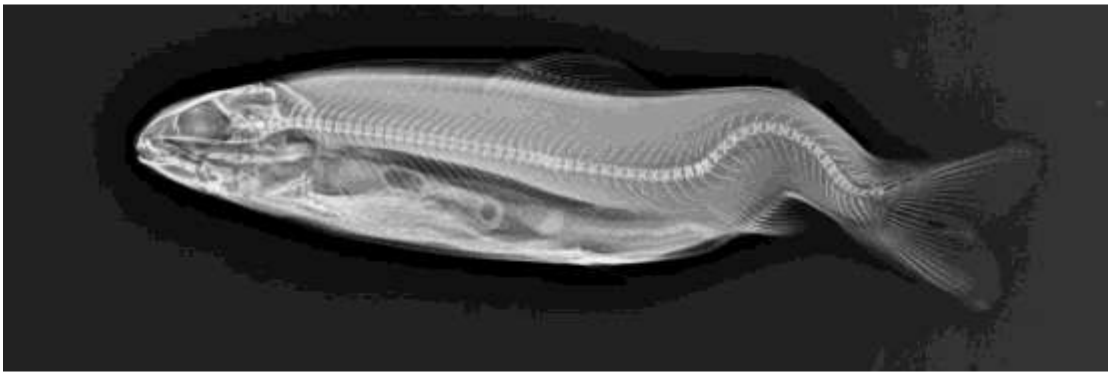


Figure 18. Atlantic salmon on 15 gram with a combination of lordosis and kyphosis.

a

b

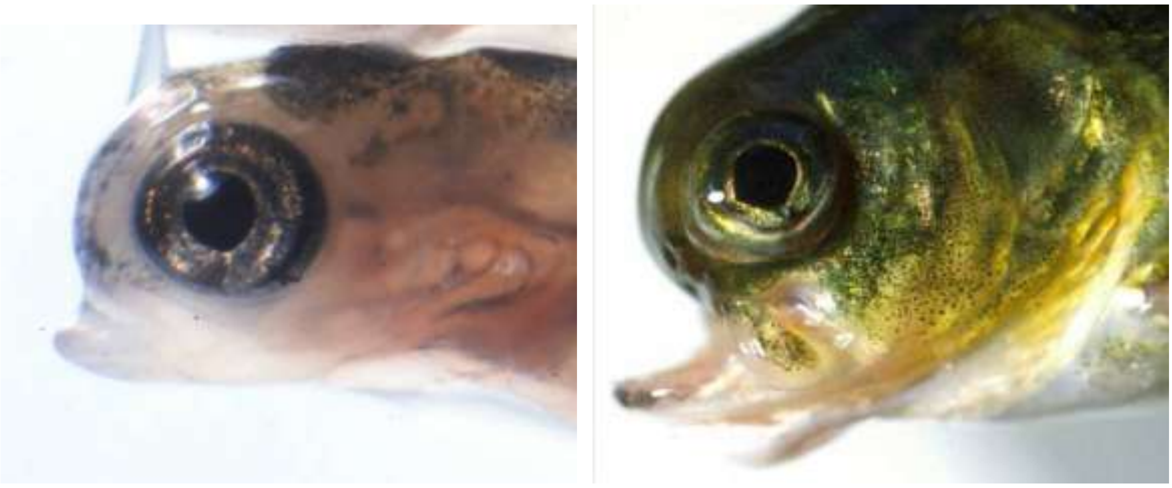
Figure 19. Multiple axis deviations, fish size approx. 30g. a)Lateral view. b) Dorsal view. Combination of scoliosis and kyphosis.

VI. Skull and jaws

Deformities of the head are less common than those affecting the spine, but may still be significant.

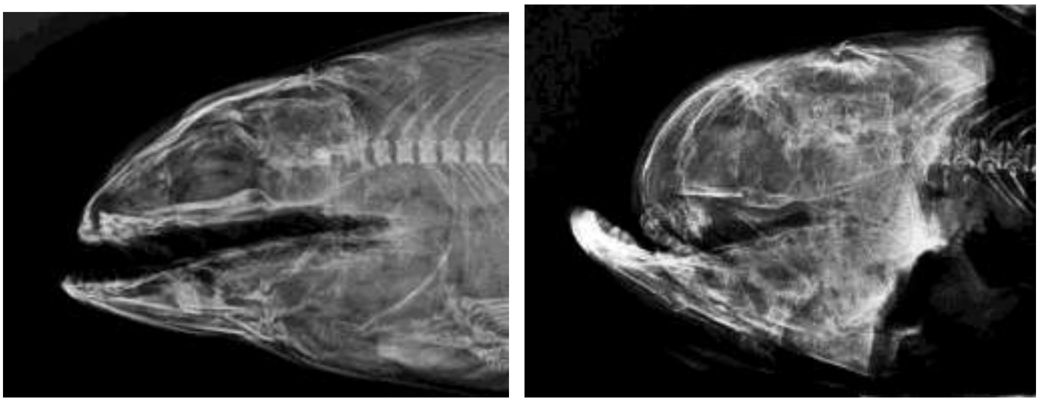
Short forehead - "Pugnose"

This term is a common name for a condition where the bones of the upper jaw and the forehead are inadequately developed. This malformation is invariably induced during embryonic development, and is easily identified at first feeding and in juveniles.



a b

Figure 20. "Pugnose" in a) first feeding fry and b) juvenile (40g)



a b

Figure 21. Upper jaw deformities. Curvature in the palatine bone (a) and severe upper jaw shortening with deformed lower jaw (b)

Malformation of the lower jaw – "Screamer disease"

This term is used to describe a condition where the branches of the lower jaw are distorted and bent downwards. The condition develops post seawater transfer. A causative relation to undermineralization in early life stages has now been established. A variety of this condition is expressed as a thickening of the mandibular branches. In these fish, the distinction between normal and abnormal is more difficult.

a b

Figure 22. Malformations of lower jaw in Atlantic salmon of harvest size. a) Severe malformation of the lower jaw ("Screamer disease"). b) Thickened mandible.

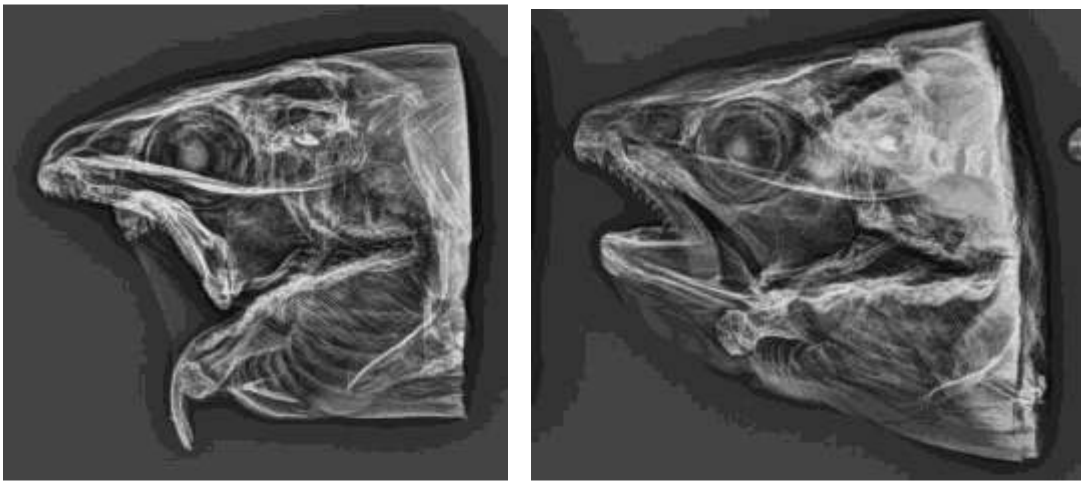


Figure 23. Radiographs of similar jaw deformities as those shown in figure 20.

Dislocation of the hyoid bone

Another maldevelopment observed in small salmon fry is a lower jaw deformity observed as a drop of the hyoid bones between the mandibular branches. In severe cases this is visible as a “double mouth”. This deformity is often sorted out early, or the fish die, possibly from problems with eating.

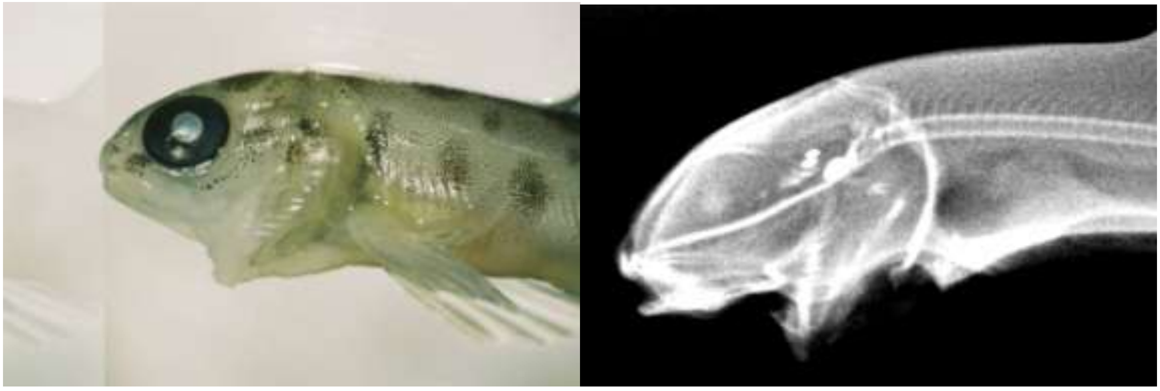


Figure 24. Picture and radiograph of severe lower jaw deformity were the hyoid bones have loosened from the mandibular arches.