FINEFISH Collective Research Project
(*Contract Nº012451*)

Malformations in farmed fish
Guidelines for classification

(*WP2: Standardisation of Environmental Fish Monitoring)*

III. Rainbow trout (*Oncorhynchus mykiss*)

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Rainbow trout (*Oncorhyncus mykiss*)

Rainbow trout has been in aquaculture much longer than the Atlantic salmon, but the causative factors for spinal deformities are less well defined in this species than in salmon. General considerations may allow the assumption that the similarities between these two salmonid species justify transfer of knowledge developed between them. Farming environment for rainbow trout is, however, much more diverse than the relatively uniform Atlantic salmon production. Thus, any cross-species reference as to causal relations for malformations must be made with caution, and should be verified through experiments or other investigation procedures.

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 to identify deformities of vertebrae. 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, fixed 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 usually 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 mammography: 22kV, 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 radiography: 32kV, 50 mAs or 40 kV, 40 mAs

Film-focus distance is variable, but approximately 70-80 cm is suggested.

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

Available material for developing a classification manual in rainbow trout is at present incomplete.

The following stages are recommended in a standardized screening program:

20 g size	Will detect malformations induced during embryonic development, and those induced during first feeding
100g size +	For freshwater production of portion-size rainbow trout
Seawater 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.

The sizes should be adapted to the rearing procedures of the farm, meaning that if juveniles are sold or restocked at e.g. 10g, this should be one of the checkpoints. It is likely that high quality radiographs (mammography) can give good images down to at least 5g. Malformations induced during embryogenesis can probably be detected at any time after first feeding, if subjected to whole mount staining, and this method should be considered if such an aetiology related to embryonic development is suspected.

Normal

The rainbow trout spine normally consists of 59-63 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, the length:width relation should be closer to 1:1. The tail vertebrae are narrower, and complete or partial fusions are commonly seen. In juvenile fish, the vertebrae are in general narrower than in subsequent life stages.

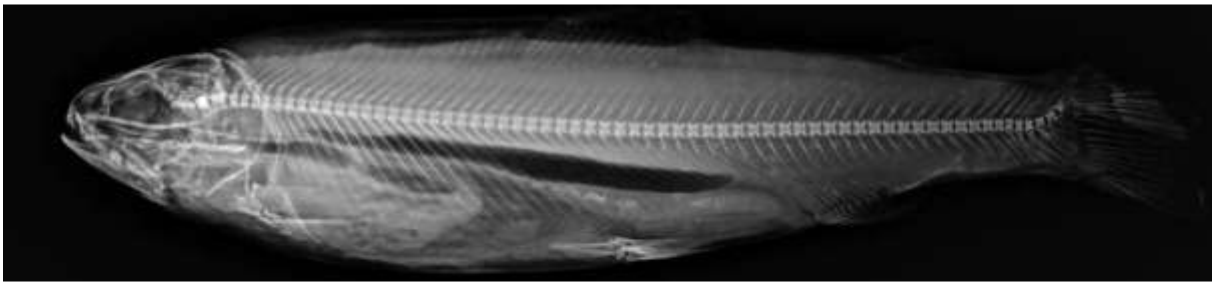

Figure 1: Normal trout juvenile laterolateral x-ray with regular vertebrae in the major part of the spine.

Malformations of the spine in rainbow trout

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

In rainbow trout, the diagnostic criteria for classification of vertebral pathology are less elaborate than in Atlantic salmon.

I. Vertebral deformities

Vertebral changes seem to be less diverse in rainbow trout than in Atlantic salmon and Atlantic cod. In general, the term fusion is commonly used to describe pathology where two or more vertebrae are more or less amalgamated. Some additional vertebral changes are associated with this process, and are assumed to be intermediate stages. The second major type of vertebral pathology is platyspondyly, in which vertebrae are flattened and appear to be compressed. Unlike fusions, the individual vertebrae are distinct, even though the intervertebrall space may be narrower than normal. The distinction between fusions on one side, and platyspondyly on the other side, is much less clear in rainbow trout, and a differentiation between the two is rarely justified based on our present knowledge. In rainbow trout, vertebral bodies are rarely completely fused, unlike the situation in other species. It is also not common to identify platypondyly, although even the most advanced fusion lesions have some features in common with platyspondyly in other species. Our impression is also, at present, that the response to different adverse stimuli gives more or less the same result in this species. Therefore, attempts to differentiate between fusions and platyspondyly at this stage are of limited value. In the following, you will find a range of different examples showing fusions of the spine and other related vertebral deformities observed in trout. For all practical purposes, they can all be labelled ‘fusions’.

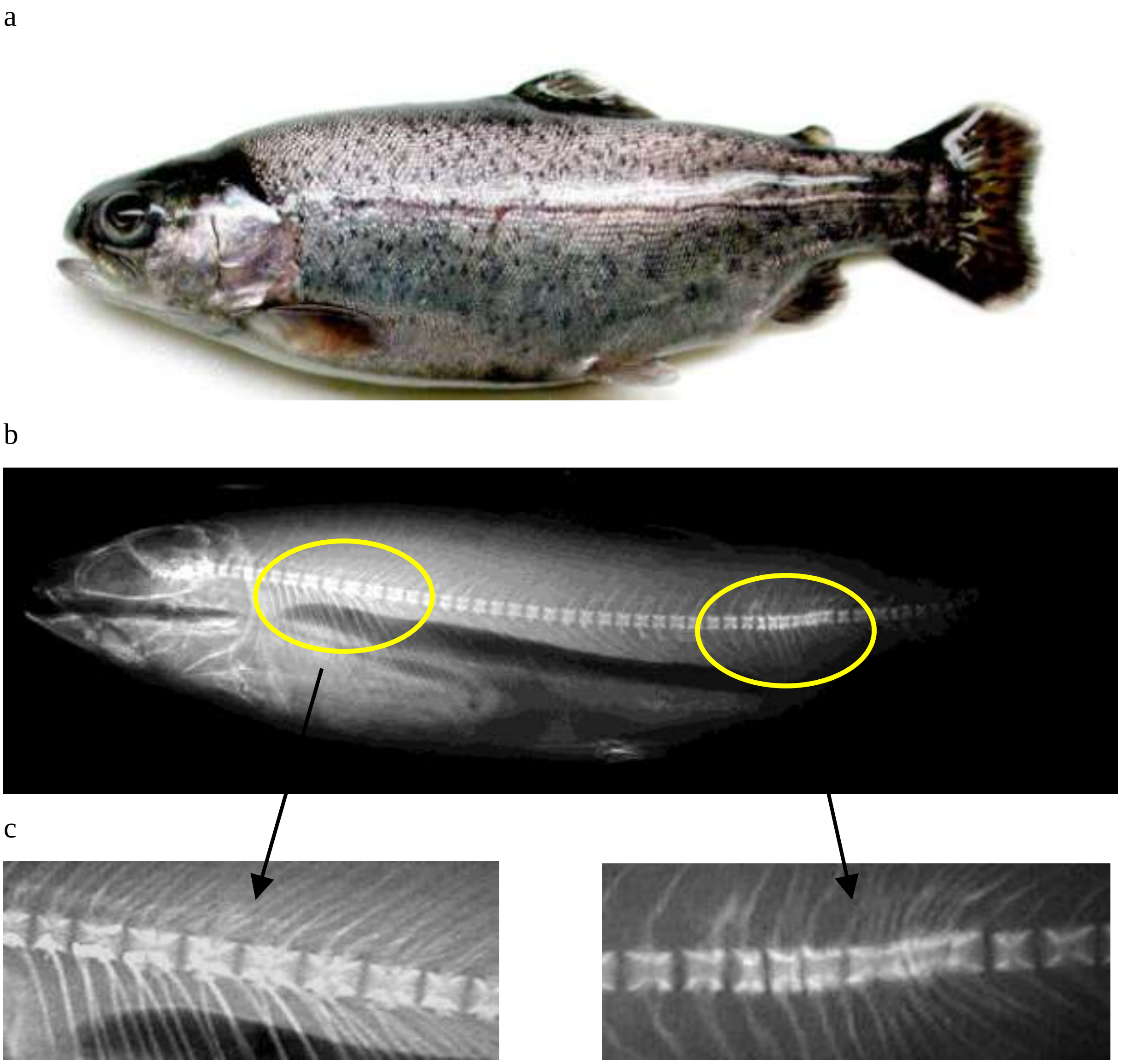

Figure 2: a) A severely shortened rainbow trout (approx. 50g). b) X-ray, demonstrating two regions of the spine with severe fusions of a slightly different appearance. c) Details of radiography shown in b.

Thus, fusions in rainbow trout appear in many different morphologies and locations in farmed trout spinal column. The following terminology is suggested:

Simple fusion:	Fusion of two vertebrae, with vertebral centra more or less 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 in contact with each other

Incomplete fusion:	A fusion process involving two or more vertebrae, in which vertebral bodies are separate, but vertebral endplates of adjoining vertebrae are flattened. Intervertebral space may be reduced or absent, indicating that the process is moving towards a more complete fusion.

Simple complete fusions affect two vertebrae and are most likely finished processes which will not progress further. If the lesion stabilises at this stage, it will probably be of no or little harm to welfare of the fish or the fish as a product.

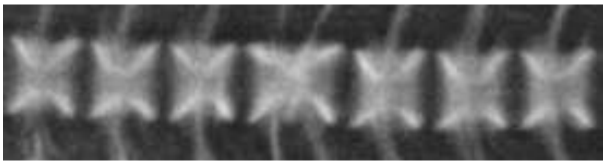

Figure 3: Simple complete fusion affecting only two vertebrae.

Complex fusions affect more than two vertebrae and vertebrae adjacent to the lesion may also be visibly affected. In other species it was demonstrated that such lesions are likely to develop further as the fish grows, and include more and more of the adjacent vertebrae.

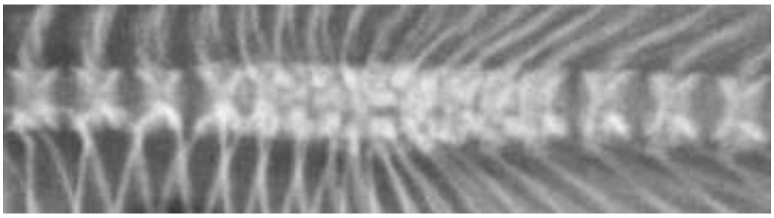

Fig 4: Complex vertebral fusion that appears to draw the adjacent vertebrae into the fusion.

The distinction between complete and incomplete fusions is less clear in rainbow trout than in some other species. An example of an incomplete fusion is shown in Figure 5.

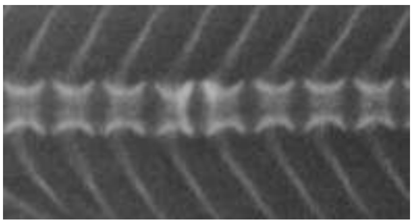

Figure 5: Possible incomplete fusion. Similar vertebral deviations in A. salmon have been shown to develop into fusions as the fish grew.

In rainbow trout, it is relatively common to find fish in which an extended part of the spinal column is affected, often combining a variety of lesions. Two examples are given below in Figure 6 and Figure 7.

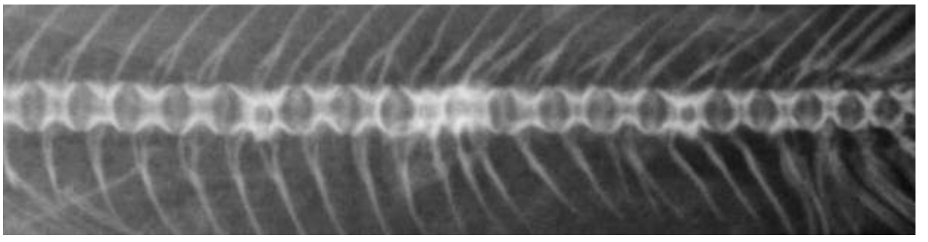

Figure 6: Complex and multiple fusions.

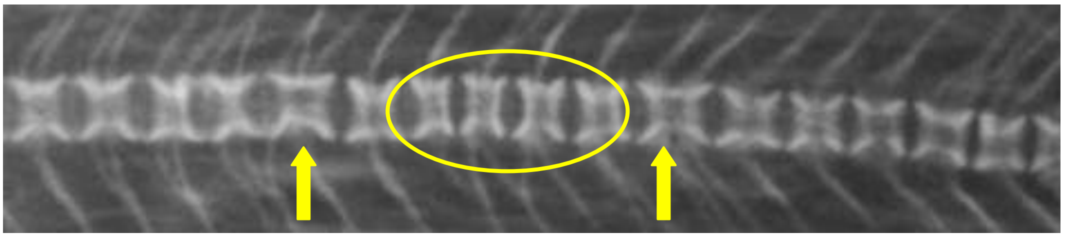

Figure 7: A group of several deformed vertebrae, including two complete fusions (arrows) and a group of vertebrae with platyspondyly-like changes (ring).

Also, it is not uncommon to fine severely affected indiviuals, in which a major part of the spinal column appear fused and compressed. Such fish will appear severly malformed and should be euthanized as soon as they are identified.

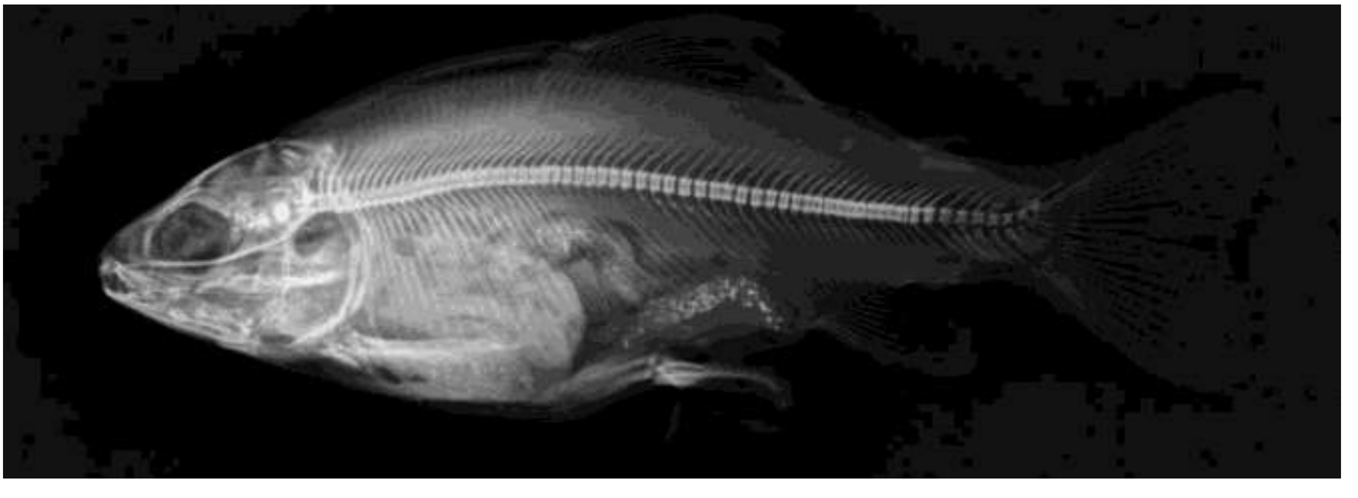

Figure 8: Severely deformed spine in 20 g rainbow trout. Only the 5-6 caudalmost vertebrae have a normal shape, and the fish has a carp-like external morphology. The vertebrae are both compressed and fused.

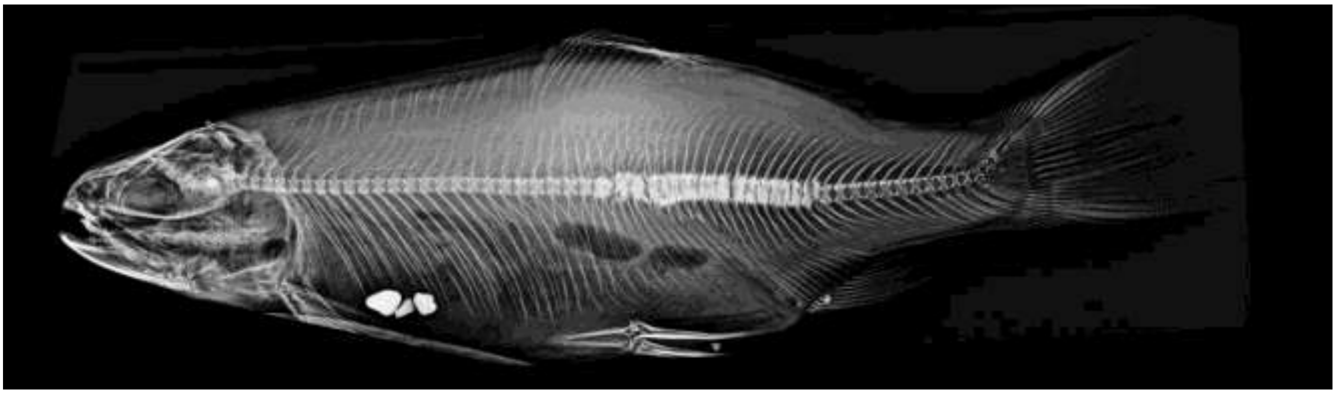

Figure 9: Extreme fusion of the spine in the region below the dorsal fin to the vent.

a)

b)

Figure 10: a) Extreme fusion of vertebrae in cranial region of the spine on X-ray. b) Gross morphology of same individual. Radiodense material in abdominal region are gravel used as tank enrichment.

In figure 11 (below) fusions at a range of different locations along the spine are shown, in fish of size approx. 5g. With this severity, changes can be identified on gross examination.

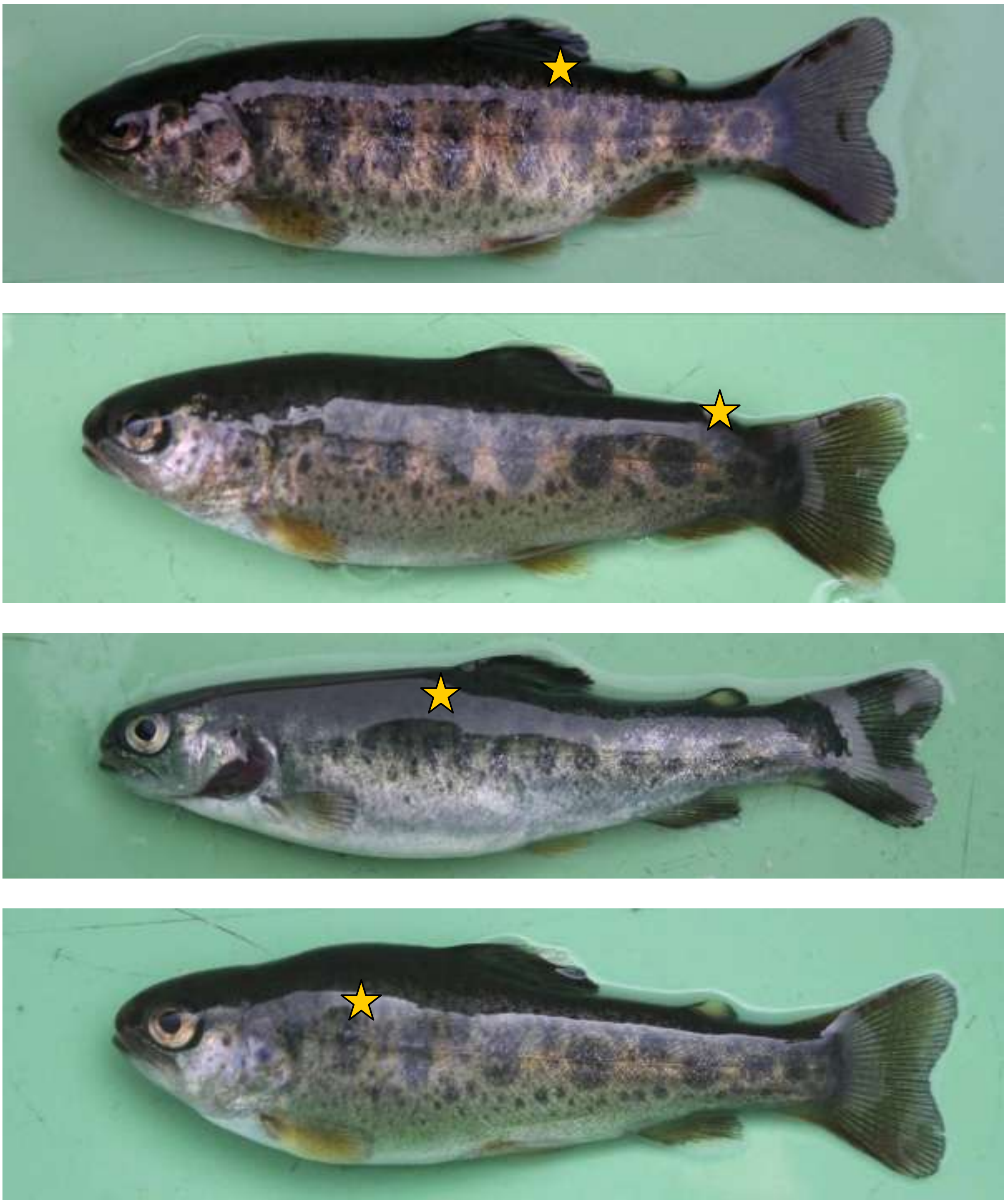

Figure 11: Fusion of vertebra in rainbow trout juveniles of approx 5g. Location of fusion indicated by asterix.

A particular phenomenon is sometimes identified in rainbow trout, in which severely fused vertebra appear hollow on X-ray. This is a type of lesion which is relatively rare, and which will typically be identified in low numbers in fish groups which otherwise include fish of a more regular type of pathology.

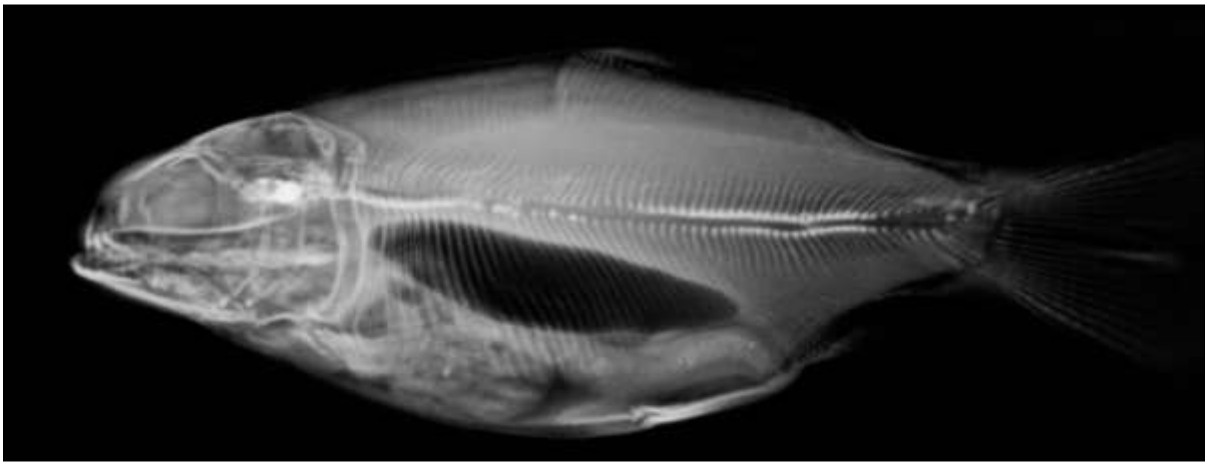

Figure 12: Severe vertebral deformity in 20 g trout. The vertebrae appear undermineralized and almost hollow in some parts, and the entire spine is fused/ compressed.

II. Axis deviations

Axis deviations can be observed both in the vertical and the horizontal plane, and seem to be more commonly observed in rainbow trout than in A. salmon. Axis deviations (scoliosis, lordosis, kyphosis) are not uncommon in rainbow trout. The axis disruption can be found at practically any location along the spine, also within a group of fish.

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, with improvements in commercial feed production. The condition is still observed in rainbow trout. If suspected, supplemental X-rays taken from above should be taken. Fixing or position artefacts in radiographs can be mistaken for being scoliosis.

Figure 13: Scoliosis in rainbow trout of approx 2 kg

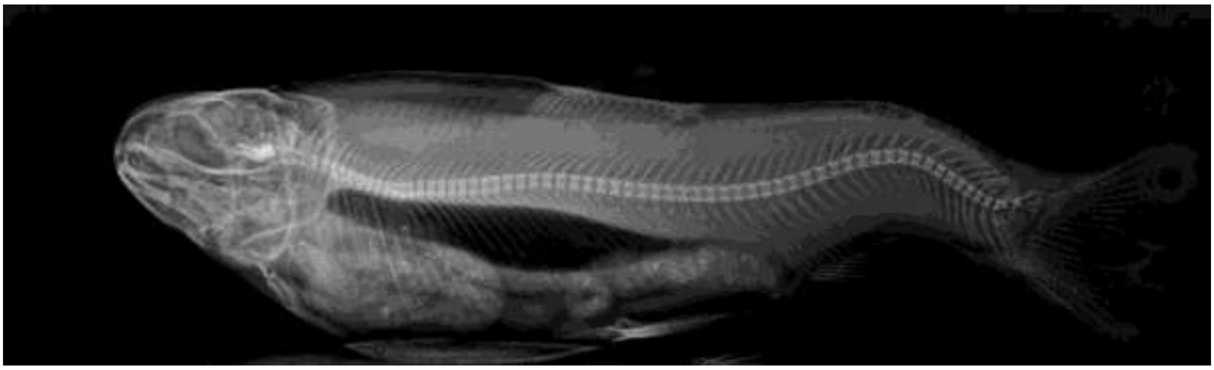

Figure 14: Scoliosis seen in a lateral x-ray of a 20g rainbow trout. Scoliosis in the entire spine, supplemented with a kyphosis in the tail.

Lordosis denotes an anterioposterior curvature of the spine in the sagittal plane, i.e. with no sideways deviation, with the convexity pointing ventrally.

Kyphosis denotes the opposite situation, with the convexity pointing dorsally.

Multiple axis deviations are not uncommon, with any combination of scoliosis, lordosis and kyphosis in the same fish. Axis deviations can be found both with and without pathological changes of vertebrae at the site of axis disruption.

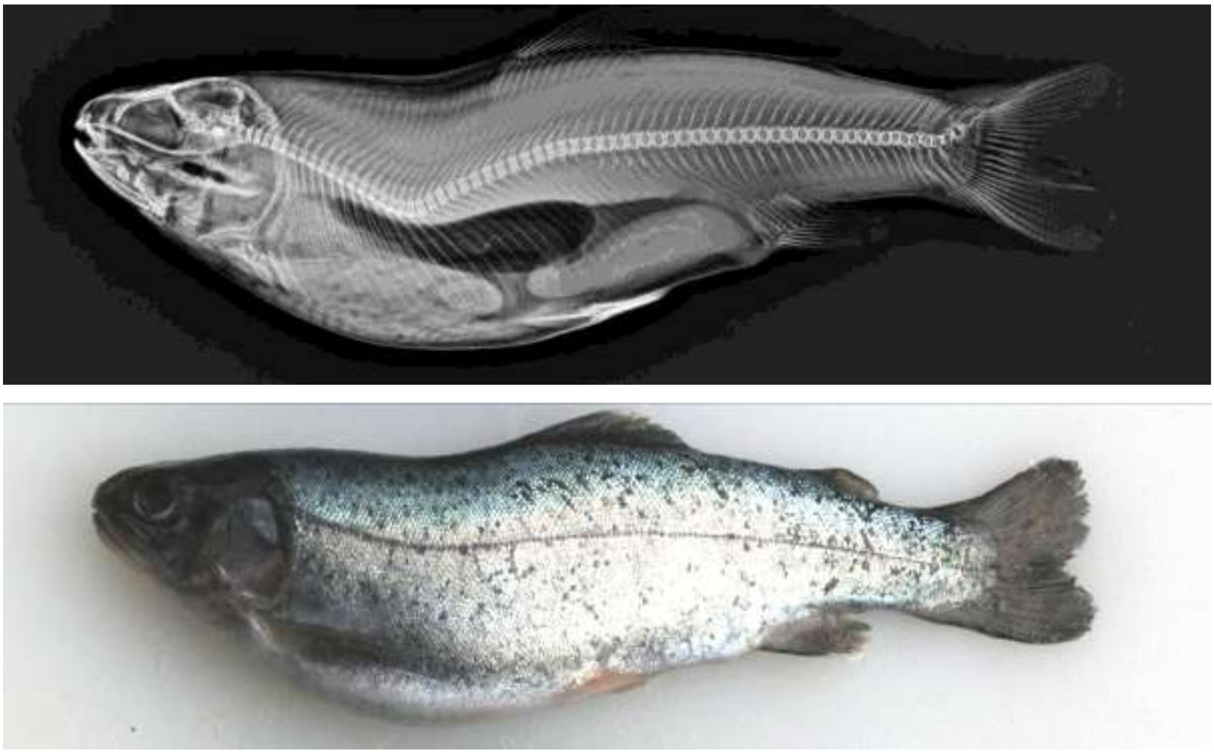

Figure 15: Lordosis in cranial region. No prominent pathology of vertebrae at site of axis disruption.

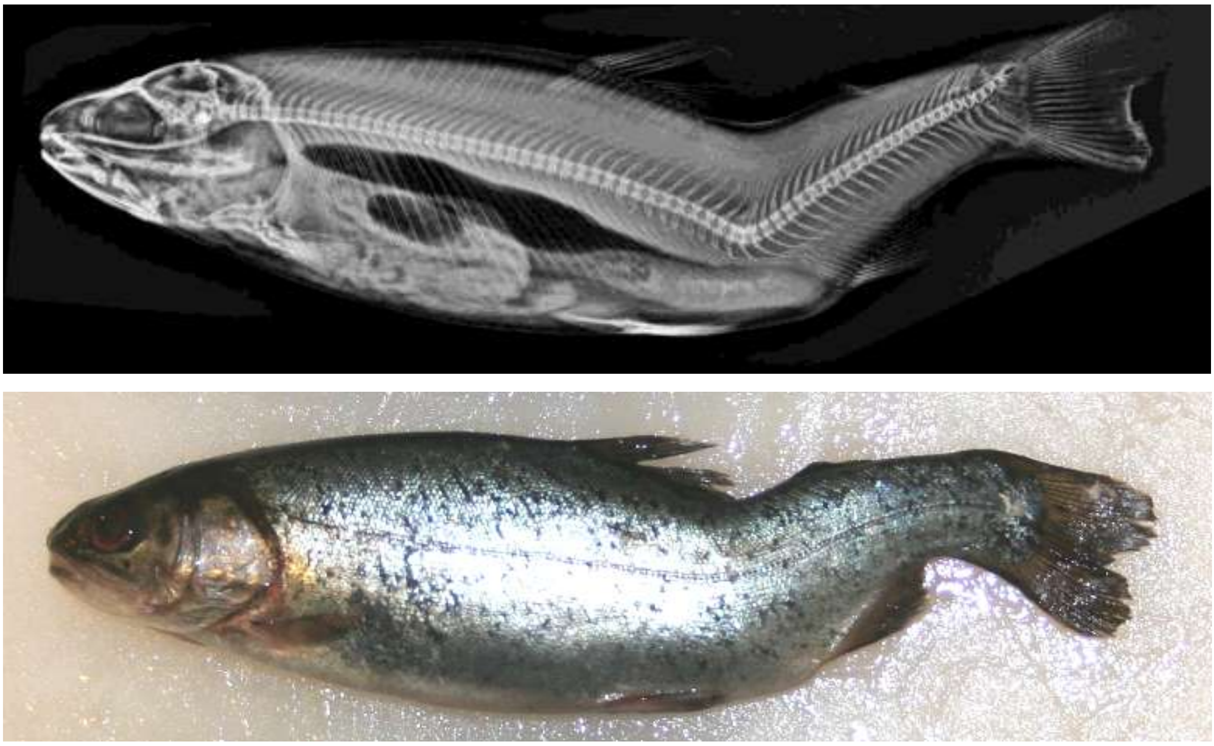

Figure 16: Lordosis in caudal region. No prominent pathology of vertebrae at site of axis disruption. X-ray and photo.

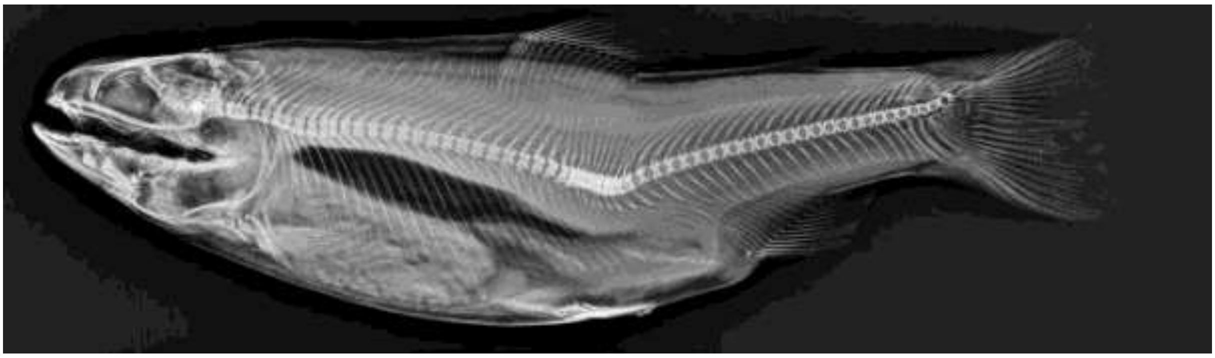

Figure 17: Lordosis in region below the dorsal fin. Vertebrae are fused at the site of axis deviation.

Figure 18: External appearance of lordosis in rainbow trout juvenile of approx 5g.

Figure *19: Lordotic neck in a rainbow trout associated with faulty swim bladder development*

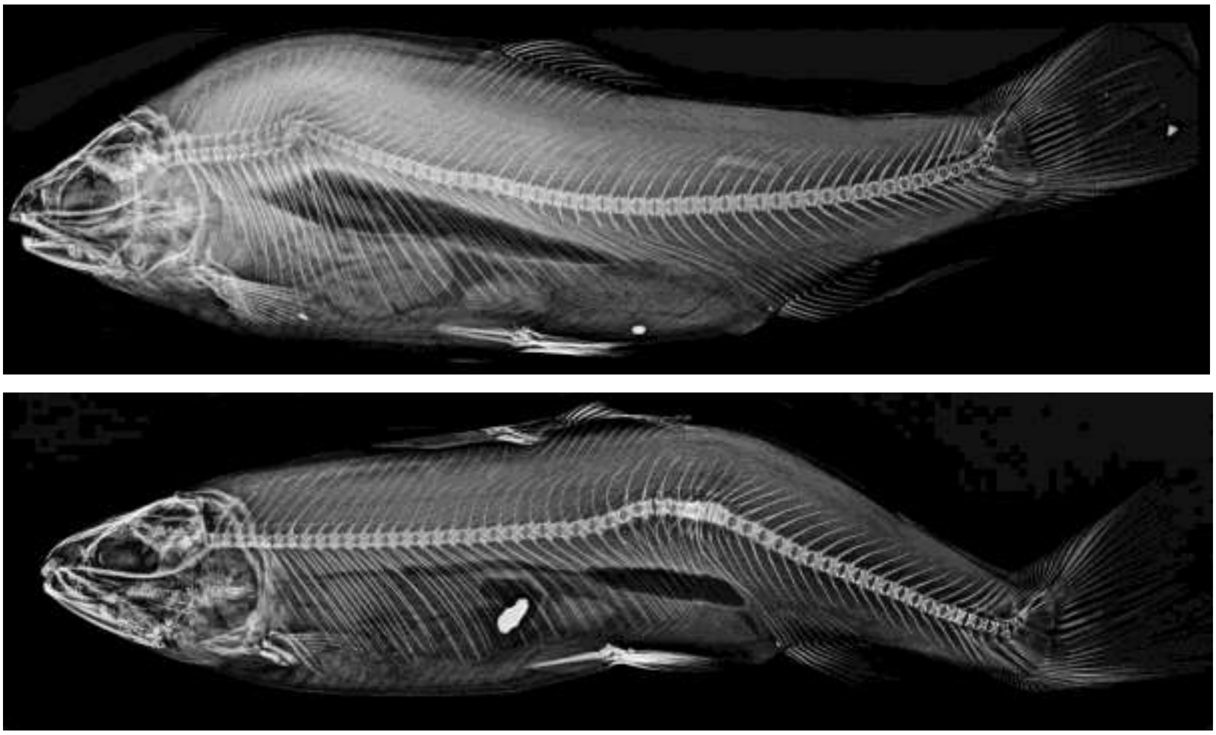

Figure 20: Kyphosis at two different locations of the spine, both with vertebral pathology at the site of axis disruption

Figure 21: External appearance of kyphosis in rainbow trout. Top: Harvest size fish (2-3kg), bottom: Juvenile of approx. 5g.

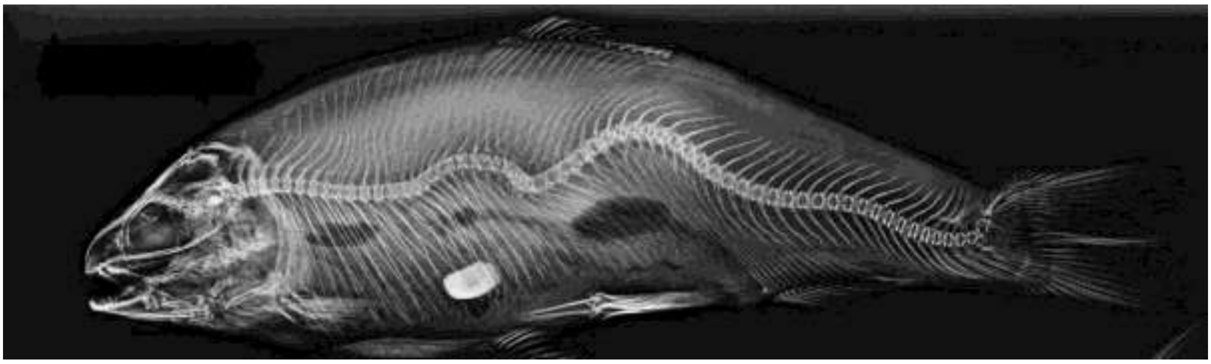

Figure 22: Multiple axis deviation (lordosis and kyphosis) of rainbow trout of harvest size (2-3kg).

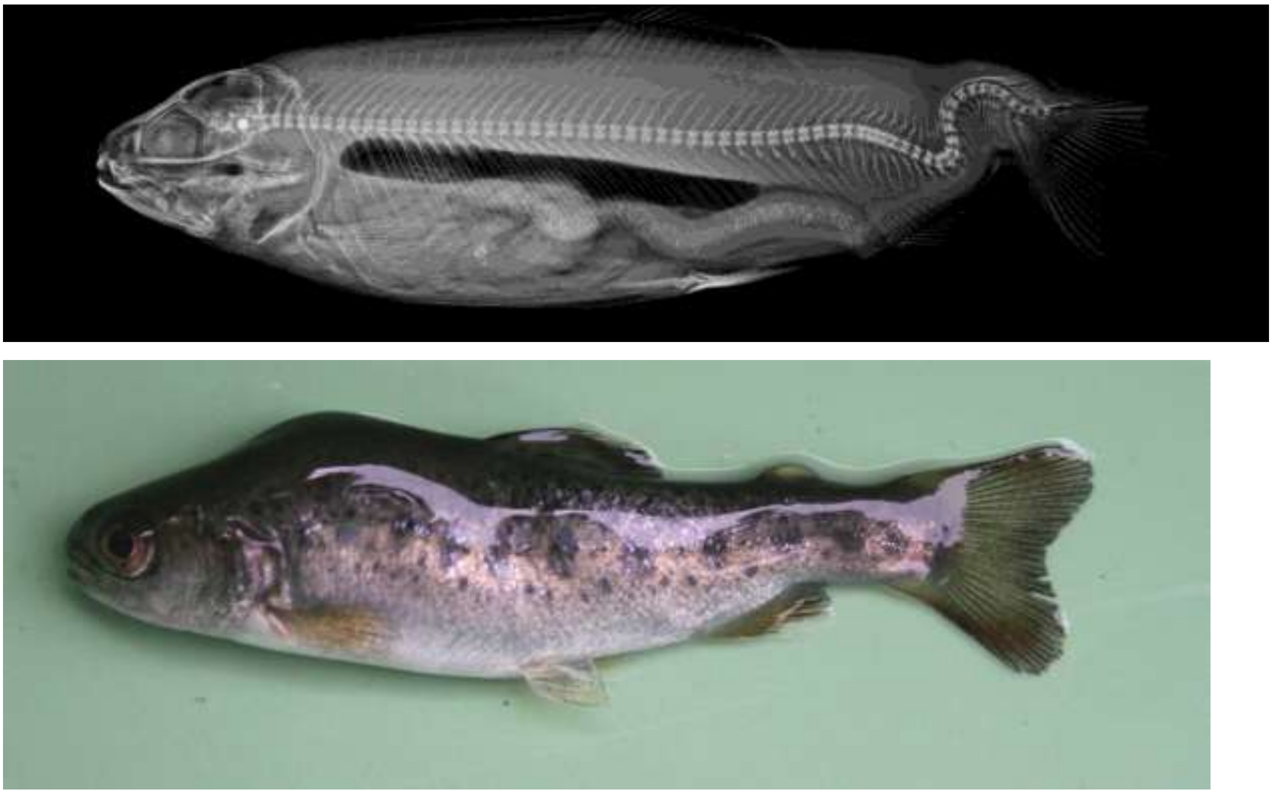

Figure 23: Multiple axis deviations (lordosis, kyphosis and scoliosis) of rainbow trout juvenile (approx. 5g)

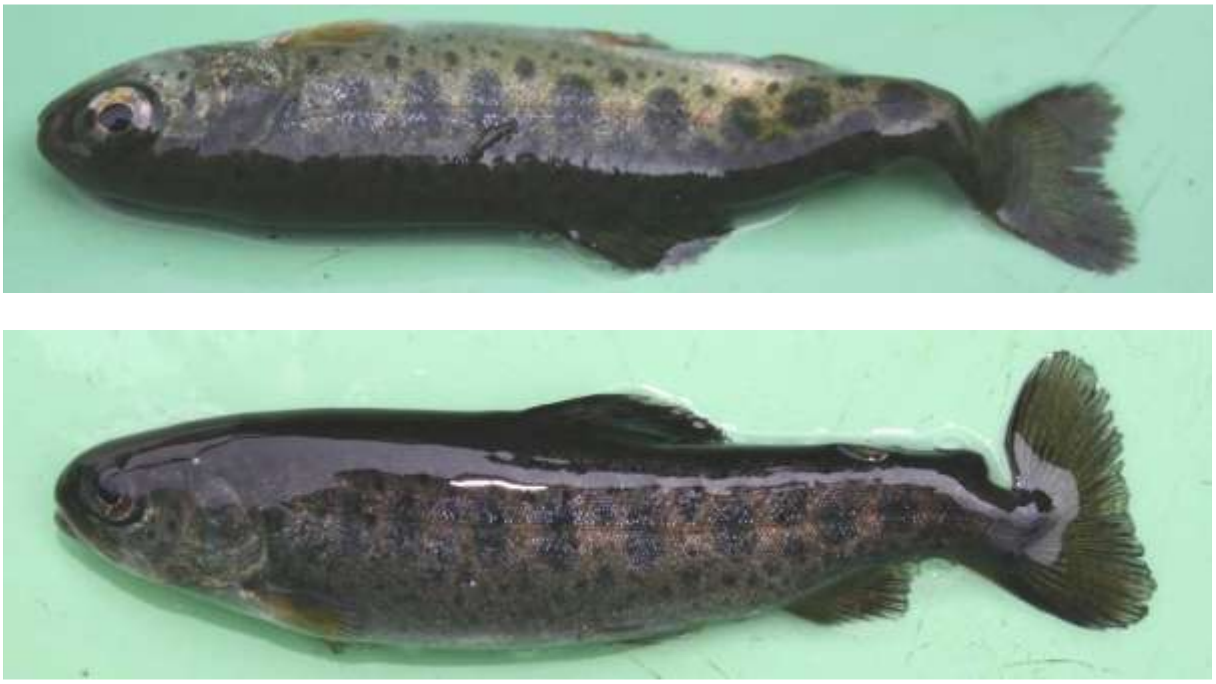

Figure 24: Axis deviation of tail. Upper: Scoliosis of tail. Lower: kyphosis of tail.

III. Developmental malformations

In the following, a number of conditions which are assumed to be caused by disturbances during embryonic development are shown. This can be a causal factor also for some of the malformations mentioned above, but both fusions type conditions and axis deviations are likely to have a more complex aetiology.

Tail fin malformations

Figure 25: Arrested development of tail fin, fin completely missing.

Figure 26: Arrested development of tail fin, fin partly missing ventral part (upper image) and dorsal part (lower image).

Figure 27: Twisted tail fin.

Strictures

In Salmonids, fish with deep indentation of the body mass are sometimes seen. Dark pigmentation of the skin at the site of the indentation is also common. The cause or causes for this condition is not known. It is sometimes assumed that they result from trauma, but field observations point towards developmental aberrations as a more likely cause.

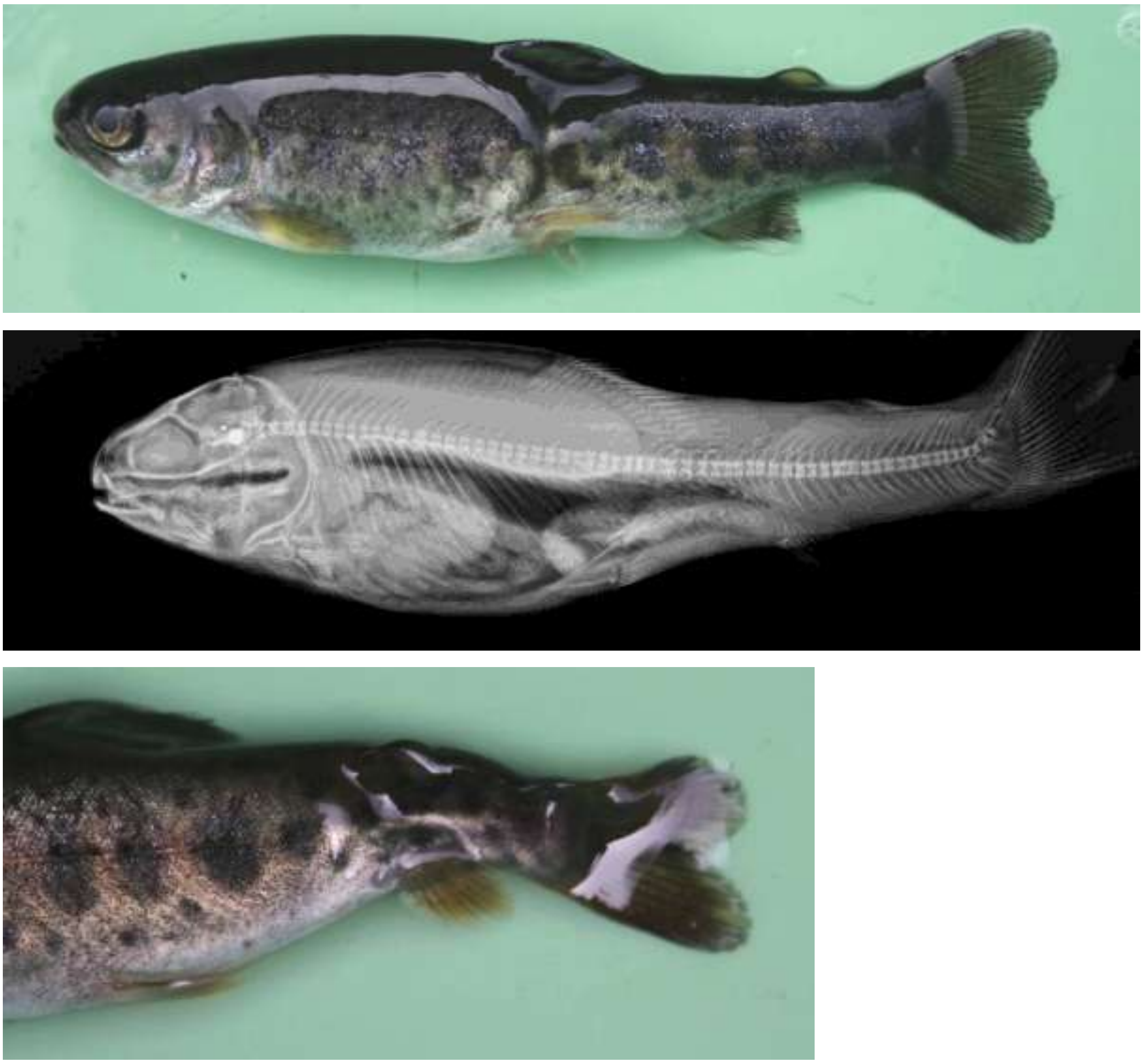

Figure 28: Body strictures in rainbow trout. Body mass is missing, resulting in deep indentations. Vertebral fusions commonly appear in severely affected areas.

Operculum

Missing development of operculum may be seen in fish as early as first feeding, indicating that this condition result from a developmental failure rather than from suboptimal rearing conditions as commonly assumed. The distinction of arrested development from erosions that arise later is not clear. The best indicator is the time of onset, and the absence of bleeding and erosion.

Figure 29: Missing development of operculum in juvenile rainbow trout (approx 5g)

Malformations of cranial structures

Malformations of cranial structures are not uncommon in rainbow trout., although rarely in high numbers. There is a considerable variation in morphology of the head and snout in farmed rainbow trout, and the distinction between a normal bluntness and an abnormal shortening of the snout is not always easy. Rainbow trout "pugnose" is, however, even more severe than the corresponding condition in Atlantic salmon, and easily identified.

Figure 30: Normal head of a 20g rainbow trout.

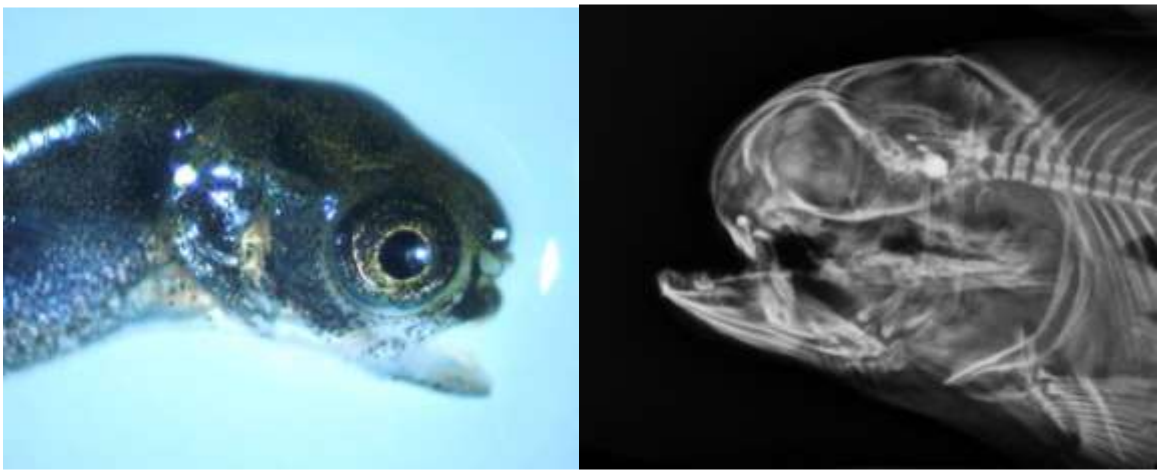

Figure 31:"Pugnose" in rainbow trout, photography of start feeding fry to the left, x-ray of 20 g fish to the right.

Figure 32: Extreme case of maxillar deformity in rainbow trout. Fish size approximately 5g.

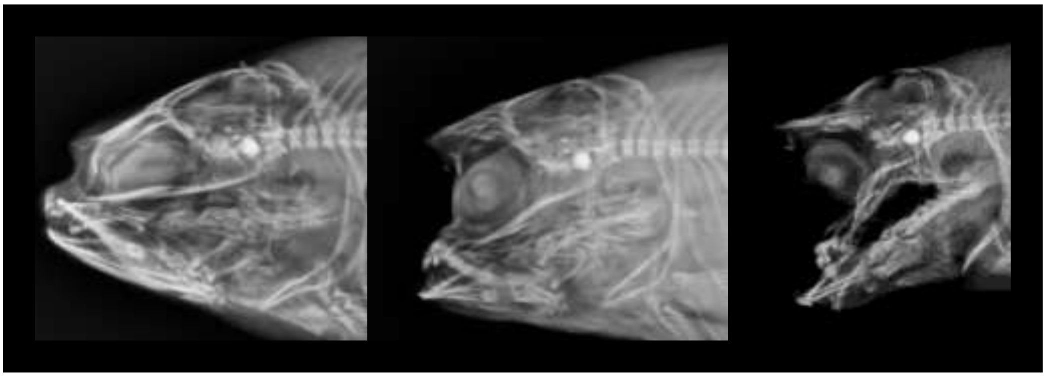

Figure 33: Three different degrees of severity of maxillar malformation observed in radiographs. The lowest degree at the left is almost normal except from a part of the premaxilla covering the nostrils is missing, in the following images more severe cases of the same deformity, all in 20g trout.

Malformations of the lower jaw, the mandible, and associated structures are also observed. A shortening or dislocation of the mandible can be identified at first feeding, but is rarely seen at later life stages. A protruding lower jaw, or undershot, can also be found, but is not common.

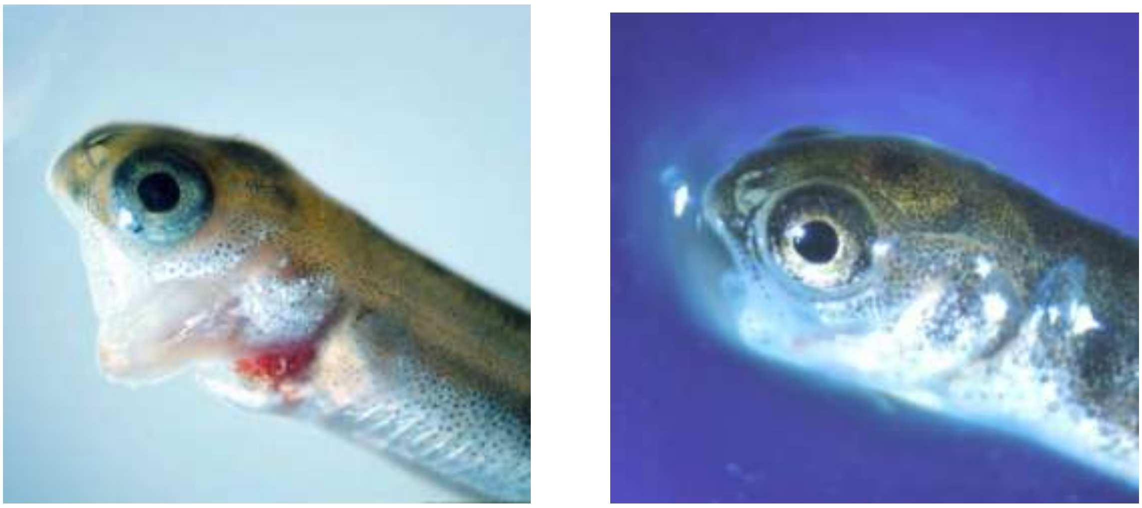

Figure 34: Malformations of the lower jaw in first feeding fry. Left: Gaping jaw. Right: Short lower jaw.

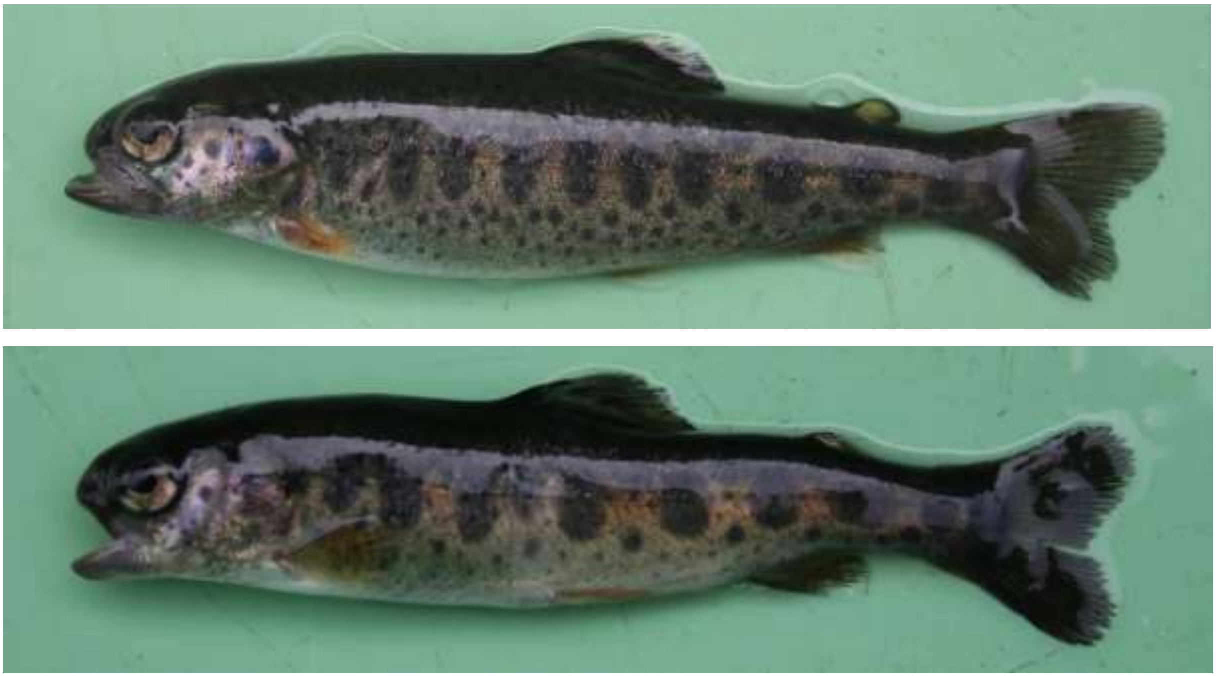

Figure 35: Malformations of the lower jaw in rainbow trout juveniles. Upper: Lower jaw longer than normal. Lower: Lower jaw fixed in a gaping position.

Twin malformation

Incomplete separation between twins is not uncommon in rainbow trout. Siamese twins will rarely survive past first feeding. In cases where one of the twins is incompletely developed, the rudiment of the incomplete twin can be present without any obvious effects on the complete twin up to harvest size.

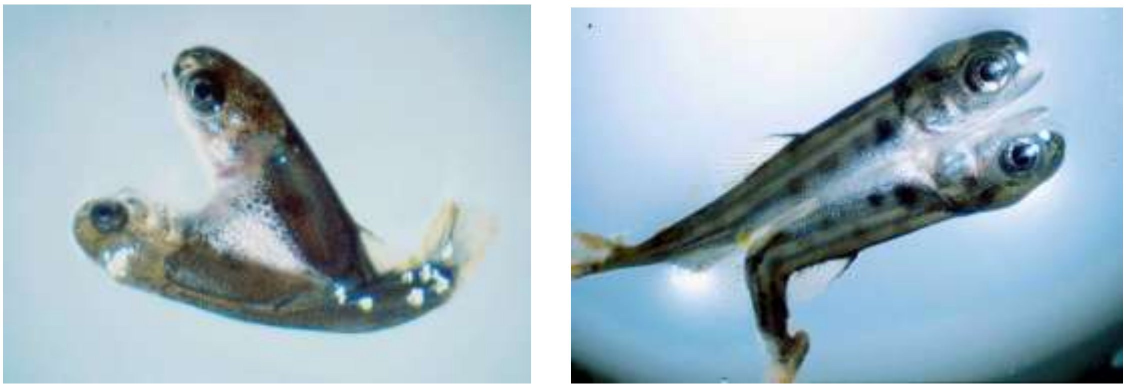

Figure 36: Siamese twins in first feeding rainbow trout fry.

Figure 37: Incomplete twins in rainbow trout. Incomplete twin can be identified by presence of rudimentary fins and excess body mass on ventral side of fish. Also pigmentation of rudimentary twin is similar to dorsal skin.

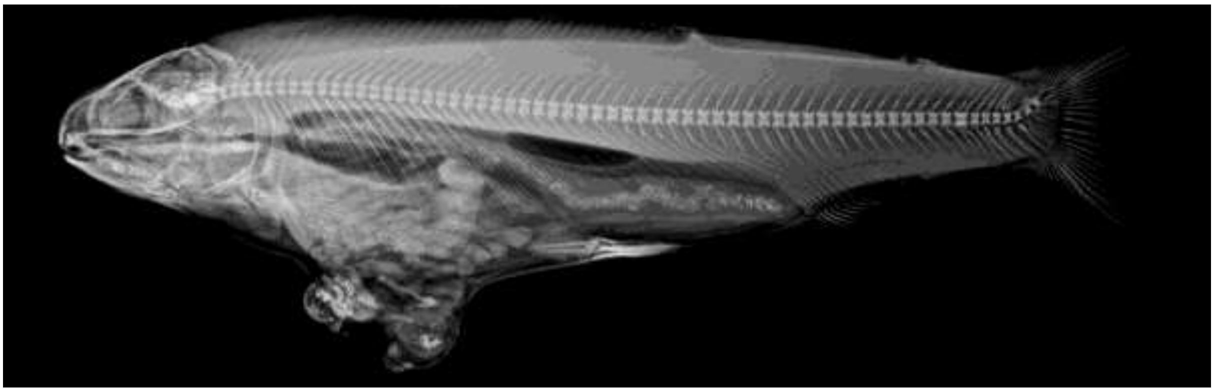

Figure 38: Radiograph of a rudimentary twin with calcified tissue and fins attached to the abdomen of a normal fish.