



**FINEFISH Collective Research Project
(Contract N°012451)**

**Malformations in farmed fish
Guidelines for classification**

(WP2: Standardisation of Environmental Fish Monitoring)

II. Cod (*Gadus morhua*)

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Cod (*Gadus morhua*)

The cod industry is less than ten years young at this point in time, and as a consequence, less is known about the prevalence of malformation and what types of malformations are likely to occur.

Diagnostic procedures

X-ray is the most reliable of the diagnostic methods available. In cod, good images can be obtained at 1g size and onwards, if using appropriate equipment and procedures. Examination of earlier life stages (<1g) is less reliable than in sea bass and sea bream, due to the fact that most of the different pathologies will continue to develop and new ones will appear with time. In cod, external examination will detect only the most severe cases. In absence of 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, 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

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

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 (taken from the Norwegian national cod malformations survey):

1-5 g size Will detect malformations induced during embryonic development, and those induced during first feeding

20-50g Before transfer to sea cages

0.1-1 kg Samples from sea cages. Valid as predictions of harvest result

Optional: Additional sampling just after weaning, staining with alizarin red.

Normal

The cod spine normally consists of approximately 50 vertebrae, and there is a certain regional and age variation in vertebral morphology along the spine. The spinal column in a normal cod is fairly straight. Seen in lateral view on X-ray, the axis continues into the head, with the palatine bone in the skull tilting slightly downwards in cranial direction.



Figure 1: Normal cod spine with the head tilting slightly down compared to the spinal axis.

Malformations of the spine in cod

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

The significance of some of the malformations is uncertain in terms of impact on product quality and consequently, value of product. The malformations must be quite severe to be identifiable on gross examination. As practically all farmed cod is sold as whole fish, and not subjected to automatic processing/filleting at this time, they may be of little consequence to the producer. This is likely to change with further development of the industry, and any vertebral pathology should be given full attention when searching for causal relations. Also, repeatedly radiographed material demonstrate that vertebral malformations induced at an early life stage may aggravate as the fish grow. Thus, any malformation represents a potential deformation of the fish and needs to be taken into account.

I. Fusions and fusion associated pathology

The term fusion is used when 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. Fusions are commonly seen in farmed cod and appear to develop as the fish grows by including neighbouring vertebrae into the fusion.

Complete fusion with shape restoration

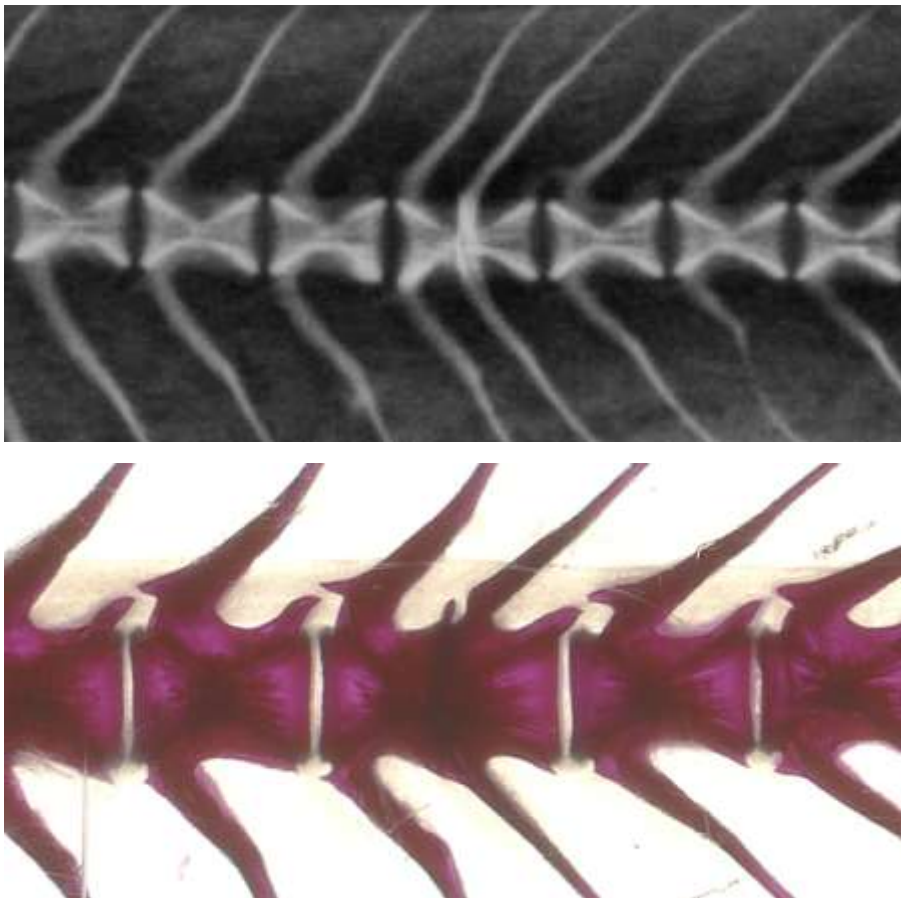


Figure 2: A well organized fusion in two cod vertebrae shown by radiography and in an Alizarin red coloured spine.

Multiple vertebrae fusion

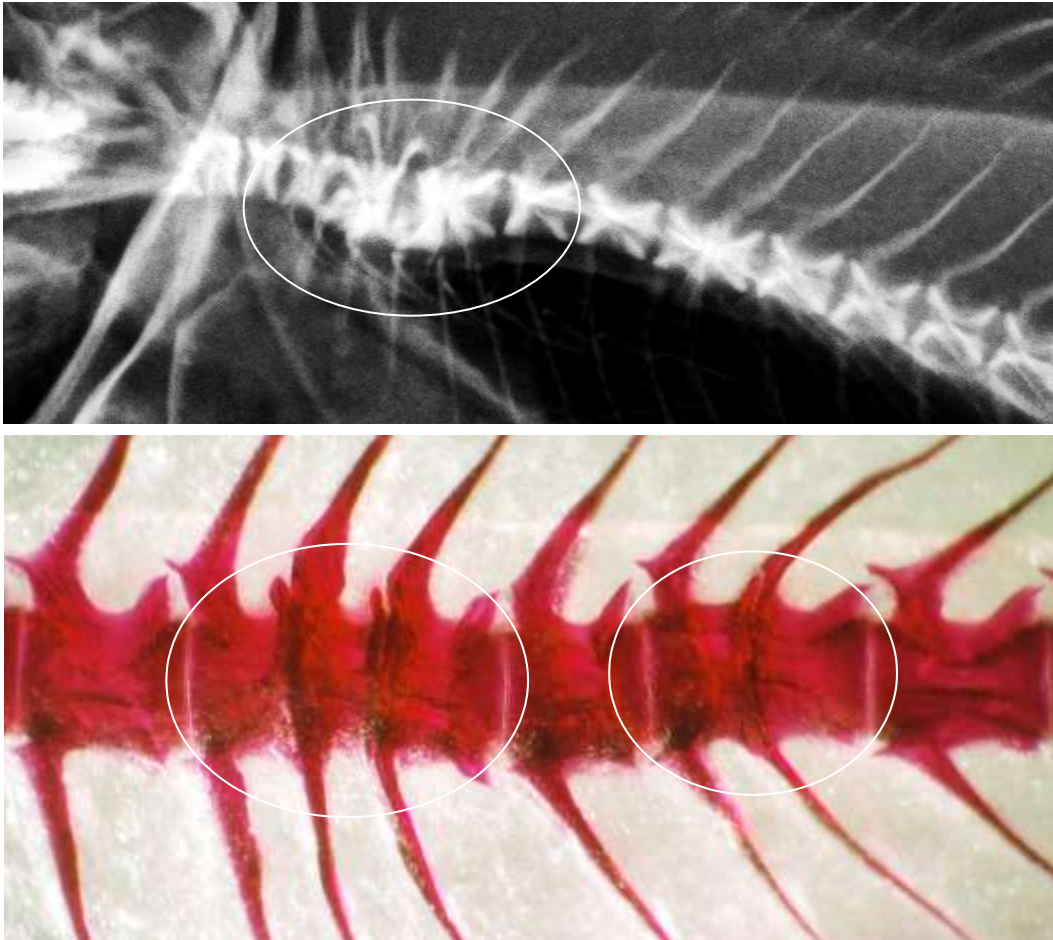


Figure 3: Examples on multiple vertebral fusions. The x-ray shows five fused vertebrae in the neck of a cod. The Alizarine red staining show three fused vertebrae to the left and two to the right.

Special case: Fused vertebrae in tail region

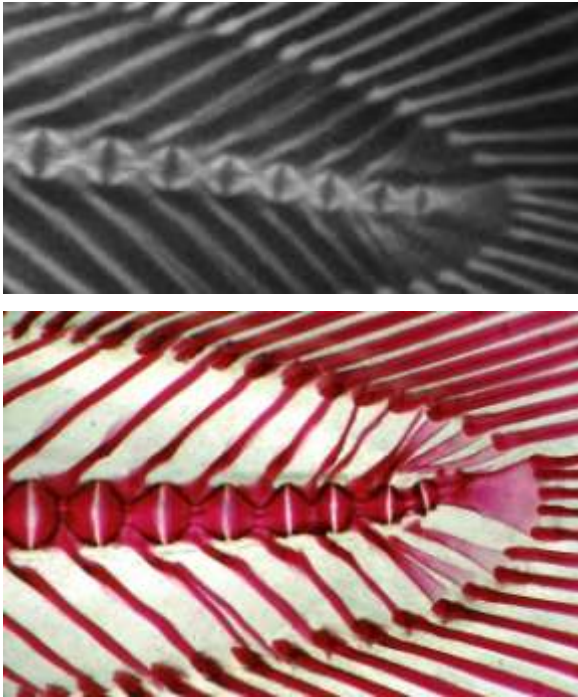


Figure 4: Fused or non separated vertebrae in the tail region of a cod. These appear regularly in many species, and do not seem to have any severe consequences. They may be a part of the normal differentiation into tail vertebrae.

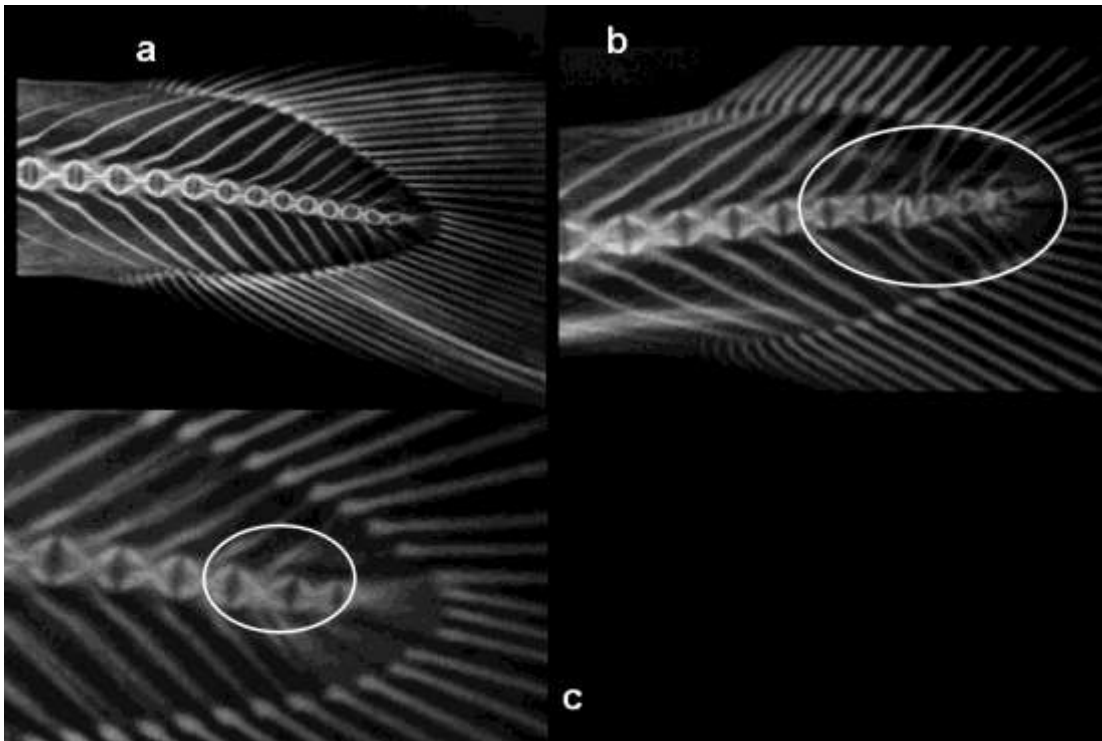


Figure 5: Normal cod tail (a), tail with two unorganized fusion processes (b) and tail with an organized fusion (c) The fusions in picture b seem to be an active process.

II. Platyspondyly

In platyspondyly, vertebrae are flattened and appear to be compressed. Unlike fusions, the individual vertebrae are distinct, even though the intervertebral space may be narrower than normal.

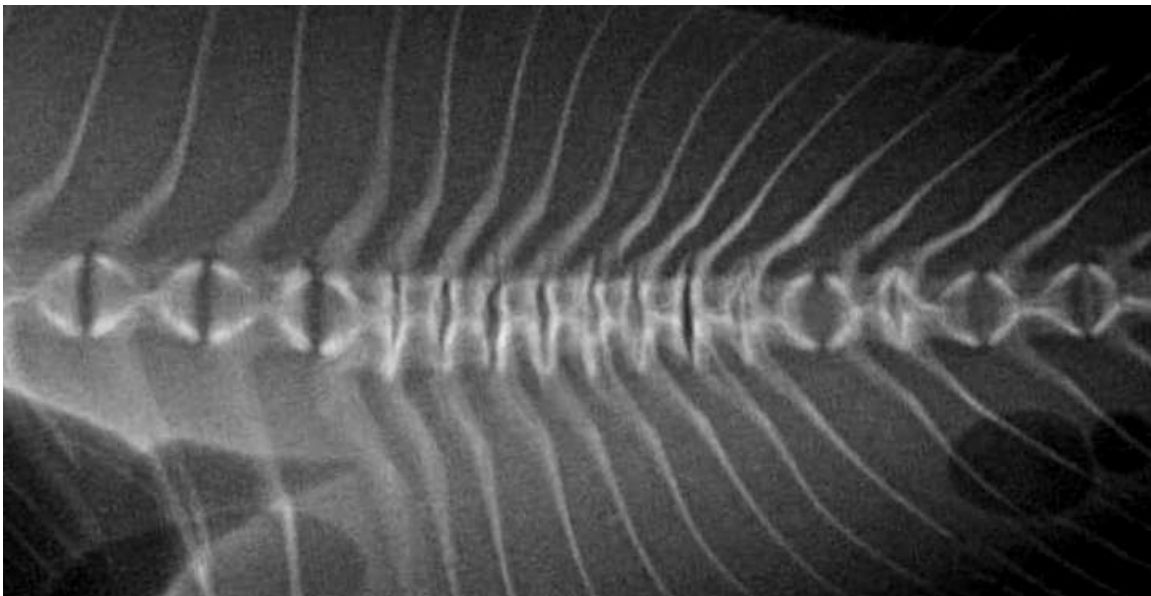
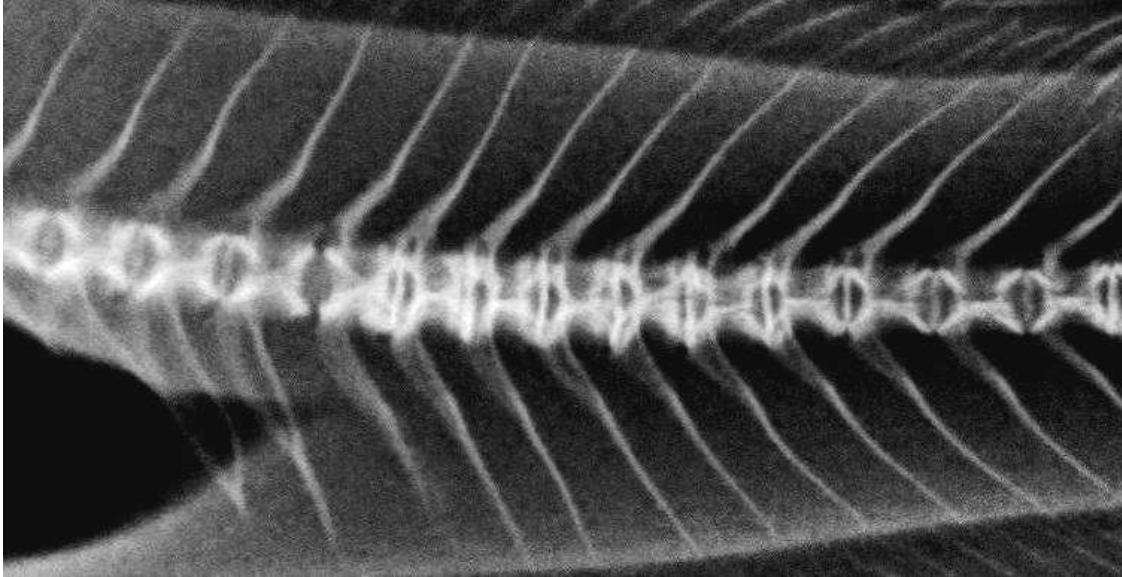


Figure 6: Platyspondyly in the spine of a cod. The pictures show two stages of development in the same fish with eight months interval. This malformation usually appears in the caudal half of the spine, and may be detected on gross examination as a shortening of the body.

III. Vertebral shape irregularities

In some cases, vertebrae have irregular morphology, without being easily classifiable. The irregularities would mainly be described as asymmetries. The final outcome of these changes may be fusions or platyspondyly, or they might normalize.

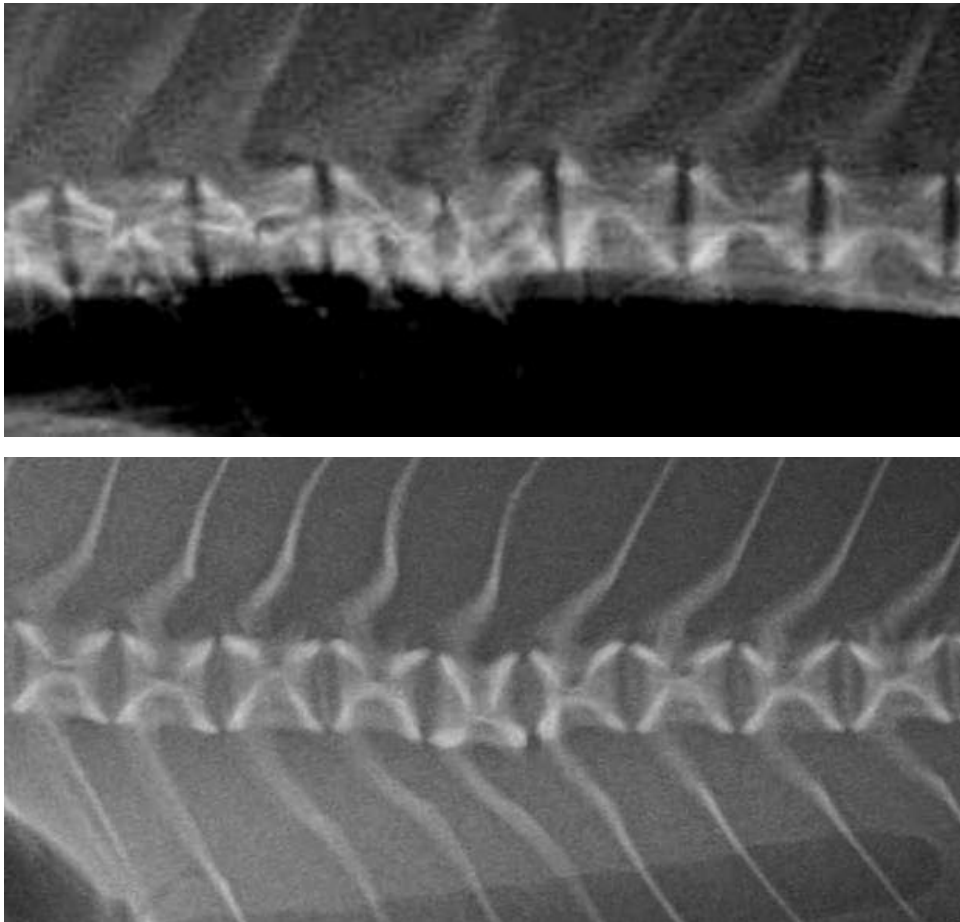


Figure 7: Irregular vertebrae in a cod spine. These may appear all along the spine, sometimes in combination with severe deformities.

IV. Axis deviations

The spinal column in a normal cod is fairly straight. Seen in lateral view on X-ray, the axis continues into the head, with the palatine bone in the skull tilting slightly downwards in cranial direction. Axis deviations are among the most common deformity problems in cod production

These malformations are the most severe in relations to current commercial production. Fish groups with these malformations included are presently marketed, but this is likely to change in future. Also, any fish group where fish with axis deviations can be detected on gross examination is likely to include additional fish with the same problem present in a less severe pathology.

Deviation of vertebral axis in neck region

Common name: Nakkeknekk (Norwegian), stargazer.

Due to a decreased upper angle between the palatine bone and the spine, the head bends upwards in variable degrees. In the least severe cases, the external diagnosis may be uncertain. This condition can be complicated by severe vertebral deformities in the cranialmost vertebrae, as well as other axial deviations such as lordosis, kyphosis and scoliosis in the cranial part of the spine. One way of measuring the palatine-spinal angle is to draw the lines from the point where the nasal bone is at its lowest, the centre point in the transmission between the palatine bone and spine, and finally to the intervertebral space between vertebra 6 and 7. The upper angle in this “V” serves as a useful measure for the neck angle. In normal cod, this angle is bigger than 180 degrees.



Figure 8. Extreme case of “stargazer”, grade IV.

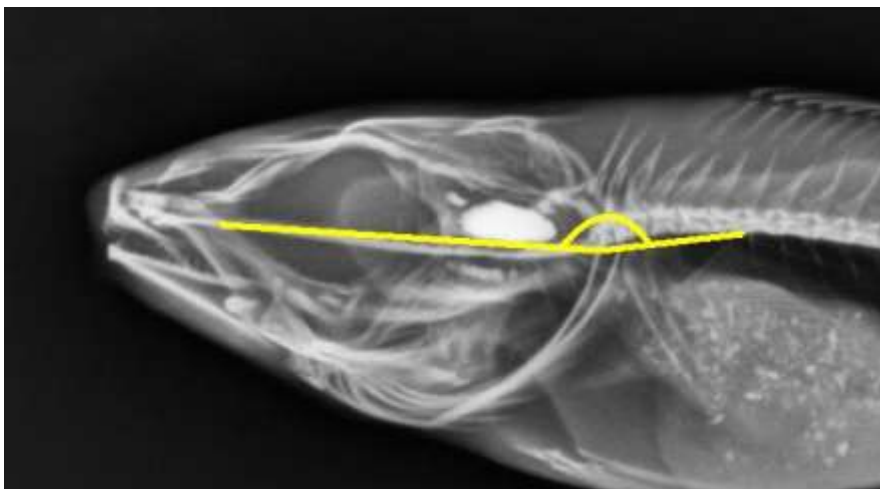


Figure 9: Measurement of the palatine-spinal angle as described in the text.

Another way of grading the degree of neck deviation is judged by subjective, visual grading, as we have used in the national survey for cod deformities.

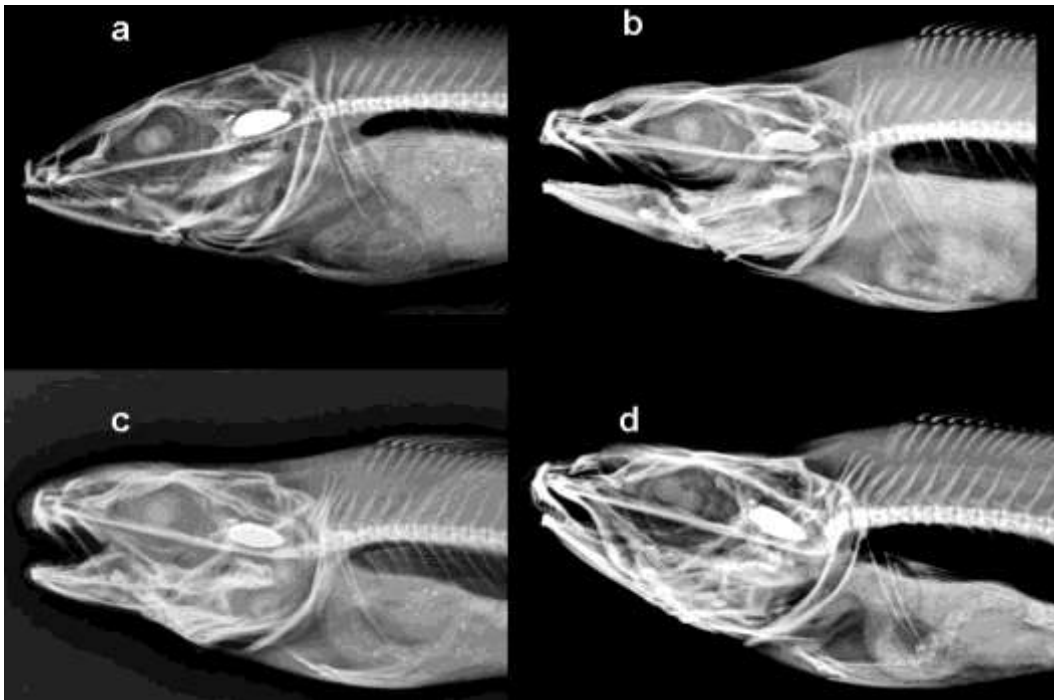


Figure 10 a) Normal. b-d) Graded according to scale used in Norwegian national cod deformities survey. b) grade I, c) grade II, d) grade IV

NB! Caution if X-raying small, formalin fixed specimen. Shrinking during fixation may cause artefacts.

A development of the condition with time has been demonstrated (Fig 11).

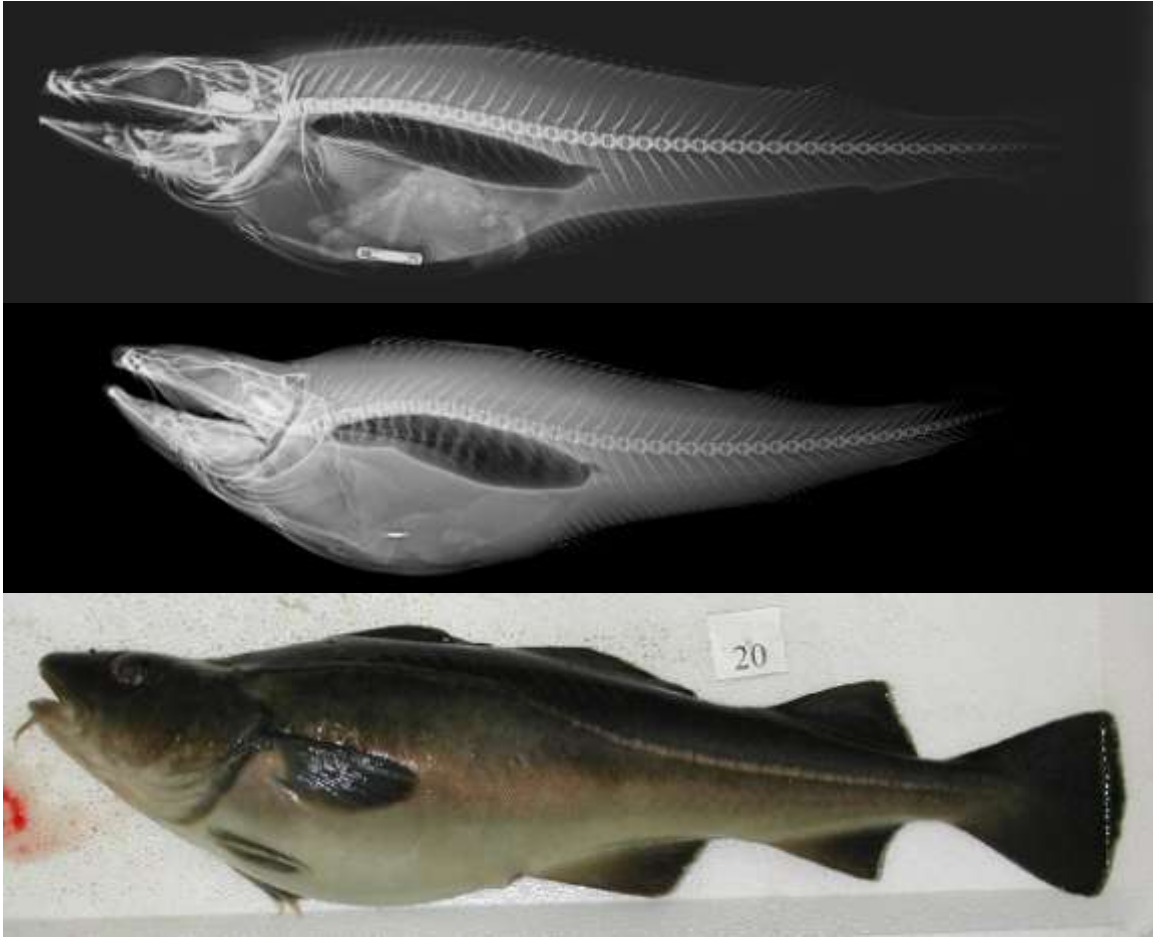


Fig 11. Development of pathology in neck vertebrae with time. Three images of the same individual. The top X-ray taken at transfer to seacage, approx. 50g size. The second X-ray was taken one year later, at approx 1 kg size, and demonstrates development from grade II to grade III. Lower photo shows external appearance of fish at 1 kg size.

Lordosis

The vertebral axis is bent upwards in caudal direction to variable degree, commonly seen in the region of vertebra no 20-25 (haemal lordosis) but also in other parts of the spine. The angle must be of a certain magnitude in order to see the deformity on external examination. This axis deviation often includes vertebral changes in the same area.

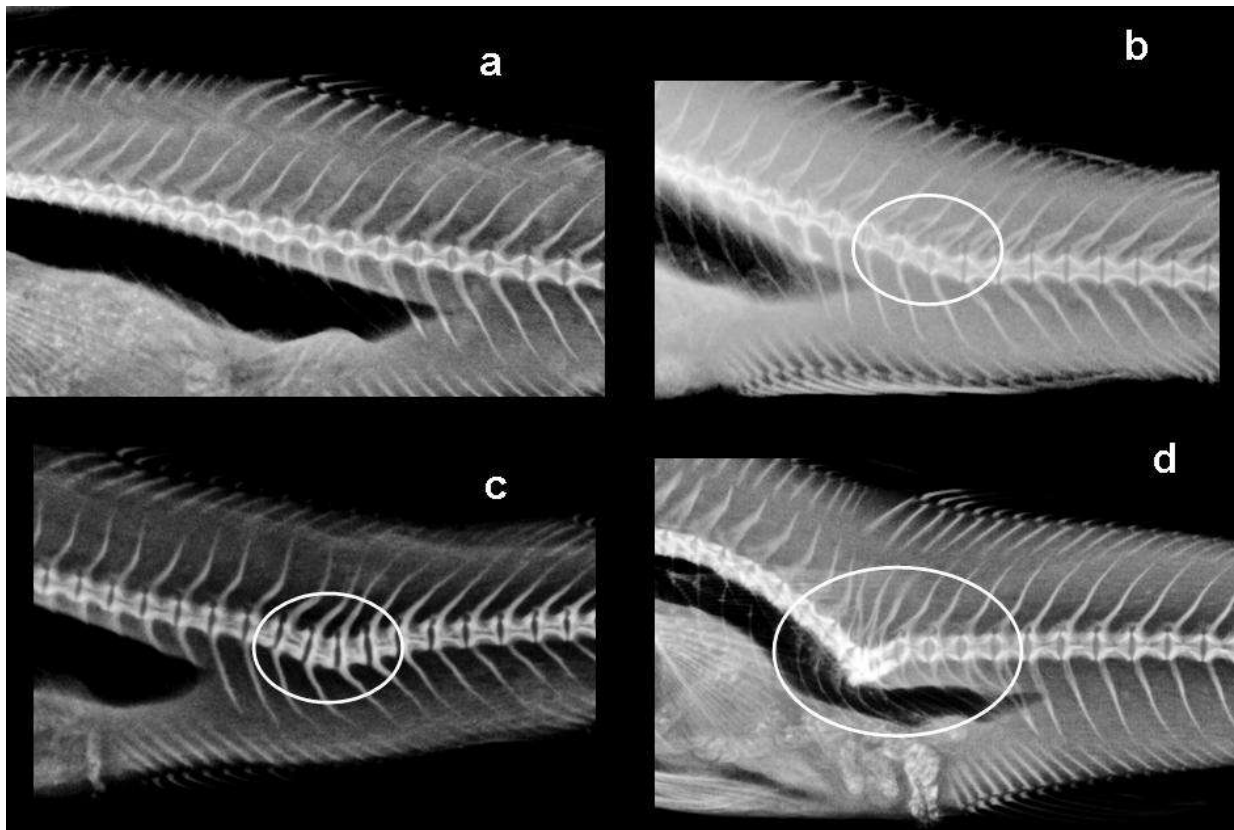


Figure 12: Haemal lordosis in cod. Normal spine (a), lordosis of degree I, II and IV respectively (b, c, d). The grading is according to the scale used in a Norwegian national cod survey. The lordosis in picture c and d also include vertebral changes.

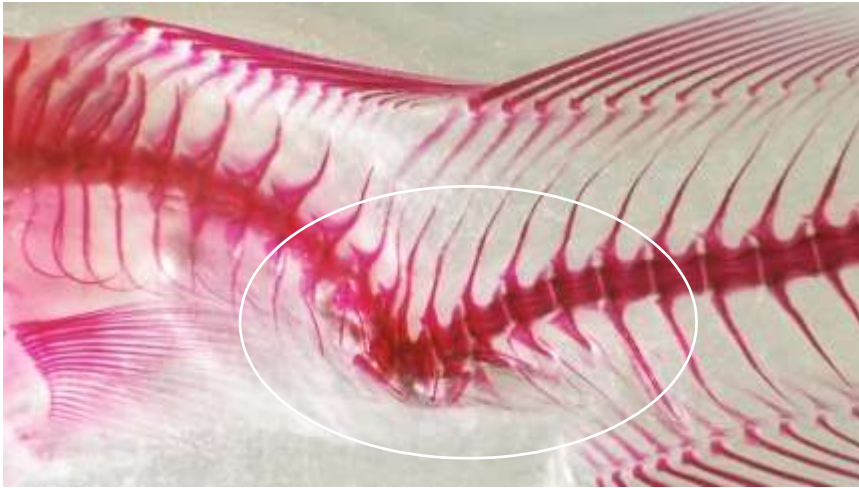


Figure 13: Alizarine red staining of the same fish as shown in figure 10 d. Severe vertebral changes are clearly visible.

Another way of measuring lordosis is by simply measuring the angle between the eight most involved vertebrae in the lordosis, centred in the intervertebral space at the “knick” point.

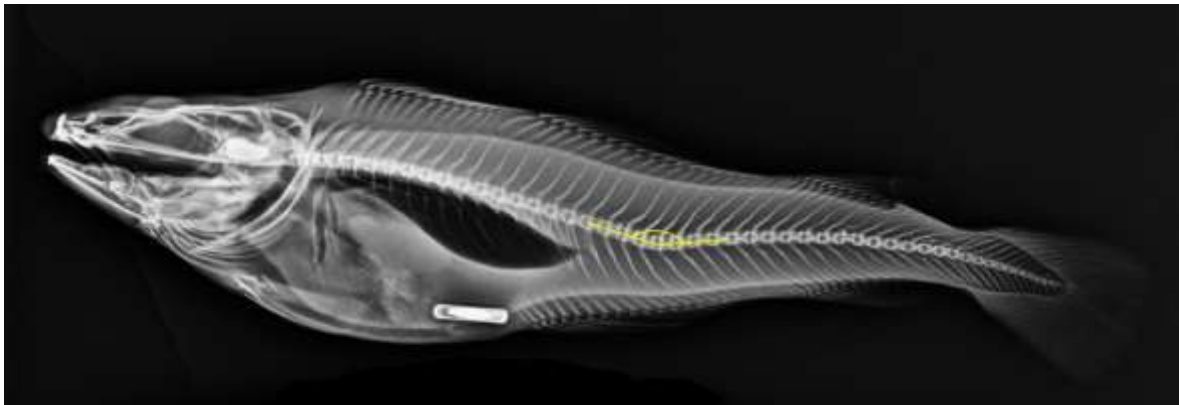


Figure 14: Lordosis measured by the upper angle, four vertebrae to each side of the centre point.

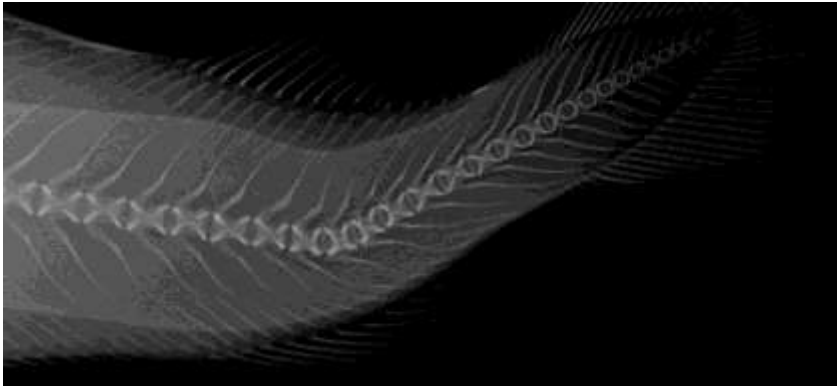
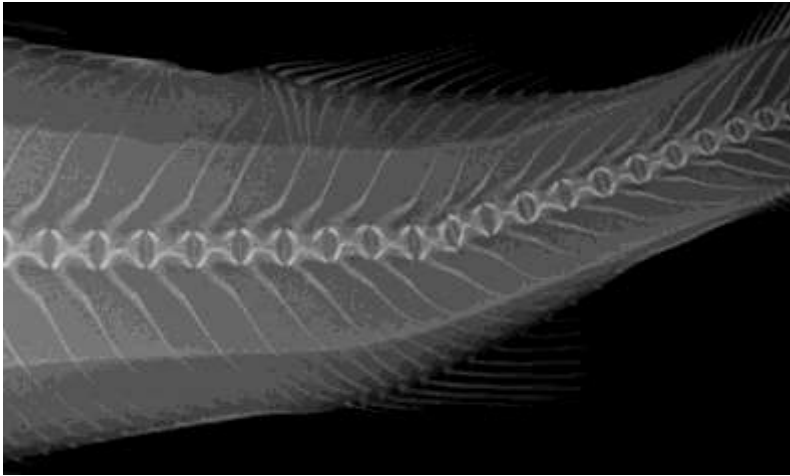


Figure 15: Two degrees of severity of caudal lordosis in cod. Both include vertebral changes.

Kyphosis

Kyphosis is a downwards bending of the caudal spine that may appear together with other deformities or alone. It is mostly seen in the cranial part of the spine, but appears all along the spine. This deformity is far less common than the lordosis.



Figure 16: Kyphosis in the cranial part of a cod spine.

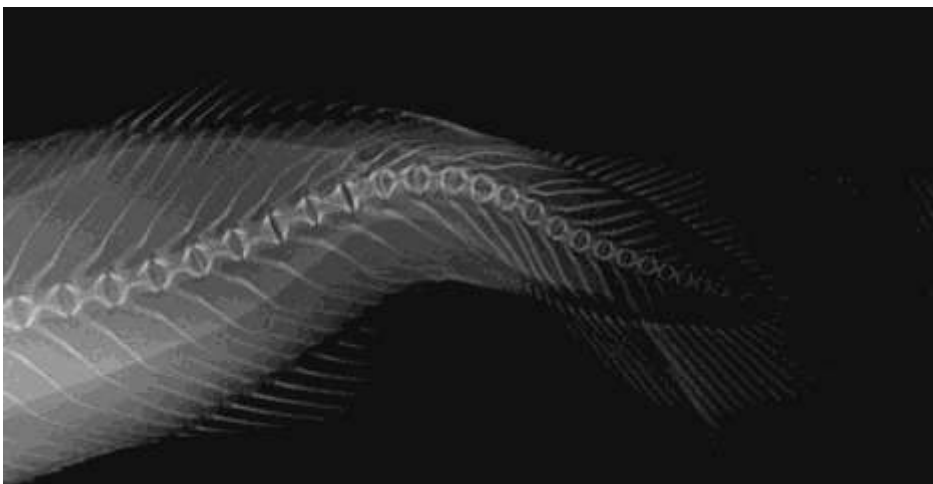


Figure 17: Kyphosis in the caudal part of the spine.

Scoliosis

Scoliosis is the sideways bending of the spine. This is easily observed in live fish, and is seen in some fish groups. It is not a very common deformity, and easily sorted out. Scoliosis is best observed from the dorsal or ventral side of the fish, alive as well as on radiographs. Since most radiographs are taken in a lateral view, less severe scoliosis can be misjudged as poor preservation of the specimen or rigor mortis in a non-flat position.

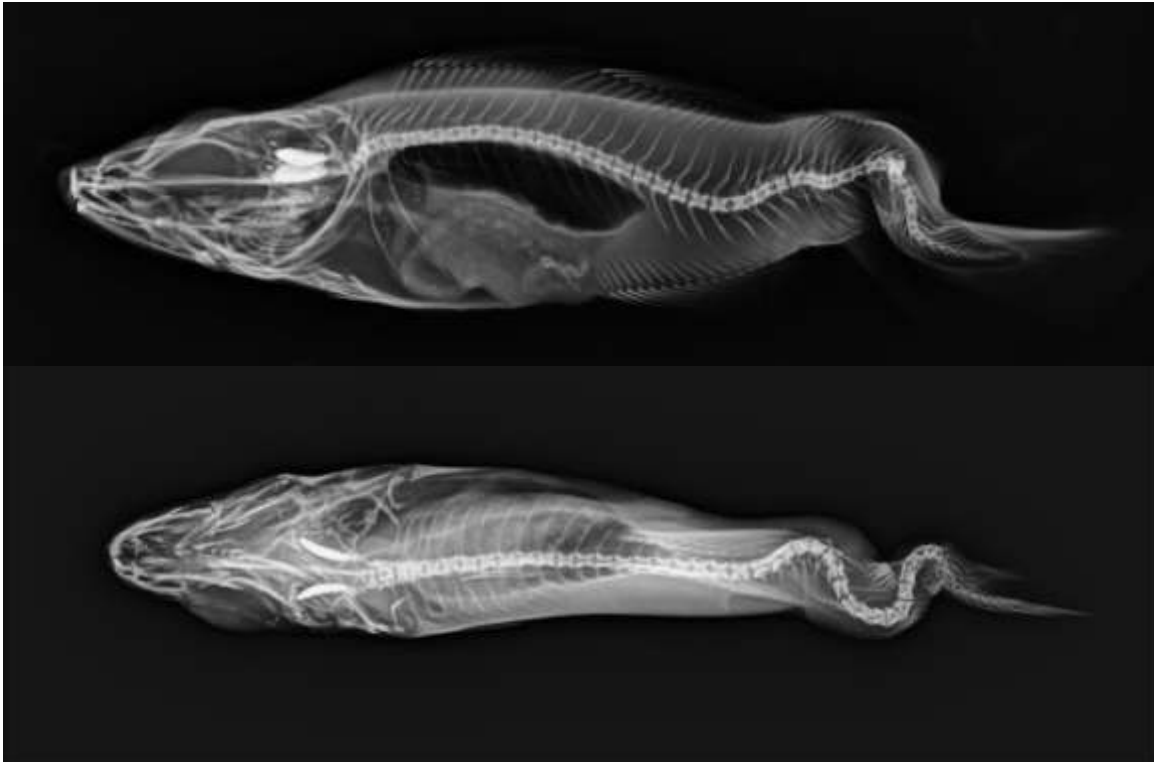


Figure 18: Lateral (top) and dorsoventral (bottom) projection of severe scoliosis in cod. As observed, the lateral image can be misjudged to be a kypholordosis with fusions.

V. Malformations of skull and jaws

Malformations of the skull and jaws is not widely recognized, but in screening of fish from experiments and from commercial productions, some deviations have been identified.

Palatine bone curvature

This is seen in radiographs as a curve in the palatine bone viewed from the side.

Pughead

Abnormal shortening of the maxilla.

Deformed lower jaw

Deviations in the shape and size of the lower jaw bones. A common variety of this deformity is a non-severe undershot, where the lower jaw and lip is protruding (figure 20) According to producers, the protruding lower lip may become sore and inflamed, probably because of trauma. This phenomenon is to our knowledge not observed in bigger fish kept in sea cages.

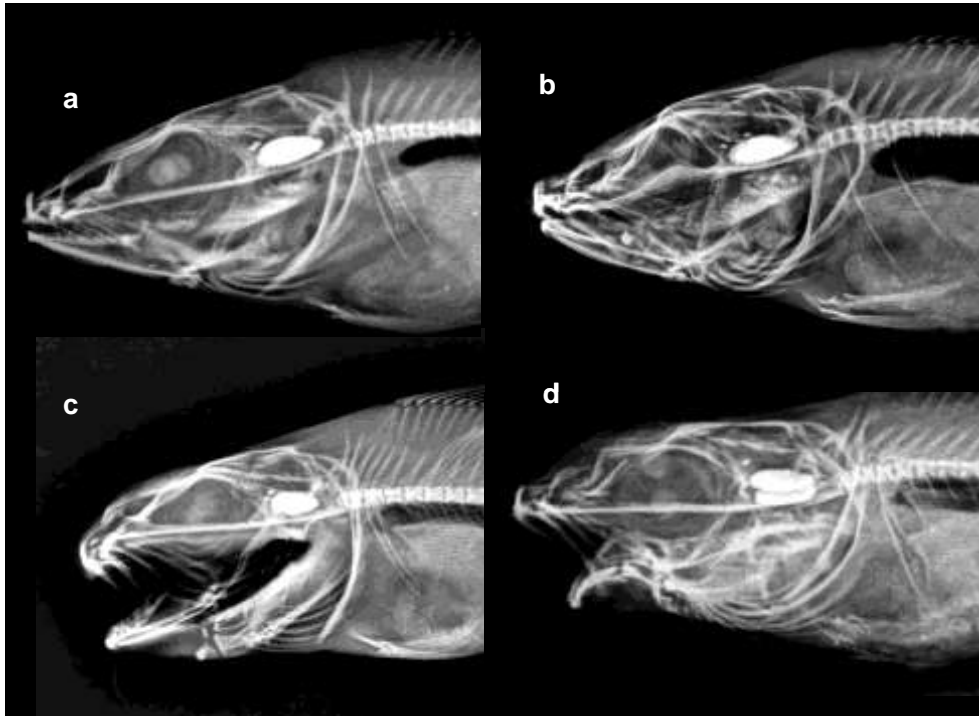


Figure 19: Normal cod head (a), curved palatine bone (b), pughead (c) and deformed lower jaw (d).

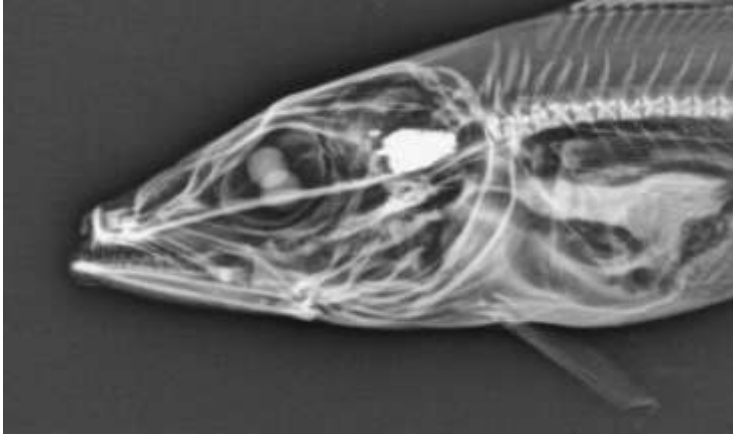


Figure 20: Undershot in farmed cod. The protruding lower lip can suffer trauma in the tank environment and be sore and object for secondary infections.

FINEFISH project

1. Protocol for sampling of larvae (day 30-35) and juveniles (up to 5 gram)

A common sampling protocol is required for the SMEs and the scientists in the FineFish project. Each partner needs to use the same protocol to minimise variation introduced by sampling. It is proposed that this protocol be used for sampling Seabass and Seabream but also for the other species in the project. In fact it could become a project “benchmarking” procedure. The procedure described below can be applied both for staining and mammography of young fish.

NB!!

Make sure that the fish is well packed and labelled before transport! The fixed samples can be sent as regular post parcels.

Please give information about:

Company/location

Date of sampling

Species

Number of fish

Fish size

Contact person (name/e-mail address/telephone number)

Fixation methods

Samples taken at day 30-35 must be fixed in 4-5% phosphate buffered formalin (pH=6.8-7.0, according to Markle 1984). This solution (phosphate buffered formalin) is commercially available through the pharmacy. After 24 hours, replace fixative with 70% ethanol.

For transport, close container lids firmly, and do not fill vials to more than 50% of total capacity.

Samples taken at 1 gram may be preserved at -20°C if they are to be radiographed locally. Samples that will be sent long distances must be fixed according to the same protocol as for 30-35 days old fish.

Sample size

Samples of small fish (day 30-35 and 1 gram) must have a size of ca 200 fish, and be randomly selected from the population (i.e. with low water level in the tank).

Anaesthetics

All fish have to be euthanized by an overdose of an anaesthetic until there is no more movement of the gills for at least a couple of minutes. This is partly to make sure the fish is humanely killed, partly to assure the fish do not gasp at the moment of death and thus ruining the image.

Any commonly used anaesthetics can be used, for example MS 222.

2. Protocol for sampling of fish larger than 1 gram for mammography or x-ray

NB!!

Make sure that the fish is well packed and labelled before transport!

Please give information about:

- Company/location
- Date of sampling
- Species
- Number of fish randomly sampled
- Number of fish sampled due to aberrant exterior
- Fish size
- Contact person (name/e-mail address/telephone number)

Sample size

Samples of fish >5 gram should have a size of 100 fish and be randomly selected from the population (i.e. with low water level in the tank).

Freezing of fish (>5 gram size)

- ✓ Fish for radiography must be killed with an overdose of anaesthetic before being frozen flat. This is important to avoid the fish “gaspings” in the moment of death, as this ruins the neck angles.
- ✓ The fish must be placed individually on a plastic surface, preferably with the right side down (not directly on Styrofoam, as the fish then will stick to the Styrofoam.), and frozen immediately thereafter.
- ✓ If there are individual recordings, please identify each fish with a number. The number should be written on water resistant paper, and the number must be stitched to the fish tail (see picture below) or put under the operculum.
- ✓ After freezing, the fish should be transferred to labelled plastic bags and kept frozen. Please avoid over filled plastic bags!

Transport of frozen samples:

- ✓ Make sure that the receiver has been notified before you send any fish!
- ✓ Include the required veterinary certificates.
- ✓ The plastic bags must be placed in air-freight Styrofoam boxes together with cooler bricks or dry ice (please check guidelines with actual freight company when using dry ice). The boxes must be sealed well with tape, and take precautions against any liquid leaking from the package by using absorbent material for wrapping.
- ✓ Make sure that the full address of the receiver is written on the box.
- ✓ The boxes should be sent by Air-Mail as quickly as possible.
- ✓ Mark the boxes with “Biological material”.



Freezing of individually labeled fish on a plastic-covered Styrofoam board



Fish in zip-lock plastic bags, ready for radiography.