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Common diseases and parasites of fish in the North Atlantic: Training guide for identification

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1 INTRODUCTION

During the past 20 years, there has been an increasing number of field surveys investigating the occurrence and distribution of fish diseases as a tool for monitoring the effects of environmental changes, including marine pollution. Fish diseases are considered to be an appropriate indicator in this context because the outbreak of a disease represents an end-point of biological significance integrating all environmental factors affecting fish health.

In the beginning of the 1980s it was felt, in the scientific community involved in fish disease studies, that it would be useful on an international basis to compare and combine the results of the different groups studying fish diseases. This would then present an overall picture and evaluation of the health status of fish populations in the study areas. However, it was soon realized that the results on disease prevalences available for different fish species and different areas were often derived from studies using non-standardized methodologies; thus, the results were not comparable.

To meet this problem, two ICES Sea-going Workshops on the Methodology of Fish Disease Surveys were held, in 1984 and 1988, initiated by the ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) (Dethlefsen *et al.*, 1986; ICES, 1989). The major aims of these workshops were to set up recommendations for standardized methods for sampling, diagnosis, and reporting of fish diseases. Such standardization would enable investigators to meet minimum requirements which would allow international comparisons of long-term trends in spatial and temporal distribution patterns.

Arising from the workshops, the WGPDMO decided in 1990 that it would be useful to produce this Training Guide for the Identification of Common Diseases and Parasites of Fish in the North Atlantic, which should be published in a way that it could be used during work on board research vessels or under other field conditions.

The objectives of this Training Guide are to summarize the recommendations and conclusions of the Sea-going Workshops and, in order to enable a proper diagnosis, to present a set of photographs showing selected examples of common diseases in major fish species of the North Atlantic.

This Training Guide gives advice to specialists and non-specialists on the following topics:

- fish species suitable for disease monitoring;
- sampling procedures;
- disease examination procedures;
- diseases useful for monitoring purposes and their diagnosis and classification;
- reporting of results;
- statistical methods applicable for data analysis.

The procedures recommended and diseases illustrated in this Training Guide are mainly based on bottom trawl catches from the North Sea. However, investigators should be able to adapt the model to their own local situations, if necessary.

2 FISH SPECIES

The fish species chosen for monitoring should be selected because it is benthic, fairly static (outside the spawning period), abundant, and exhibits diseases which are easily recognized.

For the shallow waters (< 100 m depth) of much of the North Sea, the common dab (*Limanda limanda*) fulfills those specifications. For estuaries and coastal regions as well as the Baltic Sea, the flounder (*Platichthys flesus*) is fairly suitable.

Cod (*Gadus morhua*) was chosen as an additional species for disease monitoring. In the Baltic Sea, flounder may replace dab due to limited abundance of this species in the more brackish areas.

3 SAMPLING PROCEDURES

Sampling should be accurately positioned on a nominated latitude and longitude with all repeat hauls being within clearly defined limits, e.g., radius of 2-4 nautical miles. Sampling on a station should be based on multiple hauls, even in the presence of large numbers of fish. This is necessary to reduce sampling variation, i.e., haul-to-haul variation, the problems of patchiness, etc. Therefore, at least two hauls, but preferably five hauls, per station should be aimed for.

Sampling should be conducted on a long-term basis, once a year within the same narrow time window (2 weeks to one month) or, if possible, at two periods to provide separate data from the summer and winter periods. For dab, the non-spawning period (late summer to early winter, depending on geographical location) is recommended, since the spawning season is associated with considerable migrations of the dab between their feeding and spawning grounds (Damm *et al.*, 1991; Rijnsdorp *et al.*, 1993). Sampling during spawning times may, thus, not reflect the spatial distribution patterns of the diseases typical for most of the year. For flounder, sampling times should be restricted to mid- and late-summer, when the species tends to stay close to its nursery and feeding grounds. Sampling on a long-term basis should preferably be conducted using identical equipment (ship, gear, etc.) to minimize sampling variability.

Where resources permit, the minimum recommended sampling requirement should be exceeded in terms of areas, frequency, and numbers of fish sampled.

After each haul, fish species to be examined should be sorted, either from the total catch or from representative sub-samples. The sample weight should be measured and the length-frequency distribution should be recorded (total length to the nearest cm below), separately for females and males. Measured fish should be either completely examined for diseases, or sorted according to length into different size categories prior to examination. Sample sizes are based on statistical requirements (a total sample of 250 fish allows the detection of a disease prevalence of at least 1.5% with 95% confidence limits) and also the fact that prevalence may increase with fish length. For example, the minimum sampling requirements considered appropriate for dab, flounder and cod are as follows:

Dab	Disease examination	
Size group (cm)	External	Internal
15-19	100	
20-24	100	(50)
≥ 25	50	50
Total	250	50

Flounder	Disease examination	
Size group (cm)	External	Internal
20-24	100	
24-29	100	(50)
≥ 30	50	50
Total	250	50

Cod	Disease examination
Size group (cm)	External
< 29	100
30-44	100
≥ 45	50
Total	250

If 50 fish of the largest size group of dab and flounder are not available for internal examination on the sampling station, additional specimens to make up the total should be taken from the upper range of the middle size group.

Fish examined for liver nodules should be individually aged, based on age reading of otoliths in all cases.

If possible, age/length keys should be produced for different areas of investigation. This is especially important for flatfish when comparing disease data from different regions because growth patterns and, therefore, also the age composition may vary considerably between different localities.

Remark: Although the above procedures are based on bottom trawl catches, it may be more practical to use other methods of catching fish, depending on the species and habitat in which they live. For example, fyke or entangling nets are effective for catching flounders in estuaries, creeks and other shallow-water situations not suitable for bottom trawls.

4 DISEASE EXAMINATION PROCEDURES

The fish should be examined whilst fresh, i.e., shortly after they have been landed on the ship or taken from nets (not frozen or refrigerated). An area for working should be cleared, preferably a bench or table at standing height, with good lighting and running water.

Two people, at least, are needed for examining large numbers of fish. One person makes the examination and the other records (in pencil) onto an appropriate form or directly into a computer using the keyboard. (These positions should be interchangeable, so that both workers know what is seen and transcribed.) The procedures to follow are:

1) Take the fish, with bare hands (or wearing thin gloves), rinse it in clean water and, under a good light, examine it externally, and regardless of whether or not an anomaly is observed, record the total measurement of the fish and its sex. Any external lesion should be recorded. Examine the gills by lifting up the operculum; if they are bright red they are normal, otherwise describe any lesion.

2) For internal examination of dab, flounder or other flatfish: place the fish (underside downwards) on the table and make an incision on the upper side with a sharp blade from the pectoral fin to the outer edge of the abdominal cavity. With a finger, pull out the intestine, and the liver (and spleen) will be clearly visible. Carefully dissect with a blade around any adhesions, and the liver will come free. Examine the liver on both surfaces. Any nodules or unusual changes should be recorded, giving the size of lesion (only those ≥ 2 mm diameter are recorded for ICES). For confirmation of liver anomalies, and any other changes, the affected area, including some "normal" tissue should be carefully dissected (up to 5 mm thick pieces only) and placed in a jar of 10% neutral buffered formalin or Bouin's fluid for preservation and subsequent histological examination (see Appendix). When that fish has been examined, move on to the second fish, and so on, until the quota has been completed.

Note 1: It is advisable to make the sample from the first haul a practice run, and not count the results on the final reporting form. This should sort out any problems which may arise, especially for persons not working regularly at sea. It could be useful to conduct an intercalibration using this sample if more than one person is to be involved in the disease diagnoses.

Note 2: It is advisable to complete the examination of each trawl haul before the next haul is landed. With hauls coming in close together, or of short duration, timing is critical. Additionally, most research ships work to strict timing, including meal breaks, therefore planning between the scientists and the crew is necessary to keep harmonious relations.

5 DISEASES USEFUL FOR MONITORING PURPOSES

The diseases illustrated in this Guide are those that:

(a) occur commonly in some or all of the selected fish species;

(b) are easy to recognize;

(c) have a possible response to surrounding environmental conditions;

(d) have a response that can be expressed in significant prevalence values.

For some diseases, clear "cut-off" points above the lower limits of visual detection make on-site diagnoses more uniform for the non-specialist worker. More detailed examinations may require detailed investigations which are outside the remit of this Training Guide.

In the following table, some examples of easily recognized diseases recommended by ICES for international comparison purposes are listed together with some other lesions and conditions frequently occurring in fish species of the North Sea.

Disease	Host	Minimum requirement for international reporting
Lymphocystis Figures 1, 2, and 3	Dab, flounder and other fish species	More than one surface nodule
Epidermal hyperplasia/papilloma Figures 4, 5, and 6	Dab and some gadoids	Lesions larger than 2 mm in diameter
Skin ulcers (acute and healing) Figures 7, 8, 9, and 10	All fish species	Open lesions
X-cell gill lesion Figures 11 and 12	Dab	One or more filaments affected
Skeletal deformities Figures 13, 14, 15 and 16	All fish species	Grossly or by filleting
Fin rot Figures 17 and 18	All fish species	Covering at least 5 fin rays
Pigment anomalies Figure 19	In flatfish, e.g., dab and other fish species	
Lipid metabolic disorder Figure 20	Dab and other flatfish	
Liver nodules/tumours Figures 21 and 22	Dab and flounder	Tumours larger than 2 mm in diameter
Pseudobranchial swelling Figures 23 and 24	Cod	Grossly observable
Visceral granulomas Figure 25	Cod and other gadoids	Grossly visible primarily in liver and spleen
Gear damage Figures 26 and 27	All fish species	
Tumour formation Figure 28	All fish species	

Diseases should be classified according to their degree of severity. A three-stage progression is recommended where applicable.

6 DISEASE DIAGNOSIS

6.1 Lymphocystis

DISEASE	BODY SITE	STAGES		
		1	2	3
Lymphocystis	On the skin surface and fins. Occasionally seen internally on the surface of visceral organs.	2-10 nodules including clusters of single nodules not exceeding a total of 10 mm (Figure 1)	Nodules/clusters of nodules covering a surface of up to twice the area of spread-out caudal fin (the reason for twice the area is because you check the fish for each side separately) (Figure 2)	Nodules/clusters of nodules covering a surface of more than twice the area of spread-out caudal fin (Figure 3)

Recognition: The white to reddish nodules can be situated anywhere on the body surface. Single nodules may be confused with some parasites, especially those located between fin rays. Therefore, the "cut-off" point is at least 2 single nodules. Any doubtful cases should be confirmed by histological examination. The nodules can be quite large, i.e., 2 mm diameter. They often occur in clusters, especially in flounder and plaice (*Pleuronectes platessa*).

Cause: Lymphocystis has a viral aetiology (Iridovirus) and the nodules are the result of hypertrophy of connective tissue cells, containing many virus particles. Many species of fish are affected, although mostly fish belonging to the teleost order perciformes.

6.2 Epidermal Hyperplasia/Papilloma

DISEASE	BODY SITE	STAGES		
		1	2	3
Epidermal hyperplasia/ papilloma	Body surface	At least one but less than 4 lesions between 2 mm and 10 mm in diameter (Figure 4)	More than 4 lesions between 2 mm and 10 mm in diameter (Figure 5)	Lesions over 1 cm diameter (Figure 6)

Recognition: In the dab these lesions are slightly raised (early stages), smooth, opaque, from creamy white to slightly pink. In special cases, brown pigmentation may also occur. Advanced lesions are slightly elevated from the body surface or may be spread over large areas. In other species, especially gadoids (e.g., whiting), the lesions occur as slightly raised areas, creamy white with petechial haemorrhages. The lesions easily slough off. In eel (*Anguilla anguilla*), the lesions may be large, "cauliflower-like", and are usually situated near the mouth; hence, they are called "stomatopapillomatosis". In smelt (*Osmerus eperlanus*), the lesions are usually present on the fins.

Cause: The cause is unknown, although a viral aetiology has been implicated.

6.3 Skin Ulcerations

DISEASE	BODY SITE	STAGES		
		1	2	3
Skin ulcerations	Any area	acute (Figures 7 and 10)	healing (Figure 8)	healed (Figure 9)

Recognition: Ulcerations are easily recognized at the acute stage, being rounded, haemorrhagic centres with white peripheries. Healing stages are partly open with more white and brown pigmentation forming scar tissue. Healed ulcers show complete closure of the lesion. Scale loss (or scale malformation) is evident in old lesions.

Cause: Ulcerations have been attributed to bacterial infection but can arise as the result of parasite infestation, scale loss, traumatic injury, and debilitation associated with poor nutrition, salinity fluctuations and other adverse environmental factors.

6.4 X-Cell Gill Lesions

DISEASE	BODY SITE	
X-cell gill lesions	Gills of dab*	No grading (Figures 11 and 12)

Recognition: In the dab, these lesions mostly are observed in fish $\geq$20 cm length. The fish appear thin and pale. The opercula are slightly raised. Examination of the gills in such cases reveals creamy white to light pink swollen gill lamellae.

* The disease has been recognized in other internal organs using microscopy. In the gills, the histological appearance is of masses of large cells completely filling the areas between the secondary lamellae.

Cause: The disease has been of uncertain aetiology, but most scientists now recognize the abnormal cells not as host cells but protista, possibly a species of *Amoeba*.

6.5 Skeletal Deformities

DISEASE	BODY SITE	
Skeletal deformities	Localized to the vertebral column, head region (jaws) or the fin rays	No grading (Figures 13, 14, 15 and 16)

Recognition: Skeletal deformities are observed in all fish species. The vertebral column may exhibit upward and/or downward curvatures (lordosis), sidewards curvatures (scoliosis) or compressions (platyspondyli). If the latter condition is light, it may not be clearly visible but palpation along the vertebral column will demonstrate contractions in the musculature at the affected site. Confirmation of the diagnosis may be done by cutting the overlying muscles or by X-ray. Deformities in the head mostly appear as shortened upper (mandible) or lower (maxillae) jaw. Deformities of the fin rays may also be observed, but in this context gear damage has to be considered.

Cause: In eary life stages, deformities may be due to parasitic infestation, genetic background, vitamin deficiencies or environmental factors such as low water temperature, pollutants such as heavy metals, e.g., cadmium. In later stages, electrical shock due to, e.g., lightning, may induce deformities.

6.6 Fin Rot

DISEASE	BODY SITE	
Fin rot	All fins, mostly the dorsal and ventral fins	No grading (Figures 17 and 18)

Recognition: Fin rot is observed in all fish species. Initial stages appear as thickening of the epithelium in the marginal edge of the fins. Further development results in necrosis of the soft tissue and fraying of the fin edges, followed by exposure of the fin rays, gaps in the fins and often ending with total necrosis of the fin.

Cause: In most cases, bacteria are involved in fin rot. It is mainly bacteria belonging to the genera *Aeromonas, Pseudomonas* and *Vibrio* that are isolated from the lesions. However, it is difficult to conclude whether the primary cause of fin rot is the bacterial infection or if the bacteria are secondary to traumatic damage of the fins, e.g., gear damage.

6.7 Pigment Anomalies

DISEASE	BODY SITE	
Pigment anomalies	Any area	No grading (Figure 19)

Recognition: Pigment anomalies in the form of hyper-melanization in dab appear as dark greenish colourations with uneven edges on the pigmented side of dabs and as white areas on the unpigmented side. In flatfish species, pigment anomalies may appear as varying degrees of pigmentation of the "unpigmented side" or lack of pigmentation on the "pigmented side".

Cause: The cause of hyper-melanization is still unknown. Other pigment anomalies may be of genetic origin.

6.8 Lipid Metabolic Disorder

DISEASE	BODY SITE	
Lipid metabolic disorder	Along the fin rays in dab	No grading (Figure 20)

Recognition: Appears as raised yellow to orange lumps ("caterpillar tracks") between the fleshy (distal) part of the fin rays of dab and other flatfish. The condition is only visible from the blind side of the fish.

Cause: The condition is most probably a disorder in the lipid metabolism causing deposition of lipid cells along the fin rays. The lipid cells undergo necrosis resulting in infiltration of macrophages in the area.

6.9 Liver Nodules/Tumours

DISEASE	BODY SITE	
Liver nodules	Livers, especially of dab and flounder, but may be present in other species	Measure and record sizes of nodules over 2 mm in diameter (Figures 21 and 22)

Recognition: The liver must be carefully excised from the abdominal cavity for examination. Raised nodular to tumour-like lesions ≥ 2 mm in diameter may be recorded at gross examination. Those and many smaller nodules (white spots, brown spots, etc.) should be preserved for confirmation by histological examination (see Annex 1 for a description of appropriate fixatives). This will reveal the lesions to be one or more of the following: granulomas, cysts, necrotic or inflammatory foci, foci of cellular alterations, hepatocellular adenoma and possibly hepatocellular carcinoma.

Cause: There can be many causes. In the case of foci of cellular alterations, hepatocellular adenoma and carcinoma, contaminants are likely to be implicated.

6.10 Pseudobranchial Swelling

DISEASE	BODY SITE	
Pseudobranchial swelling	Pharyngeal region in cod and certain other gadoids	No grading (Figures 23 and 24)

Recognition: In cod the lesions are occasionally observed in fish 20–60 cm length. They are fleshy to red, either laterally or bilaterally situated "growths" in the pharyngeal region, sometimes protruding through the operculum. Examine the fish by opening the mouth and observing the pharyngeal region.

Cause: The growths are xenomas formed by many amoeba-like protista, similar to the X-cells in dab gills.

6.11 Visceral Granulomatosis

DISEASE	BODY SITE	
Visceral granulomatosis	Heart, liver, spleen, kidney and intestine in cod and other gadoids	No grading (Figure 25)

Recognition: Whitish to yellowish spots or nodules (pinpoint up to 10 mm in diameter) in liver, spleen, and kidney. The manifestations of the disease vary from a few granulomas in one of the organs to disseminated conditions where all organs are affected. The condition is not visible externally, therefore, the fish need to be cut open to expose the viscera. Frequently, emaciated fish exhibit visceral granulomatosis.

Cause: The granulomas may be caused by parasites (cystic stages), bacteria (*Mycobacteria* sp., *Nocardia* sp.) or fungi (*Ichthyophonus* sp.). Clarification of the cause may be done either by microscopic examination of smears, possibly stained by Ziehl-Neelsen stain for detection of acid-fast bacteria (*Mycobacteria* and *Nocardia*), or by histopathological examination.

6.12 Gear Damage

DISEASE	BODY SITE	
Gear damage	Mainly the fins, but skin lesions may occasionally be seen	No grading (Figures 26 and 27)

Recognition: Net injuries normally affect the fin rays situated at each side of the middle third of the fish, which corresponds to the area of the fish that is retained in gill nets. Damage appears often as curvatures of the affected fin rays or possibly the loss of soft tissue resulting in a U-shaped scar formation. Especially the "spine" behind the anal region is often broken. Trawl damage may result in scrapings of the epithelium or pinpoint haemorrhages in the skin, especially visible on the unpigmented side of the fish.

Cause: When fishermen clean their gill nets, undersized fish caught in the meshes are often violently removed from the nets resulting in serious damage to the fin rays. Fish caught in bottom trawls are often towed for several hours. The fish are then damaged during the trawl movements over the bottom, especially when the codend is packed with fish. Fish also escape through net meshes by wriggling through, thus injuring themselves.

6.13 Tumour Formation

DISEASE	BODY SITE	
Tumour formation	Any area	No grading (Figure 28)

Recognition: Tumours appear most often as rounded, well-defined (benign) formations arising from the subcutaneous connective tissue (fibromas).

Cause: Unknown

6.14 Parasites

Numerous internal and external parasites can be found in all species of fish. In the following table, the most common externally visible parasites affecting dab, flounder, cod and whiting of the North Sea and the Baltic Sea are listed. Some of the parasitic infestations (e.g., *Stephanostomum* sp. and *Cryptocotyle* sp.) may be classified according to their severity: light infection (1-25 spots), medium infection (25-100 spots), and severe infection (>100 spots).

Parasite	Main host	Recognition / Body site
Stephanostomum sp. (Trematoda, Digenea) Figure 29	Dab Flounder	White spots in the skin and between the fin rays (1–2 mm)
Glugea sp. (Microspora) Figure 30	Dab Flounder and other flatfish species	White spots in the intestinal wall
Acanthochondria sp. (Crustacea, Copepoda) Figure 31	Dab and other flatfish	Whitish-yellow parasite in the gill chamber, mean size 5–10 mm
Lepeophtheirus sp. (Crustacea, Copepoda) Figure 32	Dab and other flatfish	Light-brown parasite on the skin and below the pectoral fins, mean size 5–10 mm
Cryptocotyle sp. (Trematoda, Digenea) Figure 33	Cod and other gadoids Dab	Black spots in the skin (≤1 mm)
Lernaeocera sp. (Crustacea, Copepoda) Figure 34	Juveniles in flounder, adults in cod and other gadoids	Juveniles in the tips of the gill filaments; adults S-shaped, dark red in the gill chamber of gadoids, size ≤2 cm
Clavella sp. (Crustacea, Copepoda) Figure 35	Cod and other gadoids	Whitish-yellow parasite affecting the mouth cavity, gills and skin, mean size 5 mm

7 DATA TREATMENT AND SUBMISSION

There are a number of statistical procedures commonly applied in the analysis of fish disease data. On a simple level, one can compare broad parameters, for example, males of size 20–24 cm, etc. This procedure is time consuming and does not make use of all the data. An approach suitable for analysing data on fish epidemiology is logistic regression analysis. This approach allows one to carry out a test of homogeneity across strata and also to test whether or not interactions exist in the data. This procedure can also be used to analyse unbalanced data. The software to carry out such an analysis is widely available, for example, GLIM, BMDP, SAS and SYSTAT. All of these packages are available in PC versions. Though data can be unbalanced to a certain degree, conclusions may be misleading if the data are very unbalanced.

Fish disease data collected following the described protocols can be submitted to the ICES Secretariat in accordance with the "ICES Environmental Data Reporting Format, version 2.2". A specific data entry programme is also available from the ICES Secretariat. Data will be included in the ICES database on fish diseases in the North Atlantic and adjacent areas. A series of computer programmes have been developed to facilitate the data handling. Information on these programmes can be obtained by contacting the ICES Secretariat.

Note: In terms of liver nodules, when reporting data to the ICES Secretariat only data on biologically confirmed liver neoplasms should be submitted.

8 FINAL REMARKS

A number of other diseases and anomalies will often be observed in the fish species mentioned, and other species, if examined carefully. It is not the intention of this Guide to describe the illustrated conditions in detail, but for further reading the bibliography contained in Annex 2 should be consulted.

In addition to the diseases covered in this Guide, the new observer should also be acquainted with pigment changes, including hyper-melanization (Figure 19), especially in dab, skeletal abnormalities (which may occur as vertebral compressions or skull deformities (vertebral compressions can only be quantified in samples of fish populations by filleting the fish to expose the vertebrae). In contrast to skeletal deformities (Figures 13–16), deformities due to net damage (Figure 26) have to be considered. Skin haemorrhages due to stacking in the codend of trawls (Figure 27) should not be confused with ulcers or lymphocytes. Also, fin deformities may be observed. Injuries, either recent or old, perhaps caused by previous escape from nets, may occasionally also be seen.

A glossary of terms used in this Guide is contained in Annex 3.

ANNEX 1

FIXATIVES FOR PRESERVING PATHOLOGICAL SAMPLES FOR SUBSEQUENT EXAMINATION

1 10% Neutral Buffered Formalin

The most commonly used fixative for preserving pathological samples for subsequent histological examination is 10% neutral buffered formalin.

Formula:			
	Formalin (37% formaldehyde solution)	1000	ml
	Sodium dihydrogen orthophosphate	40	g
	Sodium hydrogen orthophosphate	55	g
	Distilled water	9000	ml

Preparation: Dissolve the buffer salts in a little of the water in a 5 litre conical flask with gentle heat. When the salts are dissolved, add the formalin and the remainder of the water. Store in a hardened glass bottle which has a facility for fitting with a safe pouring (pipetting) device. Label the bottle "10% buffered formalin".

For use: Dissect a piece of the pathological tissue, with some normal tissue from the same organ attached to the affected part (no more than 5 mm thickness). Place it without squeezing, with forceps, into a jar containing the fixative. There should be 20 times the volume of fixative in relation to the amount of tissue in a jar. After 1–2 hours the jars with the tissues should be agitated in order that any tissues adhering to the bottom of the jars are separated, thus allowing even penetration of the fixative. Note that tissues can be stored for long periods in 10% buffered formalin. Precautions: Use formalin in a well-ventilated area, avoid inhaling the vapours, and wear rubber gloves when handling fixed tissues.

2 Bouin's Fluid

Some workers prefer to use Bouin's fluid instead of 10% formalin as a fixative. It has some advantages over formalin in that small, 3–5 mm pieces of tissue fix more quickly, it can decalcify small bones in tissues, and subsequent acidophilic staining techniques are enhanced. Disadvantages include the fact that tissue cannot be stored for any length of time, and after 24 hours the fixative should be replaced with 70% alcohol for prolonged storage. Precautions: As for 10% buffered formalin.

Formula:		
	Picric acid (saturated, aqueous)	750 ml
	Formalin (37% formaldehyde solution)	250 ml
	Glacial acetic acid	50 ml

Again, store in a hardened glass bottle and fit the opening with a safe pouring (pipetting) divice. Label the bottle clearly.

ANNEX 2

BIBLIOGRAPHY FOR FISH DISEASE RECOGNITION AND SAMPLING FISH IN FIELD STUDIES

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ANNEX 3

GLOSSARY OF THE TERMS USED IN THIS TRAINING GUIDE

Abrasion:	A scraping of the outer layers of the skin or mucous membrane.
Acidophilic:	Thriving in an acidic environment.
Adenoma:	A benign tumour or neoplasm composed of glandular tissue.
Adhesion:	The uniting of two surfaces or structures which should normally be apart, e.g., visceral organs attached to the abdominal wall, the result of acute or chronic inflammation.
Aetiology:	The study of the causation of diseases.
Benthic:	Pertaining to the bottom terrain of aquatic regions; describing the portion of the aquatic environment inhabited by organisms that live on or in the sediment.
Carcinoma:	A malignant tumour whose parenchyma is composed of anaplastic epithelial cells.
Compression:	The action of squeezing together, closing up.
Cyst:	The non-mobile, dehydrated, resistant, inactive, dormant stage in the life cycle of many organisms that is generally considered to serve an important role in either protection or dispersal of a species. Also, a fluid-filled sac.
Debilitation:	Weak or enfeebled condition.
Decalcify:	To deprive or rid of lime salts in tissues, especially in reference to histological technique.
Deformity:	Distortion or irregularity of formation or development in a body.
Diagnosis:	As used in pathology, the determination of the nature of a given disease.
Disease:	A deviation from the state of complete physical or social well-being of an organism involving a well-defined set of signs and aetiology and leading to an impairment of its normal function.
Disseminated:	Scattered, distributed, spread diffusely.
Dorsal:	Pertaining to the back of the body.
Emaciation:	Wasting, leanness, atrophy.
Epidemiology:	The study of diseases or health factors and their relations with the population and the environment.

Fillet:	A slice of the thick skeletal muscle of a fish.
Fixative:	A chemical solution used to preserve tissues and organs for histological examination in as life-like a form as possible.
Granuloma:	A swelling formed of granulation tissue.
Haemorrhage:	An escape of blood from the vessels, either through intact blood vessel walls or by flow through ruptured walls.
Hepatic:	Pertaining to the liver.
Hepatoma:	A tumour whose parenchymal cells resemble those of the liver; this includes any tumour of the liver.
Histology:	The science of the minute structure of tissues.
Homogeneity:	The state of being similar or uniform.
Hyper-melanization:	See Melanosis.
Hyperplasia:	Excessive formation of cells.
Hypertrophy:	Increase in the size of cells or tissue independent of matural growth.
Incision:	The action or effect of cutting into the fish.
Infestation:	Presence of parasites in or upon an organism (host) which live at the expense of the host.
Juveniles:	Young fish, especially O+ group.
Lateral Line:	A series of sensory pores of the fish skin sensitive to low-frequency vibrations, located laterally along both sides of the body.
Lipid:	Fat-like.
Lordosis:	Dorso-ventral curvature of the spine in fish.
Macroscopic:	Of relatively large size; a structure of occurrence visible to the naked eye, or with the aid of a hand lens; megascopic; cf. microscopic.
Manifestations(s):	Having signs of; to display clearly, e.g., clear signs of disease.
Melanin:	A tyrosine derived polymeric pigment responsible for the yellow to black coloration of fishes and quite often a factor in the so-called inflammations of poikilothermic animals.
Melanosis:	The abnormal deposition of black pigment in tissues.
Monitoring:	A process consisting of regular investigations and recording of findings.
Necrosis:	Dead or dying cells or tissues within the living body.

Nodule/Node: A small node, knot or lump. This is an embracing term which can include any small tumour whether it be neoplastic or not; for example, the granulomatous formation of host cells surrounding a foreign body or parasite, or the result of cellular hypertrophication associated with lymphocystis disease.

Oedema: Excessive accumulation of fluid in the tissue spaces resulting from increased leaking of the fluid from the capillaries.

Osseous: Bony tissue.

Otoliths: All three bony pockets of the inner ear. The concentric growth rings of the otoliths are sometimes counted to estimate the age of the fish.

Overt Disease: A disease apparent or obvious by gross inspection; a disease state exhibiting clinical signs.

Papilloma: A benign tumour involving overgrowth of epithelial tissue, or a papillae of vascular connective tissue.

Parasite: An organism living in or on another living organism, obtaining from it part or all of its organic nutrients, and commonly exhibiting some degree of adaptive structural modification; usually causes some degree of real damage to its host.

Pathogen: Any organism which, in living on or within another organism (the host), causes disease in the host.

Pathogenesis: The origin or development of a disease.

Pathogenic: Producing disease or pathological changes.

Pathogenic Agent: See Pathogen.

Pathological Condition: A deviation from normal of known or unknown origin.

Pathology: The study of disease by scientific methods.

Petechial: Description of small red spots or haemorrhages on or in tissues, especially the skin.

Pharynx: The cavity posterior to and communicating with the mouth.

Pollution: The introduction by human activities, directly or indirectly, of substances or energy into the environment resulting in deleterious effects on living organisms.

Population: A group of organisms occupying a specific geographic area or biomass.

Prevalence: The total number of disease cases in a population. It can be represented as a percentage of a population.

Protozoans: Sub-kingdom comprising all unicellular organisms.

Pseudobranch: The remnant of the first gill arch which often does not have a respiratory function and is thought to be involved in hormonal activation or carbonatious secretion. The pseudobranch is rich in carbonic anhydrase.

Sample: A small representative quantity of a population, making it possible to estimate the characteristics of a population.

Scar: A healed wound, especially a skin ulcer.

Spatial: Pertaining to space.

Subcutaneous: Located under the skin.

Swelling: The abnormal enlargement of any part of an organ.

Syndrome: A group of signs or symptoms which, when considered together, characterize a disease.

Temporal: Measurement of time.

Tissues: The substance, structure, or texture of any part of the fish's body.

Transmission: The transfer of a disease agent from one individual to another.

Trauma: A wound or other external injury.

Tumour: Literally means any swelling, but by common consent the term is not held to include passing swellings caused by acute inflammation, etc. It should be restricted to neoplasms, of which there are two categories: (1) benign or simple, which grow slowly at one spot pressing neighbouring parts aside but not invading them, and normally not doing any harm to the animal, and (2) malignant or invasive, in which an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of other tissues, persists in the same excessive manner after the cessation of the stimuli which have evoked the change. Malignant tumours may cause the death of the animals.

Ulcer/ Ulceration: A break in the skin or mucous membrane with loss of surface tissue; disintegration and necrosis.

Figures 1–35

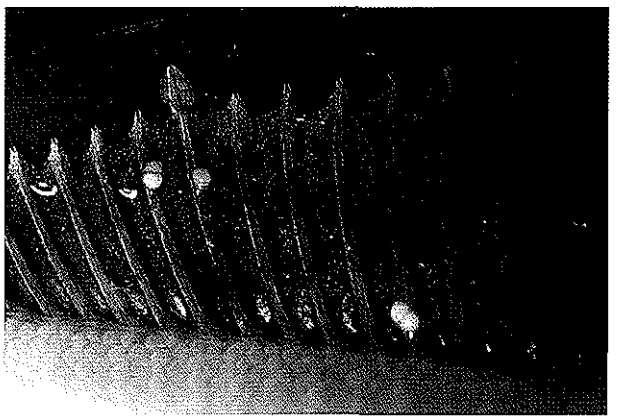

Figure 1. Lymphocystis – stage 1: 2-10 nodules on the fin ray of dab.

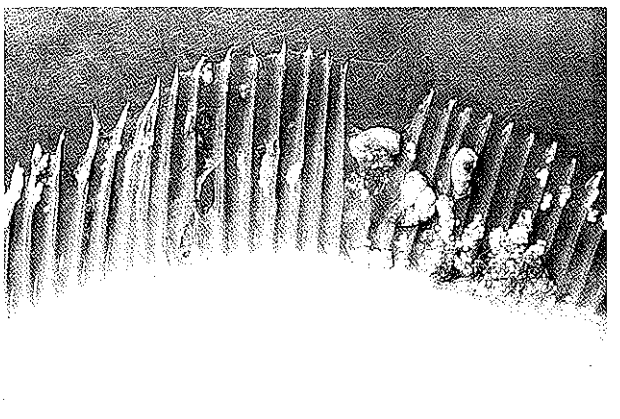

Figure 2. Lymphocystis – stage 2: clusters of nodules on the fin ray of dab.

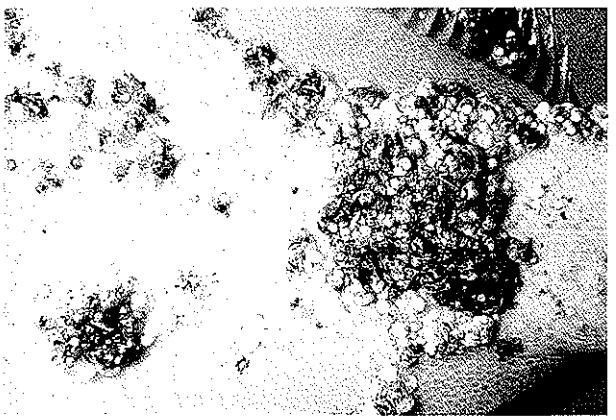

Figure 3. Lymphocystis – stage 3: multiple clusters of nodules spread over a wide area of body surface of flounder.

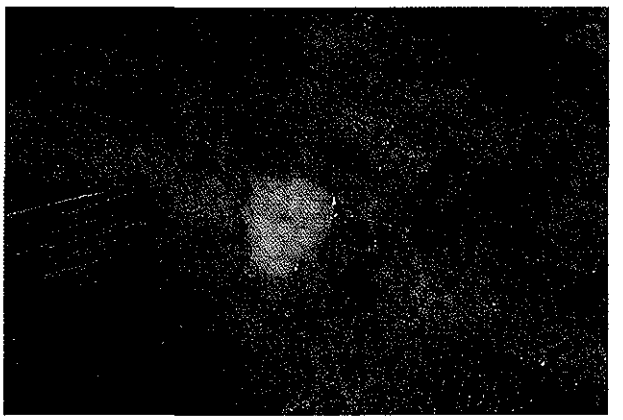

Figure 4. Epidermal hyperplasia / papilloma – stage 1: one lesion larger than 2 mm in diameter.

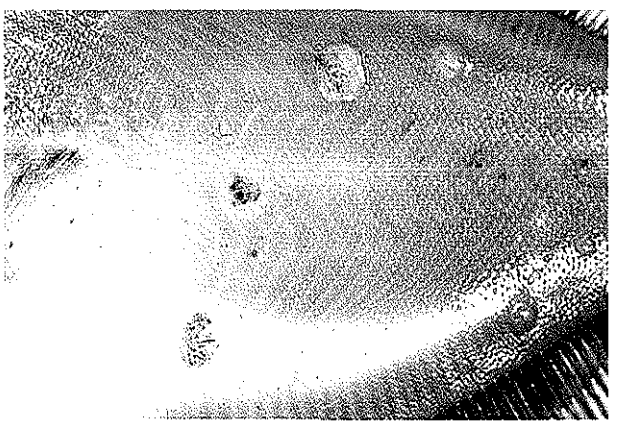

Figure 5. Epidermal hyperplasia / papilloma – stage 2: four or more nodules on dab.

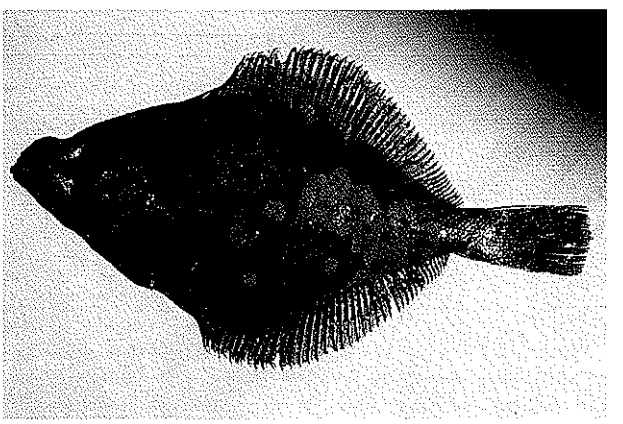

Figure 6. Epidermal hyperplasia / papilloma – stage 3: lesions over 1 cm in diameter on dab.

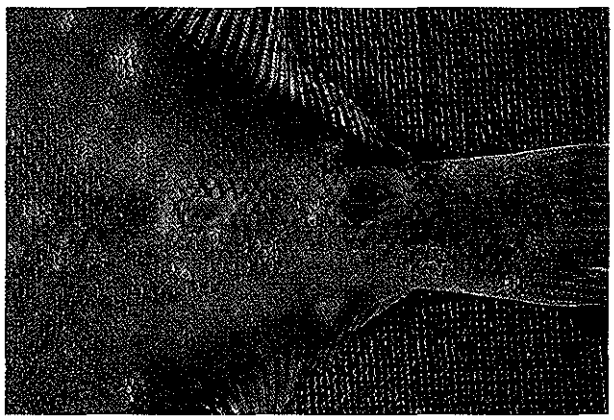

Figure 7. Skin ulcerations: acute stage of epidermal ulcers in dab.

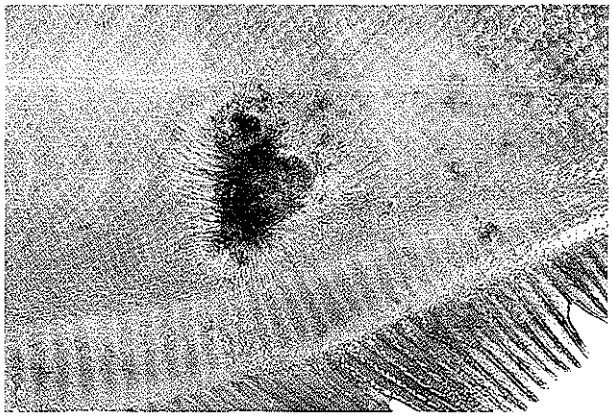

Figure 8. Skin ulcerations: healing stage of ulcer in dab.

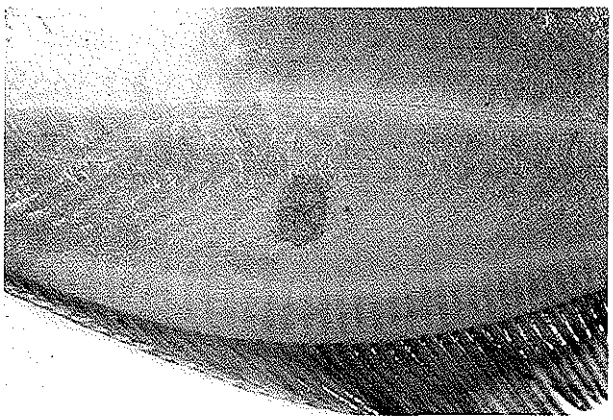

Figure 9. Skin ulcerations: healed stage of ulcer in dab.

Figure 10. Skin ulcerations in cod.

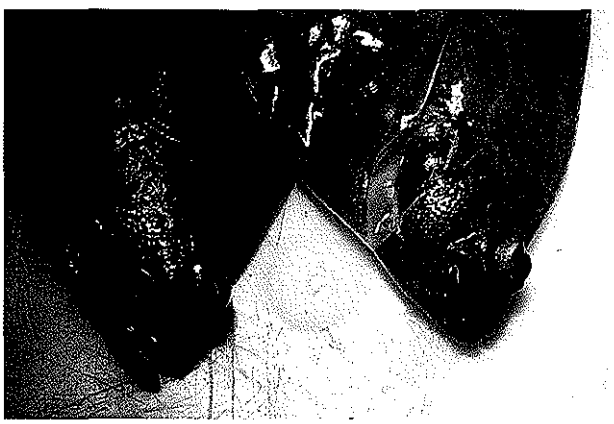

Figure 11. X-cell lesions affecting gills of dab: normal fish (left); affected fish (right) exhibiting pale gills in contrast.

Figure 12. X-cell lesions affecting gills of dab: note pale colour of gills and swollen gill lamellae.

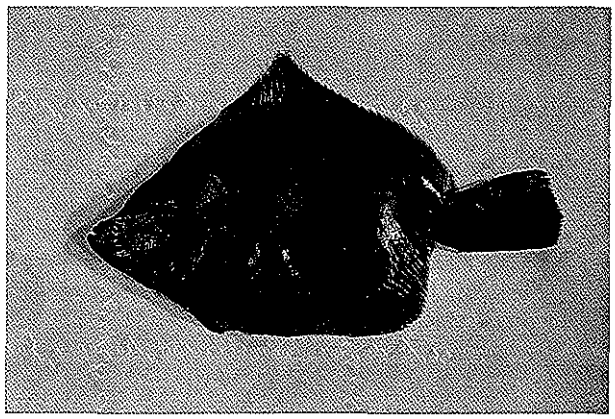

Figure 13. Skeletal deformities: curvature of the caudal vertebrae in flounder.

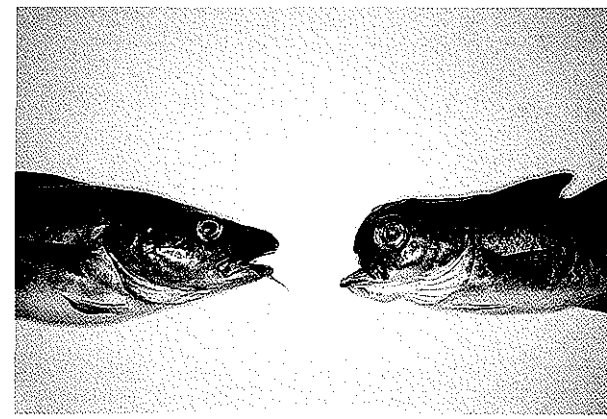

Figure 14. Skeletal deformities: "pug-head" condition in fish on the right compared with normal conditions on the left (cod).

Figure 15. Skeletal deformities: compression of the caudal vertebrae causing foreshortening in the upper fish (cod). The fish below is normal.

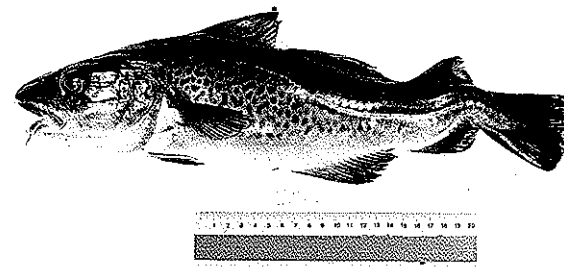

Figure 16. Skeletal deformities: vertebral curvature, scoliosis, and lordosis of the vertebrae of cod.

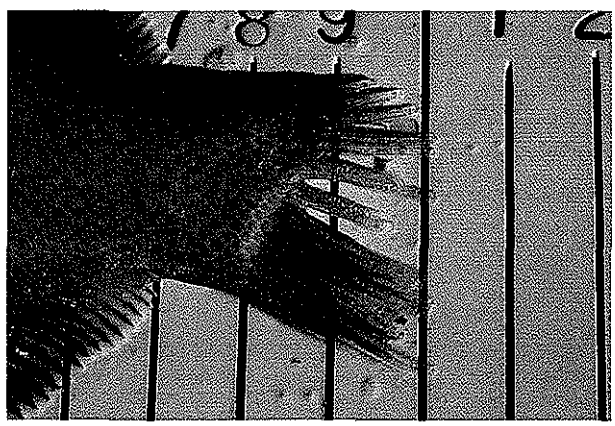

Figure 17. Fin rot: acute fin rot in dab.

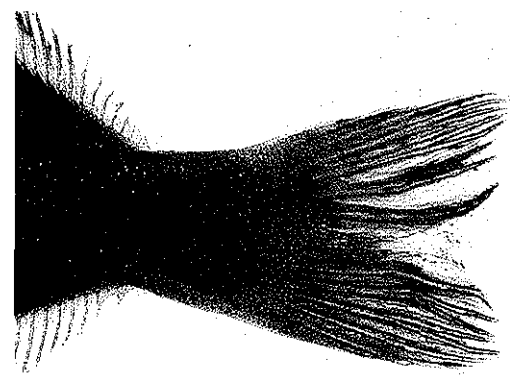

Figure 18. Fin rot: healed fin rot in dab.

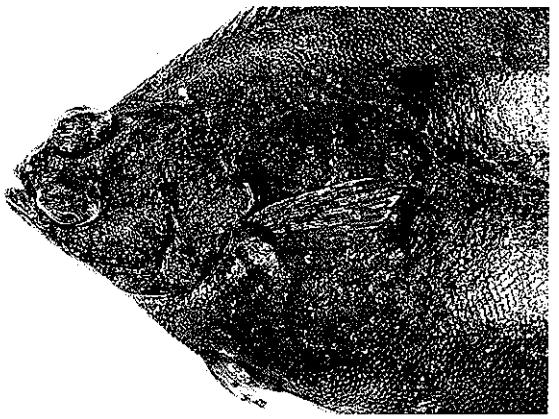

Figure 19. Pigment anomaly (hyper-melanization) in dab. On the "blind" (white) side, this anomaly appears as opaque, white, sometimes slightly elevated areas.

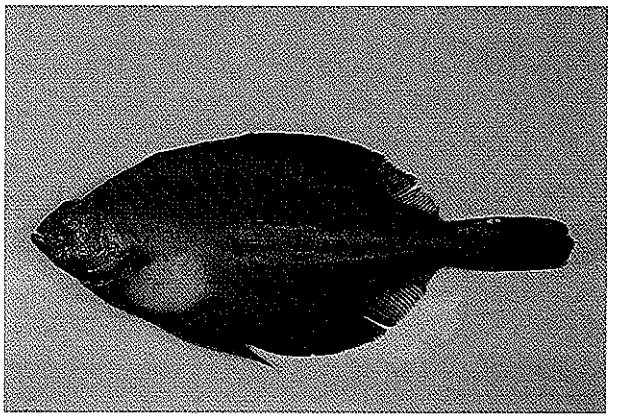

Figure 20. Lipid metabolic disorder: yellow areas at the base of the fin rays.

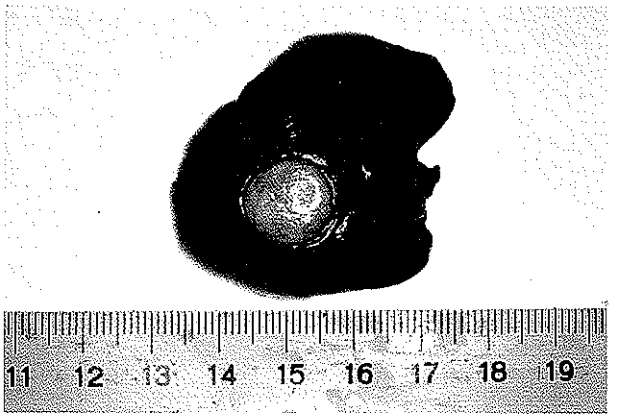

Figure 21. Liver nodule: single, raised, pale, spherical lesion in dab liver.

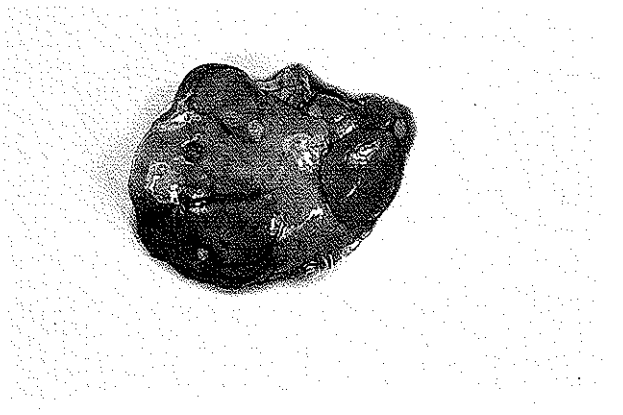

Figure 22. Liver nodule: multiple raised nodules in flounder liver.

Figure 23. Pseudobranchial lesions in cod, filling the upper part of the gill cavity.

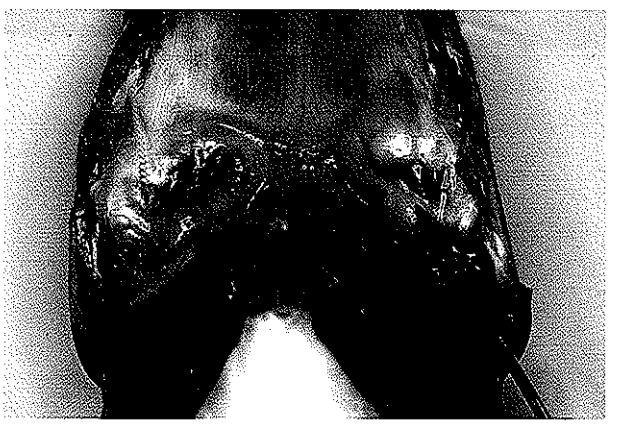

Figure 24. Bilateral pseudobranchial lesions in cod (lower jaw cut away).

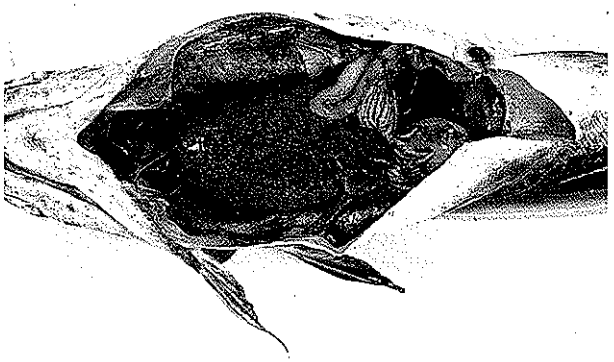

Figure 25. Visceral granulomatosis in cod. Note the multiples of nodular lesions extending over the liver.

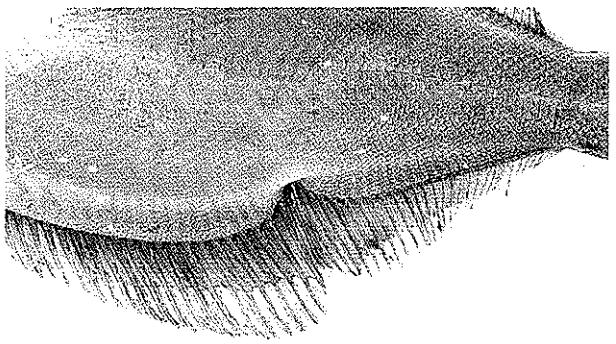

Figure 26. Abnormality in the fin rays, possibly caused by gear damage (net injury) (dab).

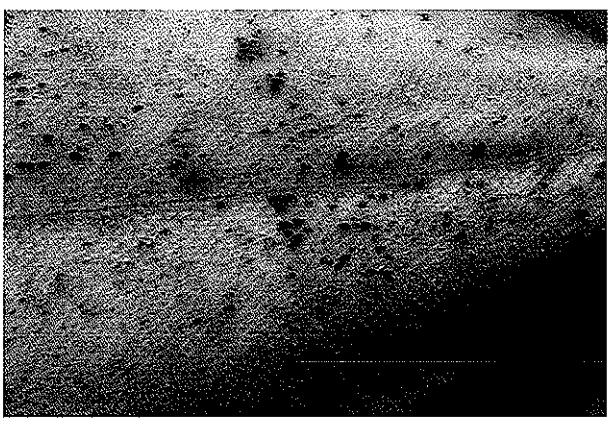

Figure 27. Extensive haemorrhaging over the body surface due to gear damage (dab).

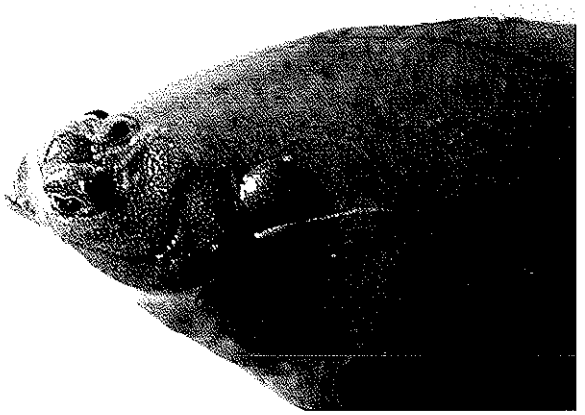

Figure 28. Skin tumour formation (fibroma) in dab.

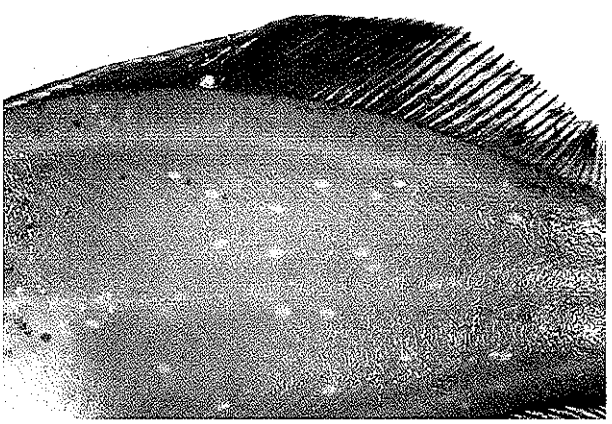

Figure 29. Parasitic infestations: *Stephanostomum* sp. Metacercariae of a digenic trematode in the skin and subcutaneous tissue of dab.

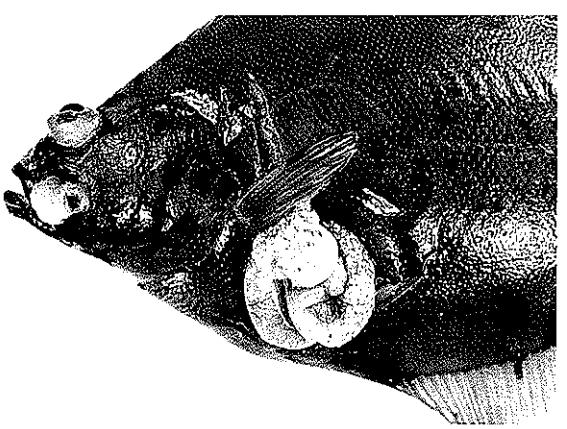

Figure 30. Parasitic infestations: *Glugea stephani*. Xenomas formed by aggregation of spores of microsporidia in the gut of dab.

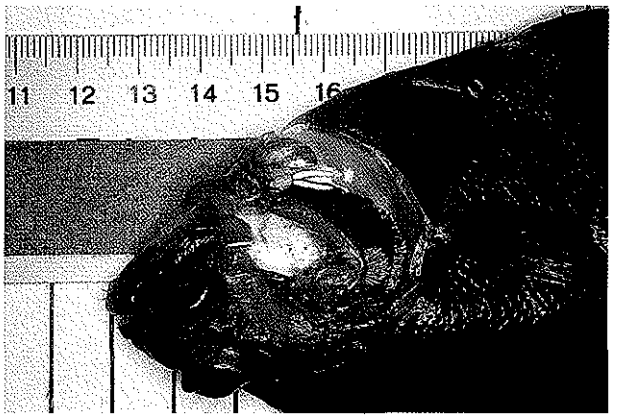

Figure 31. Parasitic infestations: *Acanthochondria* sp. Adult stage of a copepod parasite attached to the gills of a dab.

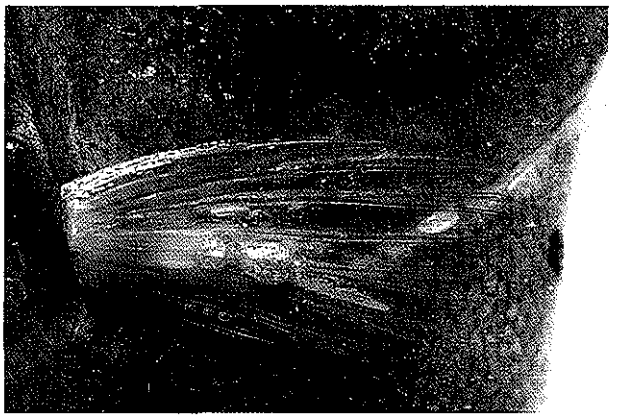

Figure 32. Parasitic infestations: *Lepeophtheirus* sp. attached to the pectoral fins of a dab.

Figure 33. Parasitic infestations: *Cryptocotyle* sp. Metacercariae of a digenic trematode in the subcutaneous tissue and in the cornea of a gadoid (cod).

Figure 34. Parasitic infestations: *Lernaeocera* sp. Adult stage of a copepod parasite attached to the wall of the gill chamber of a gadoid (whiting, *Merlangius merlangus*).

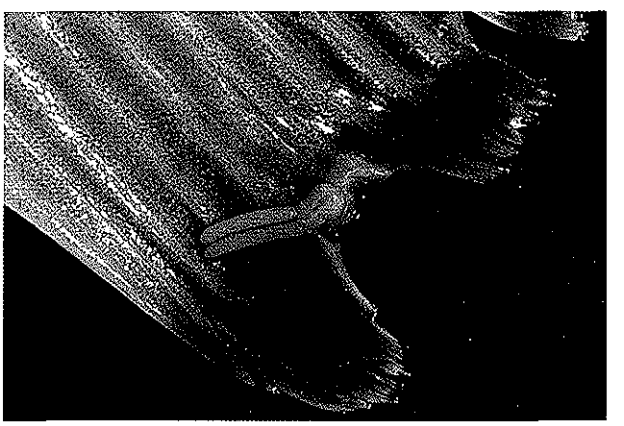

Figure 35. Parasitic infestations: *Clavella* sp. attached to the fin of a cod.