12. Fish welfare and diseases

In examining fish from an affected population, standard operating procedures must be adopted to avoid mistakes and to facilitate the exchange of information among farmers and specialists. These standard operating procedures can be summarised as follows:

- collection of environmental data (main parameters);
- observation of fish behaviour;
- *in vivo* bacteriological observation (smears of possible lesions, blood, skin, gills, spleen and kidney, either stained or fresh),
- *in vitro* bacteriological examination *(*cultures on Petri dishes of samples from spleen, anterior kidney, dorsal aorta and swim-bladder),
- identification of the pathogenic bacterium and culture of a pure strain,
- screening essays to identify which drugs are effective on the pathogen,
- selection of a suitable therapy.

In vivo tests to trace bacterial pathogens on the skin surface and in some of the internal organs, can be a quick and often decisive step to provide a first answer for many diseases. Then, the *in vitro* culture from a sample of the most infected organs may be carried out. Most bacterial colonies develop after 24 to 36 h and their examination under a microscope (x1000 with oil objective) can be helpful to prepare an antibiotic essay. In this way, it should be possible to determine the most appropriate treatment 48 hours after having observed the presence of the disease for the first time.

Minimum time required for therapeutic treatment determination

- *In vivo* examination of skin and main organs 1 h
- *In vitro* culture of sample in Petri dish +24 h
- Antibiotic assay results +48 h

To diagnose a disease, identify the responsible bacterial pathogen and determine the most efficient treatment for the fish proceed as follows:

1. sample at least 10 to 30 moribund fish;

2. examine their external surfaces and wounds;

3. obtain some skin and gill scrapings; examine (after staining) and culture samples *in vitro*;

4. examine main internal organs looking for: appearance and presence of lesions. In particular observe and culture *in vitro* samples of spleen, kidney, and stomach/intestine contents. Take samples for analysis of bacteria and viruses, to be performed later by a specialist;

5. examine results of *in vitro* cultures: type of colonies; *in vivo* microscopical study; staining;

6. perform an antibiotic test.

Several opportunistic bacteria can be found in the skin surface together with pathogenic bacteria and it is very difficult to distinguish them. It is best to reduce your study to ascertain the presence of Myxobacteria (immobile bacillus, Gram negative). For this, proceed as follows:

1. scrub the lesion with a cover slide; place the latter together with the scraping over a slide; observe the presence of bacteria under a microscope (x400 and x1000 with oil immersion);

2. take off the cover slide and dry the material over a Bunsen burner. Make a Gram stain;

3. take a Pasteur pipette, break the tip and sterilize on the burner flame; let it cool;

4. obtain a first sample for *in vitro* culture:

- using sterile tweezers, lift one margin of the skin lesion, detaching it from the muscle; introduce the pipette between lesion margin and muscle and suck some liquid;
- place one drop on a TSA/salt (Tryptophan Soya Agar with 1.5 percent NaCl) culture medium in a Petri dish;
- incubate at ambient temperature (20 to 25°C) for 24 to 48 h.

5. obtain a second sample for *in vitro* culture following the instructions given in the previous point;

6. in the two sample cultures, observe the growth of germ colonies for colour, size and shape.

Open the fish abdomen:

1. using sterile scissors, make a first cut just in front of the pectoral fin, at ventral line level;

2. cut along the ventral line, stopping just before the anus;

3. return to the first cut and continue to open in the direction of the lateral line, cutting around the pectoral fin;

4. continue to cut along the lateral line until you reach the previous ventral cut at the anus;

5. remove the piece of skin and flesh;

6. carefully observe the appearance of the swim bladder;

7. using sterile tweezers pull swim bladder out and move it forward, to give access to the kidney.

Then observe internal organs for abnormalities:

1. Loss of colour(LOC)	colour intensity is far below standard for a given organ
2. Atrophy (ATR)	significant decrease of the volume of a given organ
3. Hypertrophy (HYP)	significant increase of the volume of a given organ
4. Red Spots (RSP)	presence of small red spots, easily identified
5. Redness (RED)	presence of an abnormal red stain over part of or the whole surface of an organ
6. Haemorrhage (HAE)	presence of blood at the surface of an organ
7. Nodule (NOD)	an identifiable mass in an organ having such a consistency and a colour that it can be clearly distinguished from that organ tissue
8. Ascitis (ASC)	presence of liquid, usually clear, in the abdominal cavity

Look for the presence of these abnormalities according to the following example:

Abnormality (see above)								
Location	LOC (1)	ATR (2)	HYP (3)	RSP (4)	RED (5)	HAE (6)	NOD (7)	ASC (8)
Abdominal cavity	-	-	-	-	-	-	-	*
Swim bladder	-	-	-	*	*			
Digestive tract	-	*	-	*	*	-	*	
Kidney	*	-	*	*	*	*	*	
Liver	*	*	*	*	*	*	*	
Muscle	-	-	-	*	*	-	*	
Pylonic caeca	-	-	-	*	*			
Spleen	*	*	*	-	*	*	*	

Record all the information described following as much as possible the above mentioned procedures (see Annex 31) and contact a fish pathologist to proceed further.

Skeletal deformities

The most common skeletal deformities affecting bass and bream larvae, juveniles and adults concern jaws, gill opercula, head and backbone.

Deformities in newly hatched larvae

A fair number of abnormalities can be observed in newly hatched larvae, the most frequent ones being a form of body twisting. Affected larvae do not survive more than few hours, or few days at best, as the affected portion generally necroses. This deformity may affect from a small percentage to the totality of the population. If this percentage is above 10% it may be opportune to discard the entire batch.

The genetic origin of such anomalies is not proven, even if in trout farming it can be induced by inbreeding. On the contrary most authors believe that poor rearing conditions are a likely cause, in particular in relation to:

- nutritional deficiencies in the broodstock during ovogenesis (the most probable);
- inadequate lighting during incubation;
- excessive egg density (leading to mechanical stress and limited oxygen supply);
- handling, salinity or thermal shocks;
- pollutants in the rearing environment;
- a mix of the above mentioned causes.

Jaw and opercula deformities

Deformities can affect both the maxilla and/or the mandible, which can be either incomplete or protruding. A single operculum or both of them may be absent or be incomplete, or even be bent outwards. For larval sizes below 15-20 mm a microscope has to be used to detect them. For larger sizes they can be visually observed. Deformed jaws may be observed in larvae from hatching. Operculum deformities cannot be detected before larvae reach a length of 12 mm.

In both cases frequencies vary from 0 to 80% during the larval stage. Mandible deformities are often lethal as more than 80% of the affected larvae die, most probably due to starvation. The growth of surviving fish, although delayed, is not greatly affected (about 20% less than normal) and no additional mortality is observed later on.

On the contrary, opercula deformities severely affect growth performance (a difference of up to 60% in weight was observed in 7 months old fish) as well as they affect survival rate (over twice the mortality present in normal fish).

Backbone deformities

The most frequent skeletal deformities affect 2 to 6 vertebrae of the backbone. Scoliosis, kyphosis and fusion of several vertebrae are frequently observed, but lordosis remains the most diffused type of backbone deformity. When fish are affected, the backbone shows a typical V shape with a more o less pronounced angle. In fish without a functional swim-bladder, lordotic deformities are mainly located at the 15^{th} vertebra (counting from the tail), and at 9^{th} vertebra in other cases. As muscles involved in swimming act mechanically on the spine, in a fish with an abnormally developed spine they induce deformities in the area where the swim-bladder should be.

The first spinal deformities can be observed by transparency in larvae measuring around 15-20 mm, which corresponds to a stage in which bone calcification is sufficiently advanced. For larger fishes, soft X-rays have to be used.

The frequency of lordosis in the stock is directly linked to its origin:

- in fish without a functional swim-bladder it appears in both species. The percentage is equal to that of the non functional swim-bladder;
- in fish having a functional swim-bladder it may range from 0 to 100%.

The effects of lordosis on fish also vary according to the origin of the deformity:

- in 1 g seabass with a functional swim-bladder, the lordosis was associated to retarded growth (not well quantified), but no mortality was apparently induced by this deformity, whose angle decreased as fish grew, without disappearing completely.
- in fish without a functional swim-bladder, the lordosis is associated to growth delays and to the mortality previously described. These deformities are irreversible, even in case of late inflation of the swim-bladder (e.g., between 7 and 54 g in gilthead seabream).