

A SIMPLIFIED METHOD OF SCORING CATARACTS IN FISH

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Abstract

With the increased incidence of cataracts being reported in farmed fish it has been important to monitor the degree and progression of these cataracts. This paper describes the different methods that are used and discusses the relative merits of each.

A method of scoring the cataracts is described, 0 = No cataract, up to 4 = total opacity of the lens. The scoring method does not give detailed morphological information of the type of cataract seen but can be a useful epidemiological tool in assessing the cataract status in a population of fish.

Introduction

Since 1992 there have been reports of increasing numbers of cataracts in Ireland, Norway and Scotland in farmed fish (Bjerkås *et al.*, 1996; Waagbø *et al.*, 1996; Wall and Richards, 1992; Wall 1998;) in which some of the factors influencing the development of these cataracts have been discussed. Bucke, (1998) discussed sampling and preparation of these eyes prior to histological examination.

Cataracts may be defined as an opacification of the lens and/or lens capsule regardless of its cause (Hargis 1991). Cataracts are usually classified in various ways such as location in the lens, age of onset, cause and degree of maturation (Yanoff and Fine 1989). These methods of classification and description will often require equipment and working conditions which are not available on fish farms. This paper describes methods of scoring cataracts in field conditions in an attempt to monitor the incidence and progression of cataracts in a population of fish. This work was commissioned and partly funded by an EC FAIR CT97-3963 programme on "Cataracts in Farmed Fish".

Materials and methods

The methods of eye examinations described below are ideally carried out in darkened or shaded conditions. The reduction in light levels will prevent any reflected artefacts appearing in the eye at the time of examination.

Slit-lamp Biomicroscope

This equipment provides a range of light beams which can be directed at an angle to the observer/object axis. This enables detailed observation of any lesions in the cornea, anterior chamber or lens as well as evaluating the depth of any lesions within these organs.

This is the ideal method but it does require training as well as being expensive. An electrical supply is also necessary for most slit-lamps.

Focal Light with Magnification

This can be achieved by the use of a pen torch and a magnifying loupe. This however requires both hands. A simpler method is an auroscope with the funnel removed. This provides two to three times magnification. Only one hand is needed leaving the other free to manipulate the fish.

This method provides adequate illumination and magnification to identify most lesions but as the light source is in the same axis as the observer/object, the perception of the depth of lesions can be difficult.

Pen Torch

This method is cheap, simple and requires very little training. It is used (mainly in Scotland) to screen large numbers of fish in order to estimate the prevalence of cataracts in a population. However, even in good darkened conditions small cataracts can be missed due to lack of magnification. False positives are common due to reflected light from the cornea and lens.

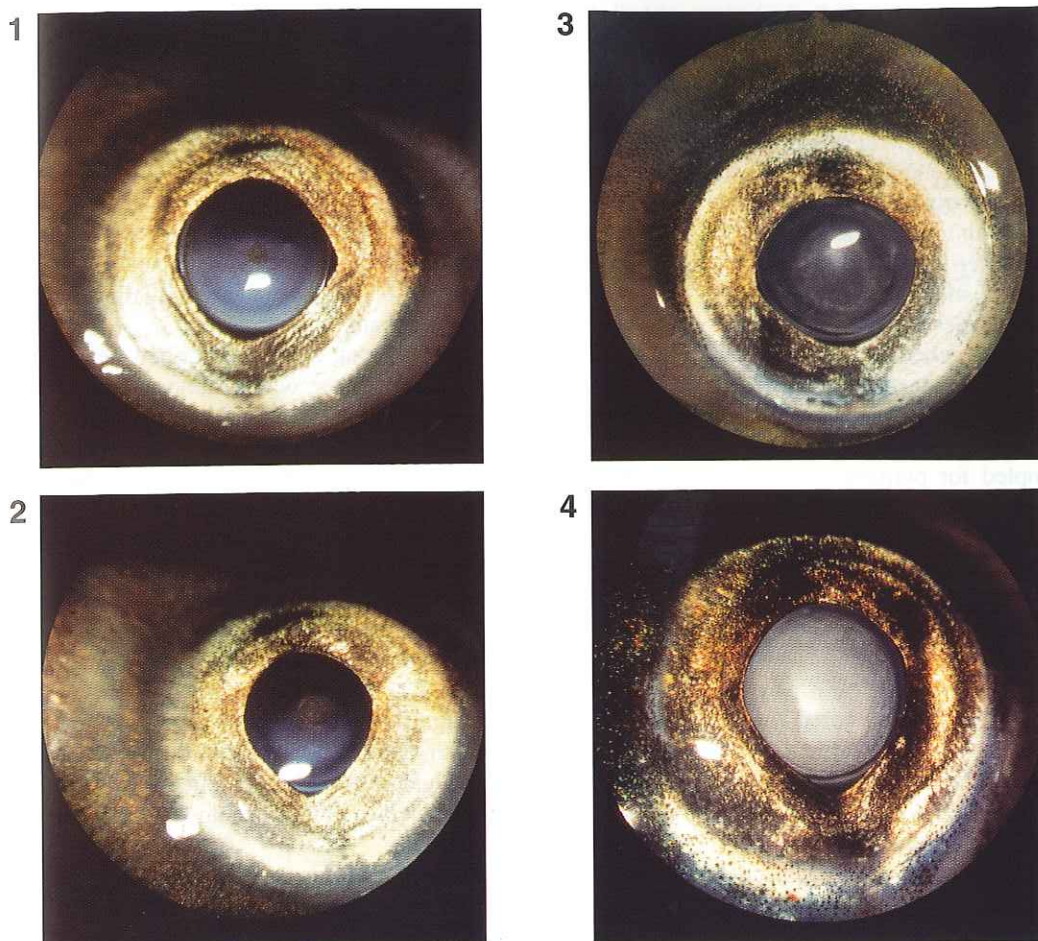


Figure 1. Diagrammatic representation of a score 1 cataract (arrow) showing a pinpoint to small opacity covering less than 10% of the lens area. The accompanying photograph in this figure shows a score 1 cataract which is situated in the anterior part of the lens.

Figure 2 Score 2 cataract – small to medium opacity covering 10 – 50% of the lens area. The photograph shows a Score 2 anterior cataract.

Figure 3. Score 3 cataract involving 50 – 75% of the lens area.

This is usually represented by a lens showing extensive changes but with some clear areas still present. The Score 3 lens in the photograph shows extensive posterior and equatorial changes.

Figure 4. Score 4 represents a complete total dense cataract involving more than 75% of the lens surface. In some of these cases a clear peripheral zone can be seen which should be noted. The accompanying photograph shows a swollen, completely opaque lens.

In most cases 30 – 50 fish are sampled from a population (cage, tank or pond). If fish are netted from the surface of the water it is likely that a high proportion of these fish will be moribund and associated with some

ocular defects. If they are netted after the introduction of food, the bias could be towards the larger healthier most dominant fish. For these reasons it is considered that the best way of achieving a balanced cross-

section of the population is using either a sweep net or a large submersible framed net which could be raised quickly. Both methods generally caught large numbers of fish thus reducing the bias in either direction.

Fish were generally anaesthetised in ethyl p-amino benzoate (Benzocaine: Aldrich Chemicals) at a concentration of 2mmg per litre water until the rate of opercular movement is much reduced and the fish were deeply anaesthetised.

Different genetic stocks, size and age of the fish, management practices and feeding regimes were noted and were used to determine which populations of fish were to be sampled for purposes of comparison. Historical differences from previous year were noted. These populations would normally be examined on a regular basis throughout the production cycle to identify any progression or increasing severity of the cataracts.

Scoring system

The cataracts are scored on a basis of the area of the lens affected when looking through the pupil. The scoring system does not take into account the density of the cataracts – only the area covered.

This scoring system does not rely on the position of the cataract in the lens. However it is usually possible to identify and note anterior and posterior lesions. If a slit-lamp is being used to describe the lesions even greater detail could be recorded as regards appearance and location.

The cataracts seen in Norway, Ireland and Scotland in one sea-winter Atlantic Salmon have often shown a peripheral area of new clear lenticular growth. This was also noted when present.

Discussion

There have been various methods reported to identify and describe fish cataracts in the field. Wall (1992) used an indirect battery powered ophthalmoscope with a 30D condensing lens. This gave good illumination and resolution but was expensive and could only be used in dry weather. Kincaid and

Calkins (1991) described the use of a light box to examine lake trout (*Salvelinus namaycush*) cataracts which presumably reduced any aberrant reflection. The method described here was devised to estimate the severity and progression of cataracts in fish by lay personnel. Unlike the mammalian eye with a rapid photo motor response constriction of the pupil does not occur in bright light and so a mydriatic (pupil dilating drug) is not necessary.

The 1-4 scoring system described here will not identify other ocular abnormalities nor will it describe in detail the position and type of cataracts. It will be necessary from time to time to describe and classify the cataracts seen, as well as other ocular lesions. This should be considered as an important adjunct to the method described here.

This method takes no account of the density of the cataracts. A lacy, less dense cataract could thus occupy most of the lens area (Score 3 or 4) and yet the visual acuity may not significantly affect the performance of the fish. However, this would be an extreme individual case and in practice examination of sufficient numbers would even out these anomalies.

Cloudy or swollen lenses which are often judged to be early signs of developing cataracts are also difficult to score using this system. These early subtle changes are especially difficult to see using a pen torch. Likewise the proximity of the cornea to the anterior lens capsule (Walls 1967) can cause confusion between anterior capsular cataracts and corneal lesions.

Given these limitations, scoring methods have been successfully used in Ireland, Norway and Scotland over the last 4 years. Especially through sequential studies they have significantly contributed to understanding the factors influencing the development of cataracts in farmed fish.

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IS THERE A CONNECTION BETWEEN RAPID FLUCTUATION IN WATER TEMPERATURE AND CATARACT DEVELOPMENT IN THE ATLANTIC SALMON (*SALMO SALAR* L)?

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Abstract

Cataract was diagnosed in Atlantic salmon (*Salmo salar* L) in sea water after a period of elevated surface water temperature with simultaneous rapid fluctuation in water temperature measured at 15m depth. The affected fish were from four different hatcheries and had been released the preceding spring. Fish from each hatchery were kept in four different nets, and all nets were affected, although not to the same extent. A possible relationship between cataract and fluctuation in water temperatures is discussed.

Introduction

During the last three summers, serious outbreaks of cataract development have occurred in salmon farms in Norway. The outbreaks have primarily occurred during periods of increased water temperature following sunny weather. Not all farms in an area have been affected, however, and not necessarily fish from all nets in the same farm. Investigations of probable causes have not revealed one single cause. It is more likely that several factors are involved, including rapid growth (Bjerkås *et al.*, 1996; Waagbø *et al.*, 1996), which is also known to be related to increased water temperature and appetite (Elliott and Hurley, 1997; Koskela *et al.*, 1997). The removal of blood meal, which was earlier a component of many fish foods, has also been suggested as a cause of cataract in modern fish farming (Wall, 1998), and a relative deficiency or excess of nutrients cannot be excluded. It may also be that the composition of the piscine lens makes it especially susceptible to cataract formation. A rapid decrease in water temperature has been shown to cause transient cataract formation in the salmon, probably by a direct effect on the lens proteins (crystallins) (Bruno and Raynard 1994). Indirectly, cataract development may theoretically also be related to an increase in water temperature, through a change in glucose metabolism in

the lens. Most glucose, which is the lens' main substrate for energy production is metabolised by anaerobic glycolysis, mainly through the hexose monophosphate shunt. In vitro studies of rainbow trout lenses have shown that the rate of glycolysis through the hexose monophosphate shunt increases up to a temperature of 33°C, after which it declines and the lenses become opaque. The development of this lenticular opacity may therefore be related to decreased energy production (Olson *et al.*, 1970). Studies in dogs suffering from diabetes mellitus with subsequent hyperglycemia have shown that the capacity of the hexose monophosphate shunt is restricted. Excess glucose is metabolised to sorbitol, which does not diffuse across cell membranes and may lead to an osmotic gradient that draws water into the lens fibres (Basher and Roberts, 1995). It has been speculated that hyperglycemia may contribute to cataract development in the salmon, although preliminary studies have been inconclusive (personal observations). Rapid fluctuations in water temperature have in freshwater been shown to be associated with increased growth rate and cataract development (Bjerkås, Bjørnstad and Waagbø, unpublished data). The present study also reports increased cataract incidence after a period of extreme fluctuations in seawater

temperature under otherwise normal farming conditions.

which until the summer of 1998 had only experienced occasional cases of cataract.

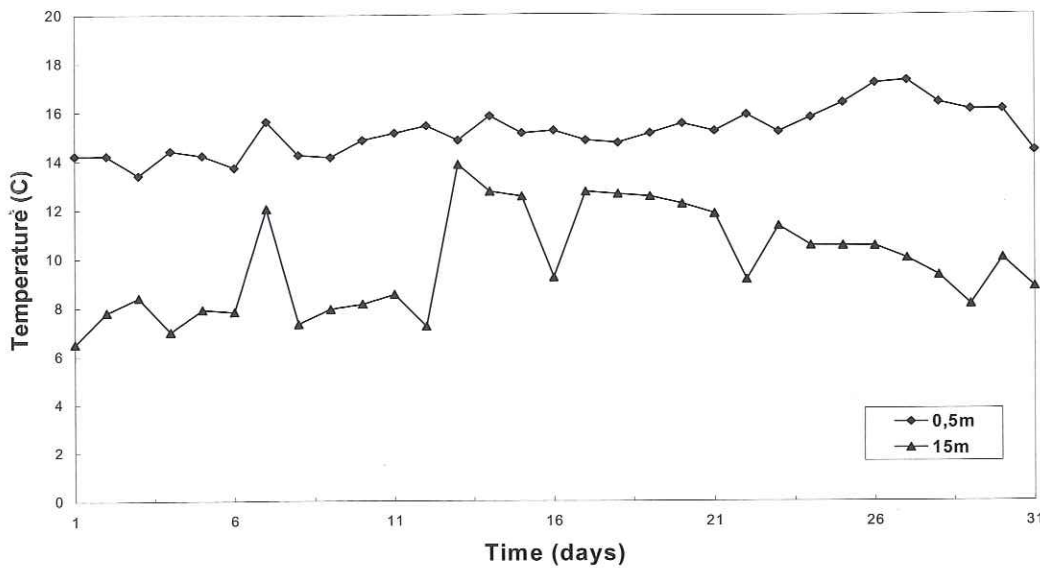


Figure 1. Fluctuation in water temperature measured at 0.5 and 15m depths

Materials and Methods

The affected farm was a commercial salmon production unit (Rødøy, Northern Norway)

The fish were of mixed strains from four different hatcheries and were kept separated in four nets, each containing between 130,000 and 190,000 fish. They weighed between 70 and 95g when released during the period April 8 to May 16, 1998. Single cases of minor cataract changes were noted at screening of all four nets in June. The fish in net 1 (which were the first to be released) were probably desmoltified when delivered, with a subsequent mortality of 60,000 of 190,000 fish. Otherwise the fish had been in good condition. The feed ration consisted of EWOS standard feed (EWOS, Norway). Daily recordings included measurement of water temperature and water oxygen concentration and observation of wind and weather conditions. Water temperatures were measured at the same time of the day, at 0.5 and 15 meter depths, and had been done so routinely in the farm for the last three years. The summer of 1998 was unusually warm



Figure 2. Partial cataract located to the anterior (arrow) and posterior lens cortex.

and sunny, and extreme fluctuations in temperature between different depths of water were recorded (Fig. 1). While temperatures were between 17.3 and 13.1°C at 0.5m depth, the temperature at 15m depth fluctuated between 14.6 and 6.6° C. The biggest difference between 0.5 and 15m water temperatures on the same day was 8.2° C. In the beginning of August the staff noted an increased number of fish with cataract, net 4 being most severely affected. Eye examinations were performed in mid September. Twenty fish from each net were caught, anaesthetized with benzocaine and examined with a Kowa SL-5 slit lamp biomicroscope under darkened conditions. The number of fish affected with cataract was recorded, and the severity of the cataract changes were graded according to the following system, each eye being scored separately:

0: Normal lens.
1: Changes affecting less than 10 % when seen straight through the lens.
2: Changes affecting 10 - 50 % of the lens.
3: Changes affecting 50 - 75 % of the lens, and with a clear nucleus.
4: Complete cataract.

Results

The number of affected fish, as well as the severity of the cataract changes, are given in Table 1. Net 1, in which the fish was probably desmoltified before transferral into sea was the least affected. There was no statistically significant difference in the number of affected fish in nets 2, 3 and 4. The severity of the lens changes was significantly more marked in net 4, which contained the fish transferred to sea last, and in net 3, compared to nets 1 and 2 ($p < 0.05$, chi square test). The difference between net 4 and net 3 was not statistically significant. Most of the cataracts only affected the lens cortex (Fig.2). The mildest changes were pinpoint- or doughnut-shaped opacities, restricted to

Table 1. Examination results

Net	Examined (n)	Normal (n)	Cataract (n)	Sum cataract scores
1	20	12	8	21
2	20	2	18	49
3	20	6	14	71
4	20	3	17	80

the anterior poles, and represented a combination of epithelial hyperplasia and changes of cortical fibres. Posterior cortical cataracts were saucer-shaped opacities extending from the posterior capsule to the posterior perinucleus. The lens nucleus was affected in only a few eyes. There were no cases of lens rupture or uveitis, indicating that the cataract development had been moderate in speed.

Discussion

The cataract development in the affected farm occurred after a period of fluctuation in the water temperature at 15m depth. Some fluctuation is to be expected and the fish would normally adapt to this situation. However, in this farm, the temperature fluctuation was more extreme than may be considered normal. It cannot be excluded that some fish were affected with cataract when transferred to sea, as minor lens changes are not uncommon in smolt and cases of minor cataract changes had been diagnosed by one of the authors in the early summer. However, cataract had not been observed by the staff until after the period of temperature fluctuation, indicating that the earliest changes had been minimal. Increased water temperature also affects salmon growth (Elliott and Hurley, 1997) and rapid growth has been shown to be related to cataract formation (Bjerkås *et al.*, 1996; Waagbø *et al.*, 1996). Experimental studies have also shown a connection between rapid fluctuation in water temperature, increased growth and cataract formation (unpublished data). Another possible factor may be a direct effect of rapid temperature elevation on lens proteins that could alter the protein structure. The normal transparency of the lens is maintained by the regular structure of the lens crystallins. The

crystallins were earlier considered to be specific for lens tissue, but is now known to be present in other tissues of the body as well. Studies in mammals have shown that one group of crystallins, the α -crystallins, may work as so-called heat shock proteins under certain conditions (Horwitz, 1993; Jakob *et al.*, 1993). Heat shock proteins are maximally synthesized at 5-15 °C above an organism's normal environmental temperature (Thomas, 1990) and act to protect other proteins against denaturation in cases of elevated body temperatures. The protecting, or chaperone-like, activity of the α -crystallins seems to be temperature-dependent, with decreased activity at higher temperatures (Rao *et al.*, 1998). The α -crystallins, as found in mammals, seem to be lacking in solely marine animals (Ahrend *et al.*, 1999; Brahma and Bours, 1972), but it cannot be excluded that other groups of crystallins take over the chaperone effect in the absence of regular α -crystallins. The process of smoltification and time of sea transferral has been suspected to be involved in cataract formation (Iwata *et al.*, 1987; Waagbø *et al.*, 1996). In the present study, the fish in net 1, which was least affected, had probably desmoltified before delivery. Although there is a possibility that the fish most susceptible to cataract formation were in the group that died shortly after delivery, unfavourable timing of sea transferral did not seem to have triggered cataract formation in this case. The difference in cataract incidence and severity between the different nets is difficult to explain. The small number of fish examined from each net does not guarantee a random sample. However, the staff had noted more severe changes in the fish in net 4, consistent with our findings. As the fish were not weighed and measured, a possible relationship to growth rate could not be studied. A difference in cataract incidence in different nets under the same farming conditions is not uncommon, and has been reported from many farms. It seems that cataract in modern fish farming is a multifactorial disease involving both environmental and nutritional

factors as well as the possibility of a special susceptibility to cataract development in the fish.

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