



Aggravating factors in the development of ocular abnormalities in farmed Atlantic halibut (*Hippoglossus hippoglossus*)

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Abstract

Cystic lesions in the eyes of farmed halibut with a number of ocular sequelae have been reported previously with evidence that the condition can be associated with increased activity of choroidal carbonic anhydrase and elevated oxygen tension in the aqueous humour of affected fish. These changes may be compared to the 'bends' in human divers which are characterized by elevated circulating levels of nitrogen together with aggravating factors such as physical exercise. It is postulated that bubble formation and subsequent development of cysts in halibut require not only high aqueous humour oxygen tension but also the imposition of factors such as handling or intraspecific aggression which lead to bubble formation. A significantly higher incidence of lesions was noted in fish handled, weighed and measured every two weeks compared with a control group of unhandled fish, suggesting that physical activity associated with handled and possibly also intraspecific aggression is a factor in generation of ocular lesions in these fish. © 2005 Elsevier Ltd. All rights reserved.

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1. Introduction

We have previously documented ocular abnormalities in farmed Atlantic halibut (*Hippoglossus hippoglossus*) maintained at the Sea Fish Industry Authority Marine Farming Unit, Ardtoe, Argyll, Scotland (Williams et al., 1995), and demonstrated high levels of oxygen in the aqueous humour in the eyes of affected fish (Williams and Brancker, 2004). However, it is necessary to investigate whether other factors may be important in the generation of intraocular lesions in these halibut. In human divers or aviators with decompression or altitude sickness respectively, not only are high circulating levels of nitrogen important in the generation of disease, but factors such as exercise are critical in generating

bubbles at endothelial surfaces (Pollard et al., 1995; Pilmanis et al., 1999; Dervay et al., 2002). The present study aimed to determine whether similar events promoting bubble formation are important in the genesis of the ocular pathology observed in farmed halibut by comparing the incidence of intraocular lesions in fish handled for measurement of size and weight with a control group of unhandled fish.

2. Materials and methods

The study was performed twice, with the first undertaken between December 1997 and March 1998 and the second between March and May 1998. In the first study, 40 halibut were randomly assigned to one of two groups; Group A1 comprised 20 halibut which were removed from their tank, weighed and measured every two weeks while group B1 was an unhandled control group of 20

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Table 1

Mean \pm standard deviation of weight and length of fish in each group

Group	Length (cm)	Weight (g)
A1 before study	39.7 \pm 3.6	993 \pm 223
A1 after study	43.6 \pm 2.8	1084 \pm 298
B1 before study	38.6 \pm 4.2	962 \pm 203
B1 after study	42.7 \pm 4.6	1054 \pm 243
A2 before study	41.2 \pm 3.0	1035 \pm 256
A2 after study	45.8 \pm 3.5	1174 \pm 275
B2 before study	40.9 \pm 2.9	1014 \pm 192
B2 after study	44.8 \pm 3.1	1082 \pm 196

halibut. Mean \pm standard deviation of weight and length of fish in each group at the beginning and conclusion of the study are shown in Table 1.

All fish were examined ophthalmoscopically, with direct ophthalmoscopy, indirect ophthalmoscopy with a 20 D loupe lens and slit lamp biomicroscopy, at the start of the study and after four months. The fish in group A1 were also examined at two months while the control fish in group B1 were not examined as this would have compromised their 'unhandled' status. In the second study, 40 halibut without ocular lesions were similarly randomly assigned to either a handled (group A2) or a control group (group B2). Fish were examined at the beginning and end of the four month study. The procedures performed on the handled group biweekly were identical to those in the first study. In addition to ophthalmoscopic examination with direct and indirect ophthalmoscopy and slit lamp biomicroscopy, the fish in this second study were examined using ocular ultrasonography as described elsewhere (unpublished data).

For statistical analysis we used the chi-squared test to compare proportions of fish affected with ocular pathology before and after handling in the two groups in each study.

3. Results

Mean \pm standard deviation of weight and length of fish in each group at the beginning and conclusion of

the study are shown in Table 1. The number of ocular lesions for fish in each group is shown in Table 2. In the first study, no fish in the group A1 at the beginning of the study showed evidence of ocular pathology. Five eyes in five fish in group B1 at the beginning of the study were noted as abnormal with three showing some degree of globe enlargement and two evidence of cataract.

After two months, nine eyes in nine fish in group A1 were noted to have ocular lesions, including iridal haemorrhage and uveitis in three eyes (Fig. 1), cortical and subcapsular cataract in four eyes (Fig. 2) and globe enlargement in four eyes (Fig. 3). After four months, 18 eyes in 13 fish in group A1 were pathologically affected. Eight eyes were enlarged, four eyes exhibited iridal haemorrhage, three were affected by cortical and subcapsular cataract and two exhibited signs of ocular surface abrasion. In summary, at the end of this first study, 65% of fish in group A1 developed new ocular lesions involving 45% of eyes. This was in contrast to the unhandled fish in group B1 in which eight fish developed new ocular lesions (resulting in 13 fish with ocular disease in all); five of these involved cortical cataract formation, two with mild iridal haemorrhage and one with both globes exhibiting slight enlargement. Thus, 40% of these fish developed new lesions, involving 22.5% of eyes. The difference between proportions of fish developing new lesions in the two groups was significant at $P < 0.027$.

In the second study, no fish in either group A2 (handled) and B2 (unhandled) were affected by ocular lesions at the beginning of the study. After four months, 13 fish (65%) and 24 eyes (65%) were clinically affected in group A2, while 18 fish (90%) and 31 eyes (77.5%) were shown to have ultrasonographically detectable intraocular bubbles or cysts. This compares to seven fish (35%) and 13 eyes (32.5%) affected clinically in group B2, while 16 fish (80%) and 20 eyes (50%) in this group were shown to have intraocular gas-filled bubbles evident ultrasonographically. The difference between the proportions of clinically affected fish in the two groups in the second

Table 2

Numbers of eyes with lesions for fish in each group at beginning and end of each study

Group	Number of eyes with cystic lesions and/or exophthalmos (percentage in parentheses)	Number of eyes with cataract and/or intraocular inflammation (percentage in parentheses)	Number of eyes with iridal haemorrhage (percentage in parentheses)
A1 at start of study	0 (0)	0 (0)	0 (0)
A1 at end of study	8 (20)	3 (7.5)	4 (10)
B1 at start of study	3 (7.5)	2 (5)	0 (0)
B1 at end of study	4 (10)	7 (17.5)	2 (5)
A2 at start of study	0 (0)	0 (0)	0 (0)
A2 at end of study	12 (30)	5 (12.5)	3 (7.5)
B2 at start of study	0 (0)	0 (0)	0 (0)
B2 at end of study	7 (17.5)	8 (20)	2 (5)

A – Unhandled fish.

B – Handled fish.

1 – First study group.

2 – Second study group.

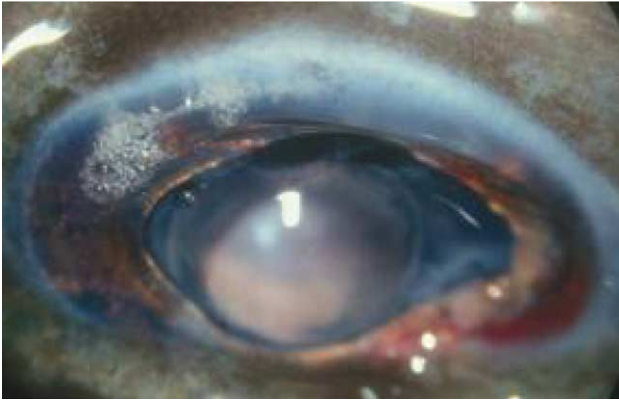


Fig. 1. Gas bubbles and iridal haemorrhage.

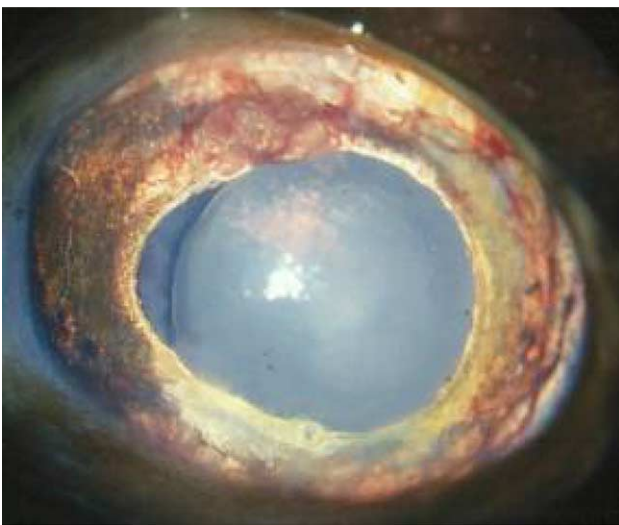


Fig. 2. Iridal hyperaemia and cortical cataract.

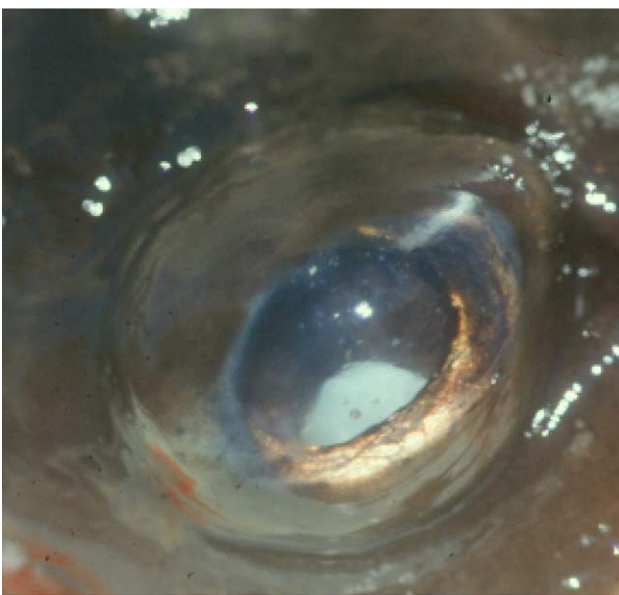


Fig. 3. Exophthalmos and globe enlargement evident by subluxation of the cataractous lens.

study was significant at $P = 0.025$, but when ultrasonographic findings were taken into account in assessing ocular normality the difference was not significant ($P = 0.64$).

4. Discussion

We have previously shown that development of intra-ocular cysts in farmed halibut is associated with increased levels of activity of choroidal carbonic anhydrase (Williams et al., 1998) and increased aqueous oxygen tension (Williams and Brancker, 2004). We postulate that oxygen tensions that would be tolerated at depth, with oxygen kept in solution under high hydrostatic pressure, result in gas bubble formation in the shallow tanks in which on-growing halibut are reared in aquaculture environments. If this is the case, there are numerous similarities to decompression sickness in human divers. In decompression sickness, pre- or post-decompression exercise is an important factor in the development of the condition (Pollard et al., 1995; Pilmanis et al., 1999) as has been noted since World War II when fighter pilots developed a similar syndrome in high altitude flight, developing more severe clinical signs after exercise. The postulated mechanism for such an association in divers is that micronuclei for gas bubble formation are to be found in all individuals after diving (Vann et al., 1980; Vann, 2003b) but that other factors such as exercise are necessary to form frank gas bubbles centred around these nuclei.

In halibut aquaculture, on-growing fish are regularly graded and moved to ensure that similarly sized animals are housed together (Brancker, 1993). Thus the protocol used in this study, whereby fish were weighed and measured every two weeks, was not an unusual regime in an aquaculture setting, although performed with a higher frequency than in most normal fish farms. In both studies, significantly more handled fish in group A developed lesions than did those unhandled fish in group B. We suggest that the mechanism underlying this difference involves trauma which is otherwise of little consequence to the fish.

Individuals taken out of the water tend to flap and writhe and at times strike their heads on the substrate on which they are lying. We have noted aqueous bubble formation (Fig. 1) during such episodes. Thus it is probable that long-term sequelae of such bubble formation resulting in peri- and intra-ocular cyst formation with subsequent exophthalmos, cataract and intraocular inflammation (Williams et al., 1995) are more common in handled than in unhandled fish. Wild-caught Pacific halibut suffer stress and mortality after handling, elevated water temperatures and exposure to air (Davis and Olla, 1988). Thus it not surprising that these captive halibut develop abnormalities after regular handling.

Two questions remain from the data given above. Firstly, why did the second study result in higher numbers of clinically apparent lesions in both handled and unhandled fish than in the first study? Secondly, why did the utilisation of ultrasonography in the second study apparently negate the difference between clinical ocular findings in the handled and unhandled fish?

The only differences between the two studies relate to the time of year at which they were conducted; the individual fish in both studies were selected from the same stock. The two potentially relevant variations associated with this time difference are a small increase in the size of the fish used in the second study and the increased temperature of the water from an average of 6.8 °C during the first study to 7.6 °C in the second. Increased size and/or temperature might conceivably have increased aggression between individuals while increased temperature may be associated with an increased propensity to bubble formation with a reduction in the oxygen-holding capacity of the water.

Aggression is an important factor in on-growing farmed flat-fish (Sunde et al., 1998), and may be a significant factor in commercial farming of halibut in the future. Ocular damage caused by aggressive behaviour between fish, so-called 'eye-snapping', is a recognised problem in aquaculture. Larger fish might be expected to exhibit more aggression, which could explain a higher incidence of physically induced lesions. However, no significant difference in incidence of ocular surface trauma was noted in the fish and the difference in size involved in these studies was not associated with marked differences in behaviour (Peter Smith, Ardtoe, personal communication).

Higher water temperatures, as seen in the second study, may explain the increased levels of pathology. Oxygen solubility declines with increasing water temperature and thus at higher temperatures oxygen will be more likely to come out of solution than at lower temperatures. This may be one of the mechanisms underlying the association between exercise and bubble formation, although our observations of sudden bubble formation after mild head trauma from flapping during handling suggests that immediate trauma-associated events are more likely to precipitate these ocular changes.

While we postulate that bubble formation is central to the ocular pathology seen in these fish, other underlying factors cannot be excluded. Diseases such as lens opacification may clearly be caused by other influences. Nutritional deficits are widely recognised as causes of cataract but such factors would not explain the differences between the handled and the unhandled groups of fish in this study in which the human intervention was the only difference between the two groups. Intraocular inflammation, focused on the choroidal rete has been seen associated with vaccination in the Atlantic salmon (Koppang et al., 2004), while protozoal parasites in

the piscine choroid have been reported by Molnar (1993). Other causes of the lesions seen in this study are theoretically possible but in the absence of evidence for other aetiologies in this group of fish, gas bubble and subsequent cyst formation caused primarily by trauma is the primary reason for ocular pathology in these farmed halibut.

Why then, were small bubbles detected by ultrasonography in both handled and unhandled fish? Our hypothesis here, arising from similar work in divers with the bends (Vann, 2003a,b), is that while microbubbles occur in the vasculature on a continual basis, it is only with the influence of other factors such as exercise in divers or handling/aggression in halibut, that these progress to larger bubbles which cause pathological lesions. Thus it is not surprising that small bubbles could be detected ultrasonographically in the eyes of both handled and unhandled fish although gross pathology was seen in a significantly higher number of the handled stock.

The final question then, is how these findings relate to commercial halibut aquaculture. Many infectious, nutritional, physical and environmental factors can lead to ocular disease in farmed fish and thus our hypothesis that the ocular lesions seen in these fish are related to the cystic changes we have previously reported might be challenged. In addition the present studies were undertaken on one halibut farm and thus may not be representative of all farms; ocular lesions such as cataract (although not to our knowledge the cystic lesions we report) do occur in halibut farmed in deep sea cages and thus such changes clearly have multifactorial aetiologies. Nevertheless, in this study we have sought to confine the differences between the study groups to handling thus showing that, quite apart from other factors, the degree of handling has a significant part to play in ocular disease in this farmed species. Is such a factor likely to have an effect in a commercial setting? Grading is not undertaken biweekly in fish farms but, as we will show in work as yet unpublished, a similar spectrum of ocular lesions does occur in commercially farmed halibut. We suggest that in commercial farming it is more likely to be trauma associated with aggression associated with high stocking density that is responsible for the ocular lesions noted. Ironically, one of the main reasons for grading on-growing fish is to prevent trauma between individuals of different sizes, which is known to be a significant problem in the Atlantic halibut. Ocular disease is clearly an important factor to be minimised in the developing industry of halibut aquaculture.

5. Conclusion

We have shown in this study that incidence of clinically evident ocular lesions is higher in halibut handled for grading by size and weight than in unhandled fish.

Ocular ultrasonography has revealed that a similar proportion of handled and unhandled fish have evidence of gas bubble formation in the choroid. We propose that ocular changes associated with handling result in the aggregation and enlargement of the small gas bubbles noted on ultrasonography, resulting in clinical signs such as retrobulbar and intraocular cyst formation with potential sequelae of cataract and intraocular inflammation. In a commercial setting, intraspecific aggression may be an important factor in causing these lesions and steps should be taken to reduce this.

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