

Intraocular oxygen tensions in normal and diseased eyes of farmed halibut

D.L. Williams^{a,*}, W.M. Brancker^b

^a *Department of Clinical Veterinary Medicine, Madingley Road, Cambridge CB3 0ES, UK*

^b *38 Streetly Lane, Sutton Coldfield, West Midlands B74 4TU, UK*

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Abstract

We have previously documented ocular abnormalities in farmed halibut and reported that the production of gas-filled posterior segment cysts appears to be central in the pathogenesis of many, if not all of the signs in these eyes. In a number of fish, gas bubbles may be seen in the anterior chamber of the eye, especially after trauma or strenuous exertion associated with handling. A knowledge of the composition of this gas is important in understanding fully the mechanism of gas and cyst production. Here we report investigations into the composition of gas in the globes of normal fish, fish in which intraocular cysts have been documented previously by ultrasonography and fish where post-mortem examination demonstrated both gas- and fluid-filled choroidal cysts. High levels of oxygen were demonstrated in samples of aqueous from all fish but fish with affected eyes had a statistically significantly higher partial pressure of oxygen than did fish with normal eyes.

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1. Introduction

In previous work on the ocular abnormalities found in farmed halibut we have documented the events occurring leading to ocular pathology (Williams et al., 1995). We postulate that the lesions seen in these fish occur subsequent to gas bubble formation, predominantly occurring in the posterior segment although also seen in the anterior chamber (Fig. 1). We have demonstrated an increased level of carbonic anhydrase activity and glycogen in the choroidal body of affected fish (Williams et al., 1998) and in that paper suggested a mechanism for aberrant gas formation in these eyes.

Briefly, oxygen is formed in the choroidal body of normal fish by a countercurrent mechanism both using the rete vessels of the choroidal body and the pseudo-branch coupled with the action of the enzyme carbonic anhydrase in these vascular beds (Wittenberg and Wittenberg, 1962). Oxygen is thus produced at relatively

high tension but stays in solution because of the high hydrostatic pressure experienced at the depth of several hundreds of meters at which these fish live (McCracken, 1958). We postulate that oxygen normally produced by the choroidal body to support retinal metabolism is produced in excess in some fish. The gas readily comes out of solution in the shallow tanks in which these fish are farmed, giving rise to gas bubbles and subsequent cysts (Dehadrai, 1966). An alternative mechanism for gas production would be that gas bubbles occur as a manifestation of classic gas bubble disease, a condition frequently seen in farmed fish (Speare, 1990). In such circumstances gases drawn into solution by faulty pumping equipment or imperfectly sealed piping produces lesions throughout the body and particularly in capillary wall endothelia. In this latter mechanism the gas would predominantly be nitrogen with only around 20% being oxygen, reflecting the proportions of gases in air entrained into the pumping system. In the mechanism we propose the gas consists only of oxygen. A key experiment required to evaluate which of these is the mechanism of aberrant gas production is to sample

* Corresponding author. Tel./fax: +44-1223-232977.

E-mail address: doctordwilliams@aol.com (D.L. Williams).

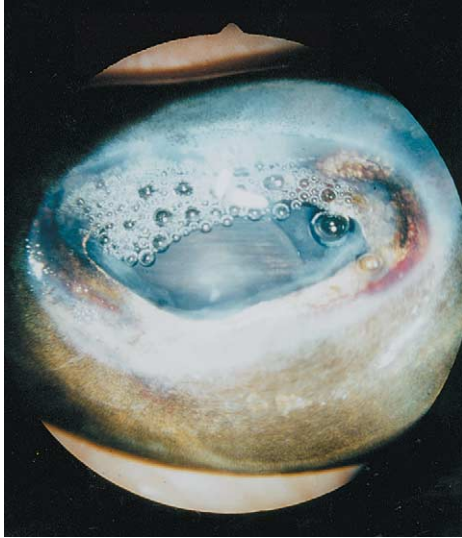


Fig. 1. Gas bubbles in the eye of an affected halibut.

the ocular fluids in affected fish and perform gas analysis on them.

Previously we had assumed that performing such an analysis of gas composition in an aquaculture setting was impractical, given the transport difficulties associated with the large size of a standard blood gas analyser. This apparent difficulty was initially overcome by use of a modern portable machine, the StatPal II (SenDx Medical). This system has the significant advantage that it uses a standard for each sample, against which the measured oxygen tension is compared. Subsequently we evaluated the changes in gas composition in samples kept on ice and transported to the anaesthesia laboratory of the Department of Clinical Veterinary Medicine, University of Cambridge for analysis. No significant change was noted over two days on ice (data not shown) and thus in further studies samples were transported to the analyser rather than using a portable monitor. In this study we aimed to obtain gas composition data in support of the hypothesis that the gas occurring in these eyes was oxygen and to document whether affected eyes were characterised by higher levels of oxygen than clinically normal eyes.

It might be argued that to confirm our hypothesis fully would also require analysis of the nitrogen partial pressures in the intraocular fluids of these fish. While this is undoubtedly a valid criticism of the work, measurement of dissolved nitrogen by mass spectroscopy (Cannon and Hatzfeld, 1977; Millis and Wood, 1977) remains impractical in the aquaculture setting in which these fish are kept. The authors consider that even without measurement of intraocular nitrogen levels, the findings presented here showing high oxygen partial pressures in eyes of affected fish demonstrate that it is aberrant production of oxygen which is likely to be responsible for the changes in the eyes of affected fish.

2. Materials and methods

2.1. Techniques of gas analysis

The StatPal II portable blood gas analysis system used in the preliminary part of this study measures pH and pCO₂ potentiometrically using ion-selective membrane electrode technology. In this system pO₂ is measured amperometrically through the reduction of oxygen on a noble metal surface at a fixed applied potential analogous to a standard Clark Cell. Before each measurement the system is calibrated using a precision-tonometered buffered calibrant solution. A microcomputer integral to the system calculates parameters such as bicarbonate ion concentration, acid base excess and oxygen saturation. Samples taken from fish in the main study group were later transported on ice to the anaesthesia laboratory, where a conventional ABL5 radiometer was used to perform gas analysis.

2.2. Fish and sampling techniques

Five 2-year-old halibut were used in the preliminary study to determine whether the small sample obtained from the anterior chamber of these fish was sufficient to allow measurement of oxygen tension. The fish were examined ophthalmoscopically with a slit lamp biomicroscope and indirect ophthalmoscope. Eyes were also evaluated ultrasonographically using a Medison Sono Ace 600 machine with a 7.5 MHz linear array probe to assess gas bubble presence within posterior segment vascular tissues. In the second study, eyes of 13 one-year-old halibut were evaluated ophthalmoscopically and ultrasonographically as above. In both these studies the fish were euthanased by decapitation immediately prior to sampling. In the third sampling session, eyes of eight aged broodstock were examined as above and then sampled after euthanasia with a captive bolt pistol followed by decapitation. These methods of euthanasia have previously been deemed humane by staff at the Sea Fish Industry Authority Research Centre, Ardtoe. In each case a sample of aqueous fluid from the anterior chamber of each eye was obtained within 1 min of euthanasia and in selected fish a venous blood sample was taken from the vascular sinus ventral to the caudal spine. Each sample was taken in an airtight syringe and stored cooled to between 2 and 5°C until analysis. Temperature correction from 37°C to the ambient temperature at the time of measurement was necessary given the temperature-dependent nature of the Clark cell current (Andritsch et al., 1981) and was performed automatically by both the StatPal II and ABL5 systems.

The anterior chamber of each globe was reinflated with Davidson's fluid and the eye enucleated and fixed by immersion in this solution for later histopathological evaluation.

3. Results

The StatPal II system and ABL5 radiometer were used to determine pH, pCO₂ and pO₂ in samples of blood and aqueous as small as 0.2 mL. Clinical signs, gas composition and pathological findings for the eyes of the five fish in the preliminary study are shown in Tables 1 and 2. Differences in gas composition and pH between normal and affected eyes in the second study are shown graphically in Fig. 2. Ocular gas composition, clinical signs and histopathological findings for the 10 fish in the third study are shown in Table 3. Throughout these studies venous blood samples showed a relatively low pO₂ and high pCO₂ with a near neutral pH. More striking were the values obtained from the aqueous samples.

Aqueous samples from the eyes in study group 1 showed a mean pO₂ of 193 ± 16 mm Hg. Vitreous samples showed a mean pO₂ of 188 ± 25 mm Hg. Comparing samples from fish from this study group with differing ophthalmic pathology, normal eyes showed a mean pO₂ of intraocular samples of 188 ± 12 mm Hg while in eyes with granulomatous change pO₂ was 169 ± 34 mm Hg. Eyes with cystic change were characterised by a pO₂ of 205 ± 5 mm Hg, this is statistically significantly higher than pO₂ of aqueous from

normal eyes ($p = 0.049$), even given the small number of samples taken in this first group.

Clinically normal eyes in the second study group showed a mean pH of 7.1 ± 0.4 mm Hg, mean pCO₂ of 20 ± 14 mm Hg and a mean pO₂ of 197 ± 28 mm Hg. Eyes affected by gas bubble formation or cystic change were characterised by a mean aqueous pH of 7.2 ± 0.4, mean pCO₂ of 18 ± 18 mm Hg and mean pO₂ of 232 ± 25 mm Hg. Differences in pH and pCO₂ between normal and affected eyes were not statistically significant ($p = 0.72$ and 0.99 , respectively) but pO₂ was significantly elevated in affected, compared with normal eyes ($p = 0.0013$).

The third group of fish, namely older broodstock, showed similar results. Clinically normal eyes in the third study group showed a mean pH of 7.0 ± 0.1, mean pCO₂ of 4.1 ± 0.9 mm Hg and a mean pO₂ of 239 ± 33 mm Hg. Eyes affected by gas bubble formation or cystic change were characterised by a mean aqueous pH of 7.1 ± 0.2, mean pCO₂ of 4 ± 0.9 mm Hg and mean pO₂ of 294 ± 39.9 mm Hg. Differences in pH and pCO₂ between normal and affected eyes were not statistically significant ($p = 0.49$ and 0.87 , respectively) but pO₂ was significantly elevated in affected, compared with normal eyes ($p = 0.0096$).

Table 1
Intraocular gas and pH findings in five fish investigated in the preliminary study

Sample	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5
pH blood	7.2	7.2	7.1	7.2	7.3
pCO ₂ blood	16	18	29	16	13
pO ₂ blood	51	48	105	68	40
pH aqueous superior eye	7.5	7.2	7.5	7.5	7.2
pCO ₂ aqueous superior eye	10	29	11	13	20
pO ₂ aqueous superior eye	195	204	196	197	158
pH vitreous superior eye	7.3	7.2	7.3	7.4	7.2
pCO ₂ vitreous superior eye	20	29	32	18	23
pO ₂ vitreous superior eye	176	201	187	186	198
pH aqueous inferior eye	7.4	7.2	7.2	7.3	7.3
pCO ₂ aqueous inferior eye	11	26	20	17	17
pO ₂ aqueous inferior eye	199	212	171	192	201
pH vitreous inferior eye	7.5	7.2	7.21	7.35	7.25
pCO ₂ vitreous inferior eye	13	27	21	16	22
pO ₂ vitreous inferior eye	188	211	120	176	201

Table 2
Clinical and histopathological findings in five fish investigated in the preliminary study

Case	Clinical findings superior eye	Pathological findings superior eye	Clinical findings inferior eye	Pathological findings inferior eye
Fish 1	Normal	Normal	Normal	Normal
Fish 2	Exophthalmos	Posterior cystic change	Exophthalmos	Posterior cystic change
Fish 3	Exophthalmos	Posterior granulomatous change	Normal	Normal
Fish 4	Normal	Normal	Normal	Normal
Fish 5	Exophthalmos	Posterior granulomatous change	Exophthalmos	Posterior cystic change

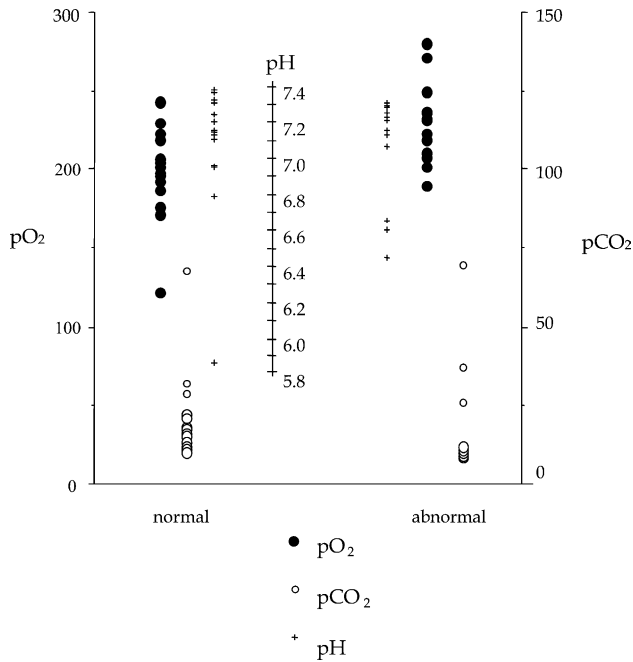


Fig. 2. pO₂, pCO₂ and pH values from aqueous samples in seven normal halibut and six with ocular abnormalities in the second study group.

Histopathology showed the eyes designated as clinically normal to have unremarkable intraocular anatomy in each study. Those designated abnormal had cysts in the posterior segment, either gas- or fluid-filled (Fig. 3) or with granulomatous change in the posterior segment (Fig. 4). There appeared no clear correlation of gas composition with the pathological state of the eyes, apart from the finding that abnormal eyes consistently had higher pO₂ than did normal eyes.



Fig. 3. Posterior polar gas-filled cyst.

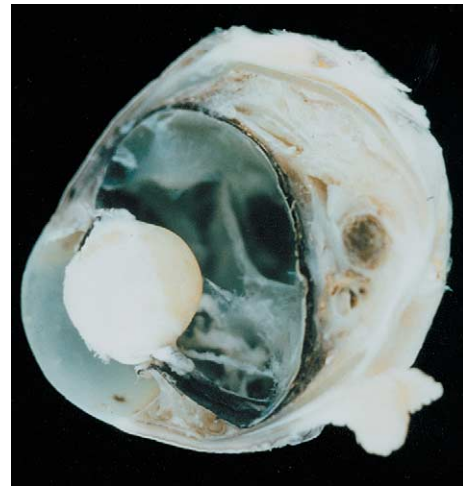


Fig. 4. Posterior cyst with granulation tissue.

Table 3
Gas values and pathological findings from the eyes of eight fish from the third study group

Eye	pH	pCO ₂	pO ₂	Clinical findings
Fish 1 superior eye	6.945	3.47	314.8	Panophthalmitis, intraocular gas bubble, keratitis
Fish 1 inferior eye	6.941	3.41	319.1	Posterior polar scleral ectasia
Fish 2 superior eye	7.338	3.71	316.7	Posterior segment cystic change, cataract, keratitis
Fish 2 inferior eye	7.265	3.05	318.2	Posterior segment cystic change
Fish 3 superior eye	7.083	3.56	219.3	Normal
Fish 3 inferior eye	6.937	5.63	311.3	Retrolbulbar cystic change
Fish 4 superior eye	7.217	5.12	318.0	Retrolbulbar cystic change
Fish 4 inferior eye	7.087	4.40	222.2	Normal
Fish 5 superior eye	6.955	3.13	228.0	Normal
Fish 5 inferior eye	6.830	3.62	219.9	Normal
Fish 6 superior eye	7.203	4.24	227.4	Normal
Fish 6 inferior eye	6.942	3.79	313.1	Keratitis, posterior cystic change
Fish 7 superior eye	7.183	4.43	232.4	Normal
Fish 7 inferior eye	7.065	5.01	234.3	Normal
Fish 8 superior eye	7.147	5.84	233.9	Normal
Fish 8 inferior eye	7.082	3.48	237.4	Keratitis but no intraocular pathology

4. Discussion

The active secretion of oxygen in fish was described nearly two centuries ago (Biot, 1807). Biot found that the gas secreted by the gas gland of the swim bladder in Mediterranean fish was 85% oxygen. Although other physiologists searched for other instance of oxygen secretion it was not until 1962 that another example of active gas production was discovered. Oxygen production was then noted in the choroidal body in a number of fish (Wittenberg and Wittenberg, 1962). The Wittenbergs found that species with small or absent choroidal retia and species which inhabit murky water had very low ocular oxygen tensions (7–34 mm Hg), values which they did not consider different from venous blood oxygen levels. In contradistinction to this a variety of actively predatory bottom-dwelling species had intraocular oxygen tensions from 250 to 800 mm Hg. These authors postulated that the choroidal rete produces oxygen to supply the metabolic processes of the retina in these keenly sighted fish.

Here we report similar findings in the Atlantic halibut (*Hippoglossus hippoglossus*). All fish investigated had partial pressures of oxygen in ocular fluids which were significantly higher than oxygen levels in venous blood and, in all probability, exceeded gill blood gas values, although these cannot be measured readily at present. A significant correlation was found between ocular lesions found on clinical examination or at post-mortem investigation and intraocular pO₂. Eyes with lesions associated with gas bubble or cyst formation had a significantly higher pO₂ than clinically normal eyes. This finding shows that the gas bubbles seen in these fish are entirely, or at least predominantly, composed of oxygen.

Measurement of oxygen tension in fluid samples requires use of standards, difficult in a field situation, and thus the provision of a calibrated standard for each sample in the StatPal system ensured that even in the testing environment of a fish farm, calibration of the machine was possible before each sample was measured.

These findings have important implications for an understanding of the pathogenesis of gas-bubble related ocular lesions in these fish. They show that the lesions are unlikely to be related to generalised gas bubble disease. Gas bubble disease is caused by entrapped gas from the atmosphere and thus 80% of the gas is nitrogen. While we have not demonstrated that nitrogen is not present, the high partial pressure of oxygen strongly suggests that it is this gas and not nitrogen which is responsible for the bubble and cyst formation. More importantly, if generalised gas bubble disease was leading to the increased oxygen in bubbles and cysts the blood partial pressures of gas would be equivalent to aqueous levels. The fact that aqueous partial pressures are three or four times mean venous blood partial pressures shows that the causative factor in the genesis

of the gas bubbles is not external supersaturation of tank water, as occurs in classic gas bubble disease, but rather localised intraocular production of oxygen by the countercurrent mechanism in the choroidal body. We suggest that while in deep water this gas remains in solution, in shallow water the high oxygen tensions produced and the low hydrostatic pressure allow the gas to come out of solution, forming bubbles.

The fact that affected eyes had, on average, a higher partial pressure of oxygen in aqueous, than did normal eyes suggests that an intrinsic factor, possibly an increased choroidal activity of carbonic anhydrase as we have previously demonstrated in affected eyes (Williams et al., 1998) is important in the genesis of ocular disease. In addition we postulate that external causative factors such as stress related to handling or interfish rivalry at high stocking densities or during particular phases of growth maybe critical in the gas bubble formation and thus the development of disease.

5. Conclusion

The finding of higher than normal oxygen tensions in halibut affected with ocular lesions associated with posterior segment bubble and cyst formation, strongly suggests that it is this oxygen, normally produced in the halibut posterior segment, which forms bubbles and causes the ocular lesions noted in these fish. The finding of increased oxygen tension in the aqueous of affected eyes confirms our hypothesis regarding the pathogenetic mechanism occurring in these fish: aberrant oxygen bubble formation is central in the disease seen in these farmed fish. Further research will be required to define the causative or contributory factors leading to bubble formation.

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