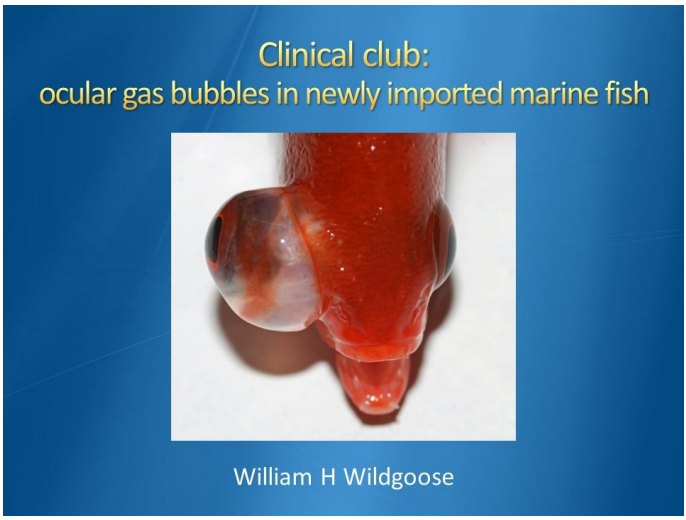
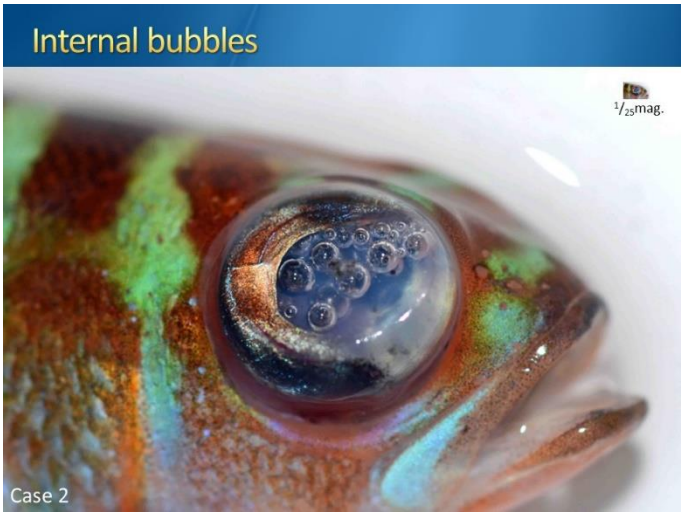
Clinical club:
Ocular gas bubbles in newly imported marine fish

WH Wildgoose, London E11 2ST



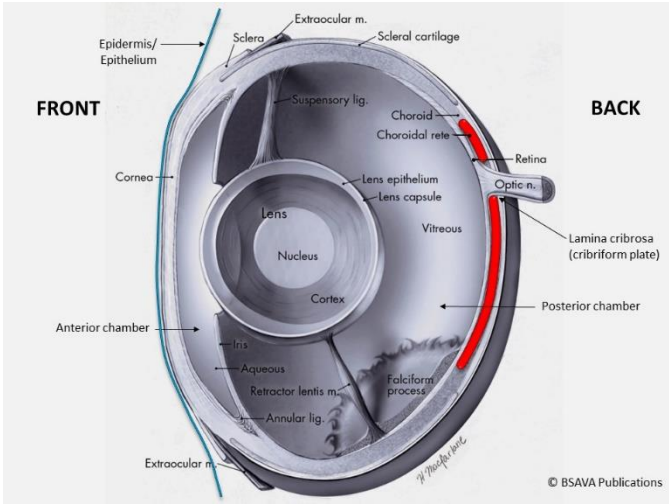
I always thought of the 'clinical club' as a part of the conference where we could bare our souls and show that we don't always get it right. Not every case is a highly polished success story and sometimes we need an opportunity to get some impartial feedback with suggestions or answers from colleagues, even if the answer is that you don't know either. So here is my problem case and hopefully the ophthalmologists among you will be able to help.



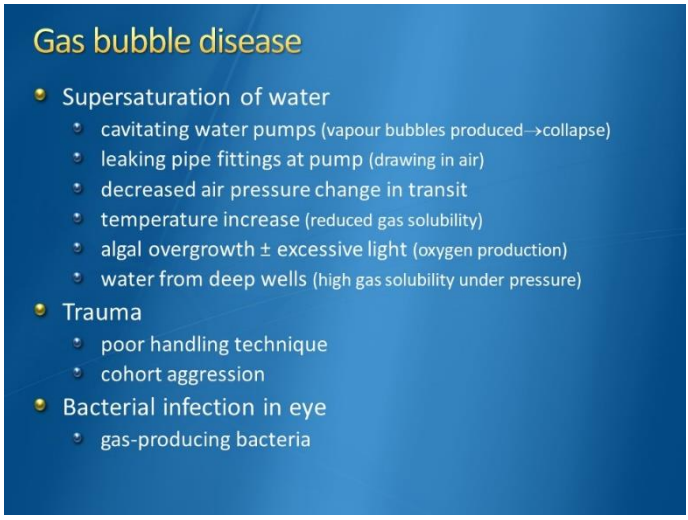
This is what I always considered 'gas bubble disease' to be, a rather classic image of bubbles in the anterior chamber of the eye but also in the gills and skin (and fins in particular). I had never seen this in any koi or goldfish, my usual coldwater fish cases, so I was keen to investigate the problem particularly because I wanted a good photo of the disease. This is a Caribbean chalk bass (*Serranus tortugarum*) from a batch that were imported a few days earlier and had shown no sign of improvement in the quarantine tanks. The fish are 6cm long and the globe of the eye is only 5mm in diameter, so visual examination was a challenge at times.

However, the small image in the top right-hand corner is a fine example showing the benefits of macrophotography where the image can be enlarged by up to 100x for closer inspection. It is at this magnification that you can really see the fine detail that is so easily missed with the naked eye. The difference between these two images is 25x but I'll often look at double that on a computer monitor.

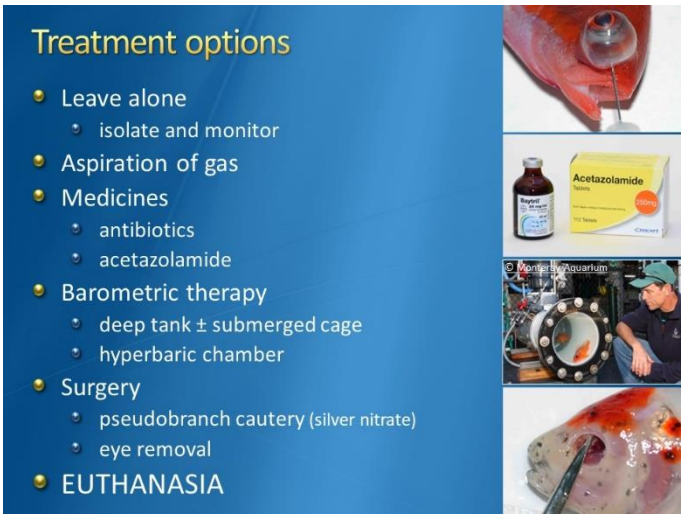
This is another Caribbean chalk bass from the same batch, and this had very impressive bubbles outside the eye and was nothing like the images I had seen before. The inside of the eye looked normal, as did the rest of the body. I wasn't even sure if it was 'gas bubble disease', which made me more inquisitive. Neither fish had visible bubbles anywhere else on the body.



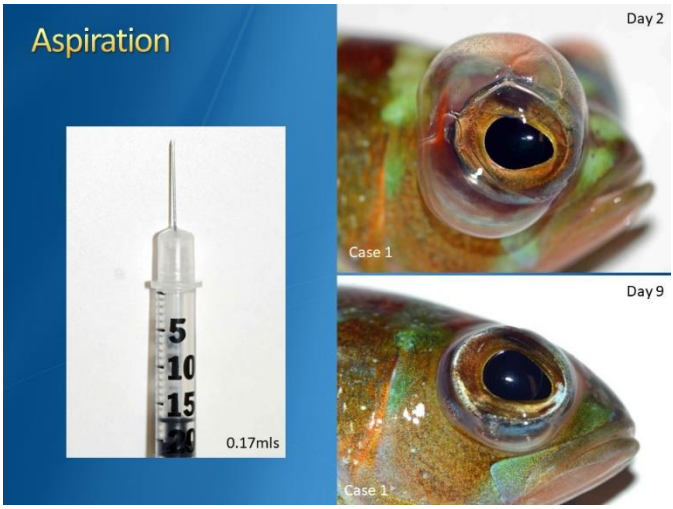
As a refresher, this is a diagrammatic cross-section of a fish eye. It is basically a fibrous balloon (sclera) with a transparent front window (cornea) and is full of fluid. It has a large round lens to help focus light on the back of the eye (retina) to improve vision. It has a large blood supply and network of blood vessels (rete) called the choroidal rete, choroidal body or choroidal gland. This rete is only found in fish and mainly in species with a pseudobranch, a rudimentary gill arch. Its purpose is to provide a high oxygen level and nutrients to the retina and lens, particularly for visual predators. The exact cell structure of the cornea varies a lot between species, but basically the whole eyeball lies under the skin or epidermis, which is fused to the front of the cornea.



This gas bubble problem is the commonest eye disease that I see in these imported marine fish, probably because it is so visible and often affects their buoyancy. But my cases only involve the eye, and not the skin or gills as seen in the classic gas bubble disease. These are some of the causes of gas bubble disease where supersaturation of the water with gases is considered to be the commonest and is caused by various underlying environmental factors that produce high levels of dissolved gases in the water. Cavitating pumps are often quoted as a cause, where vapour bubbles are produced and collapse by pressure forces acting on the water inside the pump. Other causes of gas bubble disease include intra-ocular trauma and bacterial infection.



These are the common treatment options for gas bubble disease, with an increasing level of cost and impracticality. With the two Caribbean chalk bass, I had the benefit of looking after them at the practice and monitoring them, naïvely assuming they would get better on their own. However, neither improved at all after being left alone and monitored so I had to consider other various options, including medical and surgical approaches. I had even looked at an elaborate decompression system using deep tanks, or a fish decompression chamber as used in the US. Or even resorting to euthanasia… that final veterinary solution to all our problem cases.

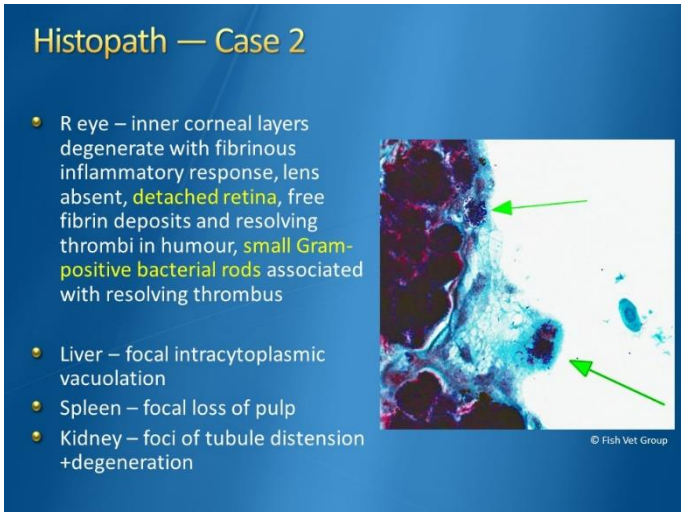


Since my conservative approach of 'doing nothing and leaving to Mother Nature' didn't work, I decided to do the obvious thing and aspirate the gas from around eye with a needle and syringe. So, 0.17ml may not seem like much gas but these are small fish with very small eyes. I performed this once and was amazed to see that almost all the remaining gas dispersed over the following 10days. Marvellous. A success, I thought…

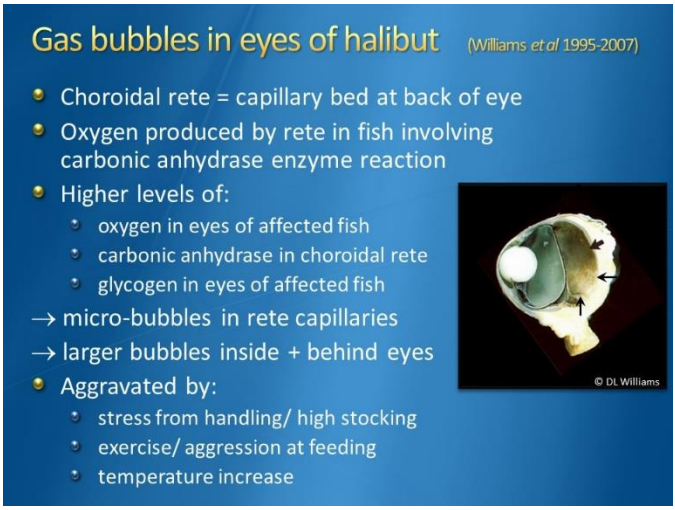
…but only to a certain extent because this was the LEFT eye of the same fish. Despite aspirating the gas around the outside of this eye, there were significant changes developing inside the eye, with small bubble formation, haemorrhage, and colour changes in the iris. Sadly, all rather disastrous and made worse when it died suddenly 10days after this last photo. Sadly, before I could salvage its body, it was half eaten by a crab also living in the tank at the time. Such are the frustrations of scientific research.

Case2 arrived with some pre-existing internal problems in the RIGHT eye which looks like a prolapsed lens and some corneal changes. Two weeks later it suddenly developed internal gas bubbles. I didn't attempt to aspirate these, and they gradually improved over the following few months. In the end, it looked ill on day 92 so I decided to euthanise the fish

before it died on me and so that I could get some histopath done. All this time, the LEFT eye appeared normal in this fish. This was 10days before the other fish died suddenly. So, was sacrificing this fish significant and perhaps a factor in the death of the remaining solitary fish (Case1)? Who knows?



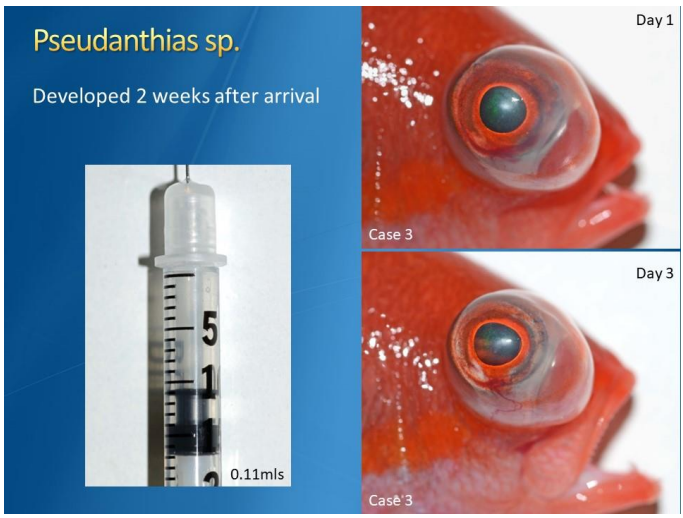
This is the histopath report on Case2, where the most significant lesions are the detached retina and the fibrinous reaction with bacterial rods inside the eye. The presence of bacteria inside the eye is not normal but was this the underlying cause of the bubbles, or was the bacterial invasion the result of bubble damage to the choroidal rete?



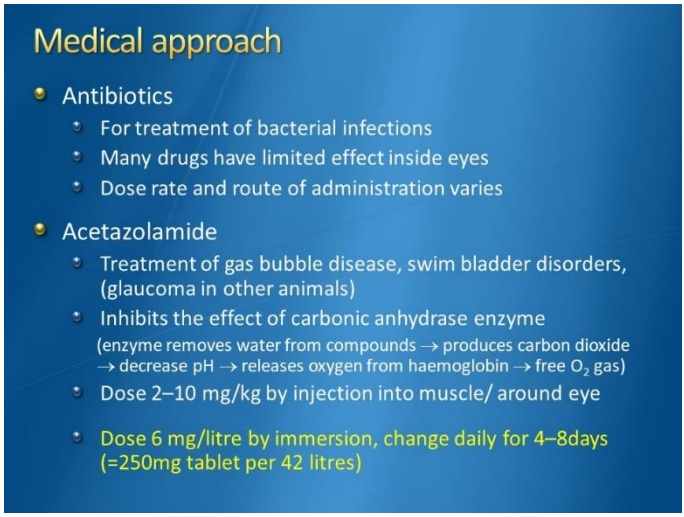
Following the disappointing results, I read a bit more about the disease and came across this interesting case in farmed Atlantic halibut (*Hippoglossus hippoglossus*) where they had similar lesions. These had extensive detailed investigations by specialists, fortunately made possible by the financial value of the fish. Unfortunately, I didn't have this luxury and even had to pay for the histology myself, so my investigations were rather limited by comparison. However, there were several interesting findings and suggestions about the disease in halibut, namely:

- they found higher levels of oxygen, carbonic anhydrase and glycogen in affected eyes and,
- very small bubbles in the capillaries of the choroidal rete and,
- large bubbles in the choroid, between the sclera and the retina (see arrows).

All this suggested a problem with the choroidal rete. They also found a few factors that aggravated the problem and that by reducing aggression at feeding for example, they prevented further cases. However, most of my chalk bass were transported individually, housed individually and even shared the same water as many other species which didn't have gas bubble problems. The bubbles would often develop up to 6 weeks after transport.



This lyre-tail coral-fish or wreck fish (*Pseudanthias* species) was one of a batch imported two weeks earlier and thought to have developed after fighting within the group. Only the right eye was affected, so trauma to the right eye was a possible cause. The gas bubble around the globe was slightly larger after a 4-hour journey and overnight isolation. I aspirated the gas like before, but over the following two days the bubble had reformed and was even larger, so I aspirated the gas again.



In view of the rapid re-inflation of bubbles, I was keen to try a simple medical approach that could be used by the importer. Since only one eye was affected, I wasn't convinced that infection was present, and I didn't want to think I would have to prescribe antibiotics to treat every case that the importer came across in the future. Fortunately, the importer has strong views about NOT using antibiotics in their fish.

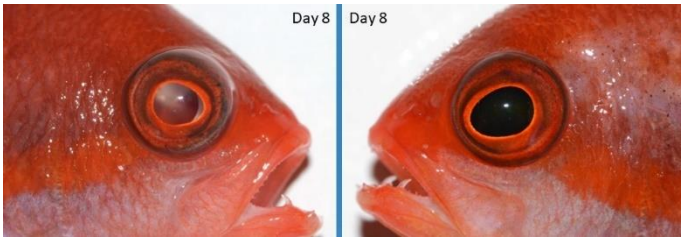
Acetazolamide is a carbonic anhydrase inhibitor and has been used for various diseases where the enzyme malfunctions in the body, including

glaucoma in other animals (fluid build-up inside the eye). Carbonic anhydrase is an important enzyme that performs various functions, and in the eye, it controls bicarbonate ions, which in turn influences the water content within the eye. Most references for using acetazolamide were as an injection at the rate of 2-10mg/kg given around the eye or into the muscle or the body cavity. However, since these very small fish weighed about 5grams, correct dosing was a problem — they only required 1/20th of a milligram at most. For example, the human injectable vials contain 500mg, enough for 10,000doses. Although it could be diluted down to a manageable volume, it was not a practical solution or particularly humane. Injecting the fish with the most easily measurable amount (0.04ml) through the finest syringe needle (30G= 0.31mm) into a 50mm fish body was still going to be the equivalent of using a thick pencil to inject 1ml into your arm… or around your eyes. It will hurt!

While looking for a more practical approach, I came across an immersion dose on a seahorse website: https://seahorse.com/topic/how-do-you-treat-bubble-disease-in-a-sea-horse/. They suggest using acetazolamide by immersion to treat a similar gas bubble problem that affects the skin and breeding pouch of seahorses. This was easier to use and works out at the rate of one 250mg tablet per 42 litres of water.

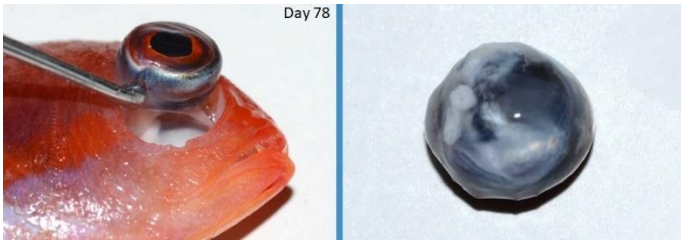
Acetazolamide is a prescription-only medicine (POM) licensed for use in humans, but it has also been used to treat glaucoma in other animals. It is relatively cheap, costing about £27 for 112 tablets (a box is enough to treat over 4,500 litres). The tablets weigh 600mg, of which 350mg are tableting ingredients such as insoluble compounds and starches which bulk up the tablet and help it 'disintegrate' in the stomach. When added to water, a cloudy suspension is produced. Here it is exaggerated at 200x normal dose. To minimise the clouding, I added the intact tablet to the filter sock in my system which filters out particles larger than 100micron in size. It saved me having to crush up the tablet and avoided the risk of fish eating fragments of the tablet. The sock is under the return pipe from my biological filer with a good flow rate, and the tablets disintegrated within a minute.

Having aspirated the gas from the fish's right eye a second time on day 3, I started the acetazolamide straight away and added one tablet daily for 5 days to my 35-litre tank. The main side effect reported in dogs is weakness, vomiting and panting (and loss of appetite in humans) but there were no obvious side effects that might be expected in fish, such as buoyancy problems.

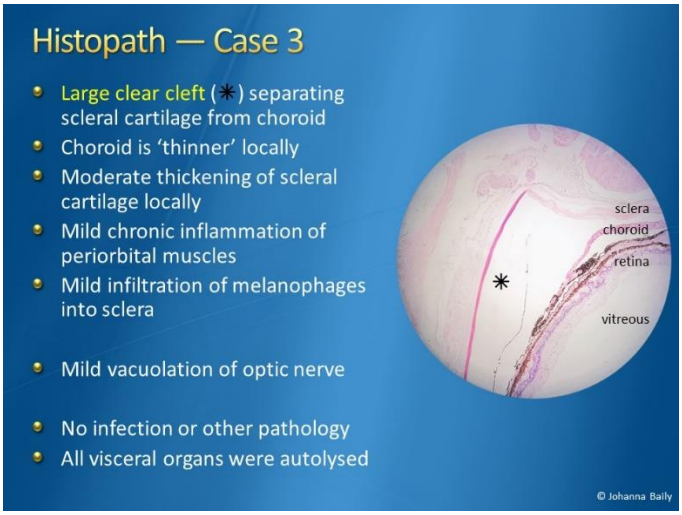


The gas bubbles never recurred after day 3, the date of the second aspiration, and this is the fish on day 8, after 5 days of acetazolamide. The left eye was never affected. The right eye improved significantly but the pale retina at the back of the eye persisted until it died suddenly 10 weeks later. This pallor was not due to the position of the camera, and I took several shots at slightly different angles on the normal left eye to see if it was an aberration or if I could create a similar 'pallor'. I couldn't and this confirmed there was some retinal lesion in the right eye.

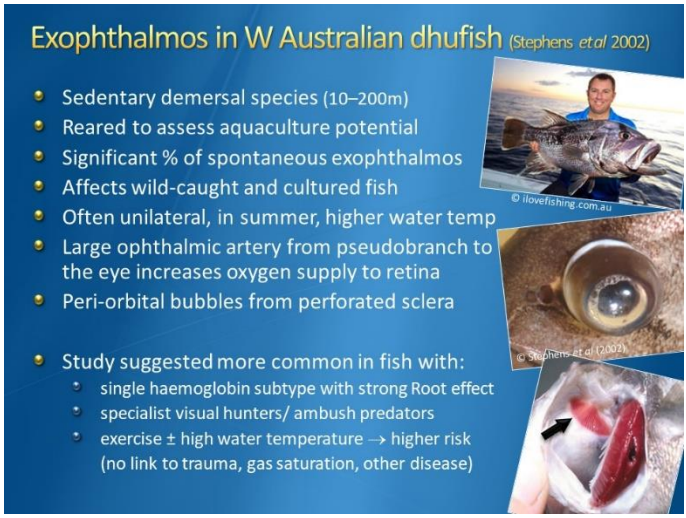
And before you ask, no I didn't try using a direct ophthalmoscope. The pupil is only 2mm in diam. and I doubt if I would know what I was looking at anyway. Apparently examining the fish retina is a challenge, even for experienced ophthalmologists, so ultrasound is more often used to study the back of the eye in fish.



The fish died suddenly and unexpectedly on day 78, so I carefully dissected out the right eye to see if there were any lesions behind the globe or in the socket. There were no obvious abnormalities. Although the fish had possibly died a few hours before being found, tissues were sent off for histological examination.



The histopathology report identified a few lesions inside eye, in particular, a large space caused by separation of the scleral cartilage and the choroid, possibly the result of an internal (intra-ocular) gas bubble. There were also a couple of other subtle tissue changes but none that I felt would account for the pallor inside the right eye, which I have assumed was the retina.



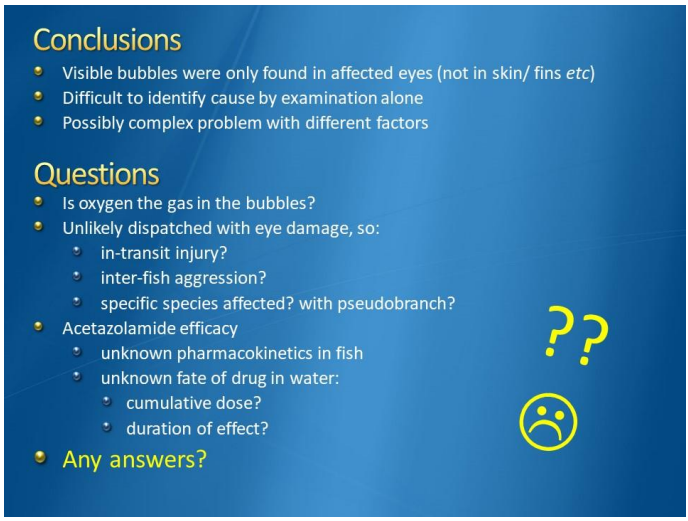
I have slowly come to the conclusion that this is a problem that only affects the eyes and not any other tissues as in the typical gas bubble disease. There are several practical difficulties that I have investigating these fish such as the cost of histopath against the financial value of the small number of these relatively low-cost fish. As a result, I have looked at similar examples in other fields and came across this study looking at the aquaculture potential of this species in Australia.

The Western Australian dhufish (*Glaucosoma hebraicum*) are large fish that have a high commercial value and excellent eating qualities. However, there is a high incidence of exophthalmos of up to 50% that affects both wild-caught and cultured fish. This impressive study concluded that the bubbles were due to free oxygen and this species has a single haemoglobin subtype with a strong Root effect. In other words, a haemoglobin that had a strong attraction and affinity for oxygen that was released as gas inside the choroidal rete. This was thought to be a problem particularly in visual hunter species where there was a high demand for oxygen by the retina. Exercise and higher water temperatures were also factors. They confirmed the bubbles contained oxygen by using a fibre optic probe passed through 16G needle (1.2mm int. diam.) through the cornea. No bacteria were found, and the bubbles resolved after several weeks. Comparing both the Caribbean cloud bass and the Pseudanthias, both these are predatory species, and their bubbles could also have a similar aetiology.

This dhufish study also found that the fibrous sclera ruptured in some cases, resulting in oxygen bubbles and localised haemorrhages in the peri-

orbital tissues. This dissection image (top left) shows the ophthalmic artery (arrow) that originates in the pseudobranch (p) and enters the globe with the optic nerve through the lamina cribrosa (cribriform plate) to supply the retina. The resolving haemorrhage (h) is also visible. In the halibut paper by Williams & Brancher (bottlom left image), oxygen-filled cysts were also found behind the globe (arrow).

It was only as I prepared this talk that I looked again at the back of the globe from my Pseudanthias that realised there are several small protrusions on the ventral aspect (small white arrows). I originally took this image to show that there weren't any obvious lesions before sending it off for histopath, but now I'm not so sure. There were no haemorrhages found on histopath, but then this is about 10weeks after the exophthalmos episode. It also doesn't help that the globe is so small in these fish.



So, what can I conclude from this rambling non-scientific approach?

This does not appear to be the typical gas bubble disease since bubbles were only found around or inside the eye and not visibly affecting the skin or gills, as is often reported. The bubbles are probably due to free oxygen and in the past has been called 'piscine ocular oxygen secretion syndrome' by one research group (Grahn *et al* 2007). Personally, I think that's a rather confusing mouthful and needs a shorter, more sexy name. A syndrome is a set of clinical signs and symptoms that are related to a particular disease.

More likely, it may be a complex problem involving several different factors, such as trauma, injury in transit or by other fish, increasing water temperatures, and aggravated by exercise or chasing during feeding.

QUESTIONS

Yes, it would be nice to know more about the cause of the problem in my fish, but what my client wants to know is, can it be treated, and can it be prevented because most affected fish die? Acetazolamide by immersion appeared to work well in one case, but it may just be coincidental, and some other questions remain such as, how does it work, and how long does it remain active in water? Obviously, more work needs to be done, but then again, this was not a cheap exercise.

This paper is based on a presentation given at the Fish Veterinary Society Conference in Livingstone on 27 March 2019.

References and further reading

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