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Fish death caused by gas bubble disease: a case report

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ABSTRACT: This work summarises the findings of an investigation on a local trout farm (Czech Republic), which was carried out in connection with the repeated deaths of salmonids (brook trout, *Salvelinus fontinalis* and rainbow trout, *Oncorhynchus mykiss*). These fishes were reared in newly installed tanks that were supplied with water from the same source as the original outdoor nature pond where the fishes had been reared without problems. The skin of dead fish was pale and covered with a thin layer of mucus. The gills had lighter colour, and microscopically, gas bubbles were visible both on the surface of gills and inside the gill filaments. No changes were found in the body cavity and parasitological examination was negative. The water in the tank was of very good quality but its oxygen saturation reached 136%. Based on the results of fish examinations and water analysis, gas bubble disease was identified as the ultimate cause of fish deaths. After making technical adjustments (technical changes to the pumping of water from the spring and ventilation of the storage tank in the building) oxygen saturation in water remained below 100% and no further cases of gas bubble disease (or fish deaths) were recorded.

Keywords: oxygen; nitrogen; air; oversaturation; asphyxiation; spring water; GBD

List of abbreviations

$\mathbf{\Sigma Ca^{2+} + Mg^{2+}}$ = the sum of calcium and magnesium, $\mathbf{Cl^-}$ = chlorides, $\mathbf{COD_{Cr}}$ = chemical oxygen demand, **DO** = dissolved oxygen, **GBD** = gas bubble disease, $\mathbf{NH_4^+}$**-N** = ammonia nitrogen, $\mathbf{NO_2^-}$**-N** = nitrite nitrogen, $\mathbf{P_{tot}}$ = total phosphorus

Dissolved oxygen (DO) is essential, and in some cases even the limiting factor, for maintaining aquatic life. Its depletion in water is probably the most frequent general consequence of certain forms of water pollution. Fish exposed to oxygen-deficient water do not take food, collect near the water surface, gasp for air (cyprinids), gather at the inflow to ponds where the oxygen levels are higher, become torpid, fail to react to irritation, lose their ability to escape capture and ultimately die of asphyxiation (Svobodova et al. 1993). Susceptibility to oxygen deficiency depends on the oxygen requirements of the respective species and to a lesser extent on fish adaptation.

On the other hand, damage caused to fish by excess levels of oxygen dissolved in water (oversaturation) is seldom encountered. However, it may happen, for example, when fish are transported in

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plastic bags with an oxygen-filled air space. The critical oxygen level of water is 250 to 300% of the saturation value. In response to oxygen oversaturation, the gills of affected fish have a conspicuously light red colour and the ends of the gill lamellae fray. Later, they may suffer from secondary fungal infections and some of them may die (Svobodova et al. 1993).

However, the condition described above should not be confused with the supersaturation of water with dissolved gases, which can cause gas bubble disease (GBD). Water may become supersaturated with atmospheric gases through several different processes, either natural or man-made. Supersaturation is caused either by an increase in the amount of dissolved air or by a decrease in the amount of air that can be dissolved in water (Weitkamp and Katz 1980). Lindroth (1957) discussed four ways by which water may become supersaturated: (1) water contains dissolved gas coming from a gas mixture containing a higher percentage of that gas than is normally found in air. This mechanism is probably of little importance as it is likely to be encountered only under experimental conditions. (2) Water contains gas that was dissolved under a pressure higher than that of the atmosphere. This mechanism has been involved in many of the documented cases of supersaturation. It is caused, for example, by air injection, operation of hydroelectric plants (spillways mix water and air and carry the air into the depth of the plunge basin. The increased hydrostatic pressure in the plunge basin increases the gas solubility. As this water, frequently in large volumes, flows away from the plunge basin into areas of less hydrostatic pressure, supersaturation develops), pumping and the use of underground water in fish farms. (3) Water contains gas dissolved at lower than ambient temperature. This mechanism accounts for supersaturation when water is heated for fish culture, during the cooling of water in power-generating facilities, and in geothermal heating of natural waters, because the solubility of gases decrease with increasing water temperature. Therefore, the heating of water causes release of gases if their concentration in water is higher than their solubility at a given temperature. (4) Two bodies of saturated water at different temperatures are mixed. This mechanism may cause supersaturation but is unlikely to produce levels high enough to cause GBD under most circumstances.

Problems with the supersaturation of water with air and deaths of fish due to GBD in fish farms have been known for over 100 years. An historical overview of this problem is given in the paper by Weitkamp and Katz (1980). The first records already originated in the years 1857, 1873, and 1884 and described external signs in fish that apparently represented gas bubble disease (Weitkamp and Katz 1980). Bouck (1980) even documented that research related to gaseous supersaturation of water has existed for over 300 years since Robert Boyle published his gas law in 1670.

Bouck (1980) described GBD as a non-infectious, physically-induced process caused by uncompensated hyperbaric dissolved gas pressure, which produces primary lesions in blood (emboli) and tissues (emphysema) and leads to subsequent physiological dysfunctions. Emboli and gas bubbles can form only when the sum of dissolved gas pressures or cavitation pressure exceeds the sum of the hydrostatic and other compensating pressures. GBD can occur only when and where cavitation pressure exceeds compensating pressures. Bouck (1980) also codified the three stages of GBD: (1) a period of gas pressure equilibrium, nonlethal cavitation, and increasing morbidity; (2) a period of rapid and heavy mortality; and (3) a period of protracted survival, despite lesions, and dysfunction that eventually terminates in total mortality.

GBD is outwardly manifested by the formation of bubbles under the skin of the fish, especially on the gills, fins and eyes (Engelhorn 1943). Dawley et al. (1976) found bubbles in the gills of dead fish but seldom in live fish; they concluded that these signs are directly associated with death. The blood of exposed fish equilibrates with the excess pressure in the water. Bubbles form in the blood and these can block the capillaries; in sub-acute cases the dorsal and caudal fin can be affected, and bubbles may be visible between the fin rays. The epidermal tissue distal to the occlusions then becomes necrotic and cases are known where the dorsal fins of trout have become completely eroded. In severe cases, death occurs rapidly as a result of blockage of the major arteries, and large bubbles are clearly seen between the rays of all the fins. A similar effect of gas bubbles forming in the blood can be experienced by deep-sea divers when they return quickly to the surface (Svobodova et al. 1993).

Uni- or bilateral exophthalmus is a further classic subacute and chronic clinical sign of GBD in juvenile and adult fish. However, in some experimental and natural cases of GBD, exophthalmia was not evident (Edsall and Smith 1991) or was

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not described as a significant feature (Pauley and Nakatani 1967). In some cases, juvenile fish and adults dying of acute GBD do not manifest any visible lesions at all (Machado et al. 1987).

Although gas bubble disease produces a variety of signs and lesions, the cause of death has generally been attributed to anoxia resulting from stasis of the blood. Weitkamp and Katz (1980) described the role played by emboli in death caused by gas bubble disease but also mentioned other factors such as blindness and stress which can lead to death from other causes, such as predation. Grahn et al. (2007) also indicated that the eye and the gill are uniquely sensitive to gas bubble precipitation. GBD lesions can also increase susceptibility to other diseases. Weitkamp (1976) found that fish suffering from GBD can apparently die due to secondary fungal infection. Marsh and Gorham (1905 as cited in Weitkamp and Katz 1980), on the other hand, concluded that the disease was caused chiefly, if not solely, by excessive dissolved nitrogen gas. This conclusion was based on the analysis of bubbles from tissues and blood vessels of animals with GBD. These bubbles contained 92% to 97% nitrogen, the remainder being oxygen. Bubbles formed in the supersaturated water had nitrogen and oxygen in the same ratio as found in air (Weitkamp and Katz 1980). In blood, nitrogen is held in simple solution, while oxygen is primarily bound to haemoglobin.

Safe limits for gas supersaturation depend on species tolerance and on factors that differ among fish farms and natural habitats, between continuous and intermittent exposures, and across the ranges of temperature and salinity. Fish of different ages and sizes react differently to elevated total dissolved gas pressure (Weitkamp and Katz 1980). Differences among species have also been described, either due to anatomical differences such as ability to regulate swimbladder volume (Chamberlain et al. 1980), or apparent ability to detect and avoid supersaturated regions (McCutcheon 1966; Gray and Haynes 1977; Stevens et al. 1980). Bouck (1980) concluded that extreme levels of supersaturation, or periods of prolonged exposure to mild supersaturation, would be needed to lead to the death of all members in a population. Therefore, it is also difficult to define upper limits for the safe saturation of water with air. Weitkamp and Katz (1980) argued that chronic exposure (weeks to months) to even a total dissolved gas saturation of 102% can cause GBD in at least some fish in a population. Higher levels are associated with a more rapid rise in mortality rates. Similarly, Wedemeyer (1996) suggested that for salmonids, supersaturation limits should be less than or equal to 103% for hatchery stages and less than or equal to 105% for on-growing stages.

Case description

Anamnesis. In May 2014, our department was asked to perform a local investigation in a small hobby fish farm breeding brook trout (*Salvelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*). The fish had been originally bred in a small natural pond (area of about 0.02 ha, average depth of 1.5 m) under flow-through conditions using spring water. Under these conditions breeding took place without problems. However, problems arose when the capacity of the facility was extended by plastic circular tanks (diameter 3 m, depth 1 m) that were placed in the hall of a former factory. Fish stocked in these tanks repeatedly perished shortly (within several hours) after stocking, although the water supplied to the tanks was of the same origin as the water in the natural pond and did not show oxygen deficits. The newly included tanks were made of plastic commonly used for fish breeding purposes, and had been thoroughly washed before use. Therefore, a negative impact of substances that may have been released from the tank walls was excluded. Previously conducted parasitological examination of dead fish was negative. The results of the basic physico-chemical water analysis showed a very good quality of water. There were no cases of death recorded when the plastic tanks were supplied experimentally with water from the nearby river Eger. However, once the tanks were supplied with spring water again, fish deaths followed.

On-site examination. A local survey was conducted on 13th November 2014. At our request, 10 specimens of brook trout were stocked in one of the circular tanks the day before our arrival as we wanted to observe clinical signs of damage, and if possible, to perform autopsies on dead fish. All these fish died on the same day (within 6 h after stocking). Immediately upon our arrival, the autopsy of dead fish and their parasitological examination was carried out. Field surveys followed.

A spring located about 50 m above the breeding facility on the opposite bank of the river Eger at a distance of about 4 km, was the source of water

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for the fish breeding facility. Water from the spring accumulated in an underground concrete tank that was drained by overflow in the form of a suction basket (strainer) into a plastic pipe. Water was then supplied by this pipe to the breeding facility. Excess water was retained in a small pond, which was located about 50 m away from the spring. In the breeding facility, the water was carried to a retention tank on the second floor, from where it was piped through a strainer by gravity flow into rearing tanks on the lower floor. Some of the water was drained away into a natural pond located about 200 m away from the building. At several sites (i.e., spring, natural pond, rearing tanks), water pH, temperature, and dissolved oxygen concentration were measured using a WTW multiline P4 and water samples were taken for physico-chemical analysis. Zooplankton samples for hydrobiological examination were taken from the pond below the natural spring and the pond next to the facility.

RESULTS

Analysis of water

Physico-chemical indicators of water samples are summarised in Table 1. For a site plan with depiction of the sampling sites see Figure 1.

Zooplankton

In the sample taken from the pond located close to the spring area, harpacticoids (*Canthocamptus staphylinus*) and cyclops (*Cyclops insignis*) were found. In the pond located close to the facility, cyclops (*Cyclops insignis*) were noted.

Table 1. Physico-chemical indicators of water

Sample	1	2	3
Temperature (°C)	9.5	10.0	10.0
pH	7.56	7.80	7.61
Dissolved oxygen (mg/l)	8.34	10.50	14.9
Oxygen saturation (%)	77	96.7	136
COD_{Cr} (mg/l)	< 10	< 10	< 10
NH_4^+-N (mg/l)	0.04	0.05	0.06
P_{tot} (mg/l)	< 0.05	< 0.05	0.05
NO_2^--N (mg/l)	0.005	0.006	< 0.005
Cl^- (mg/l)	6.4	6.4	6.4
$\sum Ca^{2+} + Mg^{2+}$ (mmol/l)	1.8	1.8	1.8

1 = underground concrete tank, 2 = small natural pond, 3 = plastic circular tank

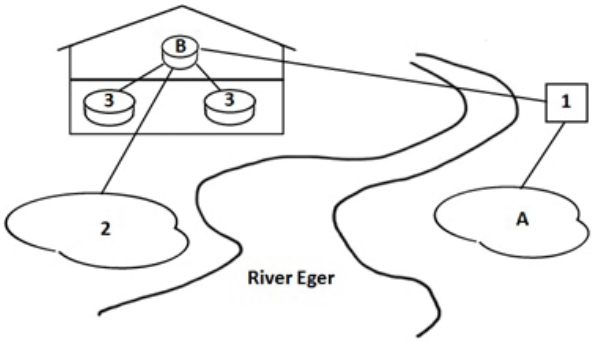

Figure 1. Water sampling points (1 = spring, underground concrete tank; 2 = small natural pond; 3 = plastic circular tanks; A = small retention pond; B = retention tank)

Fish examination

The mouths of dead fish were convulsively opened, with the opercula set conspicuously apart. Tiny gas bubbles were visible on the body surface, mainly on fins and around the mouth. The skin of fish was pale and covered with a thin layer of mucus. The gills had a light red colour and gill filament edges were pale. No changes were found in the body cavity. Microscopically, gas bubbles were visible both on the surface of gills and inside the gill filaments (Figure 2). Parasitological examination was negative.

Diagnosis

Gas bubble disease was the ultimate cause of the fish deaths.

Figure 2. Gills arch of brook trout – gas bubbles on the surface of gills (rectangular mark) and inside the gill filaments (oval mark; Photo Ch. Steinbach)

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DISCUSSION AND CONCLUSIONS

Microscopic examination of water samples collected in the pond at the spring and in the pond at the fish farm revealed a low biomass of oligosaprobic zooplankton. Since invertebrates are markedly more sensitive to the effects of contaminants (especially to pesticides and metals Svobodova et al. 1993) when compared to fish, it was possible to rule out the possibility that these substances were present in concentrations which would be acutely lethal for plankton and fish. The qualitative composition of plankton in both ponds was indicative of low trophic and saprobic status of the waters.

The results of physico-chemical analyses confirmed a very good quality of water supplied to the fish farm. Low COD_{Cr} values (< 10 mg/l) showed that the water was not loaded with organic substances. Very low concentrations (or below the limit of quantification) of ammonia, nitrites, chlorides and total phosphorus, together with the low values of COD_{Cr} confirmed that there was no contamination by sewage or agricultural wastewaters. The results of physico-chemical analyses corresponded well with the results of hydrobiological examination and confirmed the low saprobic and trophic status of the water. Based on these findings, acute damage to fish due to the presence of contaminants could be excluded.

However, we did identify an issue with oxygen saturation. Spring water which exhibited oxygen saturation of 75% was piped through a strainer, where a whirlpool was formed, into the building. This means that the water had been vigorously saturated with air while sucked into the duct. During water transport, undissolved gas could not be released. Moreover, water was further enriched by the air directly in the fish farm, where the water was drained, in a similar manner as in the spring area, from the (non-ventilated) storage tank on the upper floor into rearing tanks situated in the lower part of the building. Given that the water was transported in pipes, the excess gases could not be released from the water at this stage either. The gases then produced microscopic bubbles directly in the rearing tanks. The water in the rearing tank where the fish deaths occurred within 6 h, showed a marked supersaturation of oxygen (136%). Since the water was not saturated by gaseous or liquid oxygen, it is obvious that other gases present in the air, especially nitrogen, were dissolved in this water as well. Speare (1998) highlighted the potential risks of groundwater use in fish farming. Groundwater is frequently saturated with nitrogen, which begins to come out of solution as the water naturally depressurizes when pumped to the surface. If this water is delivered to a fish farm through closed pipes, release of this excess nitrogen is not possible until the water reaches the fish rearing vessel. Equilibration here leads to GBD in the fish.

Data on gas saturation in connection with GBD cases are usually presented either as the saturation of water with nitrogen or as saturation with air. Only in rare cases, is it expressed as oxygen saturation. To be able to compare our results with data from the literature, it is necessary to consider nitrogen/air saturation levels. Under our conditions, i.e. at normal atmospheric pressure, we can assume that the saturation of water with air and gases contained exhibits a directly proportional relationship. Thus, if oxygen saturation was 136%, nitrogen and air saturation would reach the same values. This level of nitrogen/air saturation unequivocally poses a risk for fish as documented, e.g. by Clay et al. (1976); Marcello and Fairbanks (1976); Beiningen and Ebel (1970); Raymond (1970 as cited in Weitkamp and Katz 1980). Marcello and Fairbanks (1976) described mortality of Atlantic menhaden (*Brevoortia tyrannus*) due to GBD at 130% and 140% of air saturation. Fickeisen et al. (1976) found that the dissolved gas level required to produce a 50% mortality in a test population of black bullheads (*Ameiurus melas*) over 96 h, was about 125% with slight differences depending on the water temperatures. However, as reported in the literature, damage to fish may occur even at markedly lower saturation levels (Clay et al. 1976). In 96-hour tests on Atlantic menhaden held in shallow tanks at 110% of nitrogen saturation, two fish showed signs of the disease and died within this timeframe. All fish tested at 120% of nitrogen saturation died within 24 h, apparently due to bubbles in the bulbous arteriosus and gill arterioles.

In the present study, the results of water analysis and examination of dead fish (presence of bubbles on the surface of the body, microscopic evidence of the occurrence of bubbles between the gill filaments and in the bloodstream) both indicated GBD. The observed symptoms were in very good agreement with literature data (e.g. Westgard 1964; Beiningen and Ebel 1970).

Based on the results of all the above-mentioned examinations and analysis, GBD was identified as the ultimate cause of fish deaths.

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Corrective measures reducing water supersaturation with air were implemented immediately upon the identification of the cause of fish deaths. Firstly, we removed the cover of the holding tank, where the water from the source accumulated prior to distribution to tanks. This led to improved de-aeration of the inlet water. Secondly, plastic plates were installed below the water inflow. As a result, water was sprayed on the plates and flowed in the form of a thin stream into the tank. Thirdly, boxes with plastic clips were installed at the mouth of the inlet pipe. As the water was passing through the boxes, the efficiency of de-aeration further increased. Immediately after the implementation of these measures, the oxygen saturation decreased from 136% to 110%. Under these conditions the fish stopped dying and started ingesting food spontaneously. Subsequently, technical adjustments were carried out directly in the spring area. Namely, the strainer was placed deeper under the water surface to prevent formation of the depression cone and to limit air suction into the water. Since the introduction of these measures oversaturation of the water with oxygen has not been recorded and the oxygen saturation levels have been ranging between 85 and 100% in fish breeding tanks.

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