Fish death in lakes

An incidence of mass-scale fish mortality in Bangalore was reported recently in the local newspaper as front-page headline[1]: 'Five tonnes of fish die in Ulsoor Lake. From being a clear, tranquil water body, the recently restored Ulsoor Lake has become a sea of dead fish'. Among the views expressed as the cause of this tragedy were[1–3]: (i) Chemicals flushed into the lake, following a cleaning of the BCC-owned Ulsoor swimming pool; (ii) lowered Biological Oxygen Demand; (BOD) level due to the approaching summer [BOD, expressed as mg O_2 per l, is the amount of dissolved oxygen needed to oxidize organic materials to carbon dioxide and water at a particular temperature and pressure. If there is a large quantity of organic waste, there will be a lot of bacteria working to decompose this waste. The greater the polluted organic waste, higher the BOD]; (iii) introduction into the lake, of a variety of fish known to be a prolific breeder to contain mosquitoes and (iv) death due to phosphorus load. A similar incidence of fish mortality in Bangalore had occurred in June–July 1995 in the Sankey Tank and Lalbagh Lake. These episodes have been reconciled with organic pollutants discharged into the lake. The purpose of this correspondence is to (i) inform that incidences of fish death are not unique to water bodies in Bangalore; (ii) review the available microbiological and biochemical explanations of fish death observed elsewhere, and (iii) apprise of a biological control of fish death proposed in the literature[4].

It is common knowledge that fishes in an aquarium live long if kept with photosynthetic aquatic plants such as *Hydrilla* or *Vallisneria*, or other plants under illumination and with air constantly bubbled into the water. The basis for this is a classic experiment by Joseph Priestley, who showed that a lone plant in a closed jar dies and a lone mouse in another closed jar dies, but when the plant and the mouse are together in the same jar, both live – experiments that led to the discovery of oxygen and photosynthesis. Animal life is dependent on photosynthesis. If the 'chemicals' discharged into the lake killed the suspended microscopic animals that are primary source of food of the fishes (zooplanktons), or if the lake was cleared of plants, it would have upset the 'ecosystem' and the fishes, like the mouse in Priestley's experiment, would die. Plants not only synthesize carbohydrate, but also do an

$$6CO_2 + 6H_2O \xrightarrow[\text{light}]{\text{chlorophyll}} 6O_2\uparrow + C_6H_{12}O_6 + ATP \quad (\textit{Photosynthesis by plants})$$

carbon dioxide water oxygen carbohydrate

$$C_6H_{12}O_6 + 6O_2 \xrightarrow{\text{light or dark}} 6CO_2\uparrow + 6H_2O + ATP \text{ (energy)} \quad (\textit{Respiration by all aerobic organisms})$$

Scheme 1.

additional biochemistry which animals are incapable of, that of purifying the environment by producing oxygen (Scheme 1).

Though fishes live in water, they are strict aerobes. Obtaining sufficient oxygen is a greater problem for fishes than for air-breathers for two reasons: First, oxygen has a low solubility in water, constituting only about 0.5% compared with approximately 21% in air. Second, the diffusion of oxygen is many thousands of times slower in water than in air. If the fishes or the water remained still, oxygen in the vicinity of the exchange surfaces would not be renewed by diffusion fast enough to sustain the animal. Fish moves water into the mouth, across the gill filaments, and out behind the operculum. This requires energy which is produced by constant burning of their intracellular fuel, be it a carbohydrate, lipid or protein, by the process of respiration. Fishes, unlike yeasts, do not have the option of deriving energy by anaerobic respiration.

The aquatic ecosystem comprises of two kinds of bacteria: aerobic bacteria which require oxygen and anaerobic bacteria which do not need oxygen to exist; indeed many are obligate anaerobes that cannot tolerate oxygen. Given that air contains 20% oxygen, one might expect the anaerobes to be rare. But this is not so; anaerobic bacteria are common in water bodies and in sediments, as they are in the piles of garbage and manure.

Often fish death is correlated with a stinking odour resembling rotten eggs, characteristic of hydrogen sulphide (H_2S)[5,6]. Its odour is perceptible in a dilution of 0.002 mg/l. H_2S, a colourless gas produced by respiration of certain bacteria in waters, is highly toxic to most respiratory organisms: it can kill animals, plants and microorganisms in micromolar range by coming into contact with the respiratory enzyme cytochrome *c* oxidase[7]. Certain animals, for example, tube worms, mussels and clams in the deep oceans around the hydrothermal vents from which an emission of hydrogen sulphide occurs, avoid poisoning by H_2S only because they have sulphur bacteria, in their guts which oxidize H_2S to less toxic or nontoxic forms[7,8]. The energy derived from oxidation is used for synthesis of ATP, and reducing power and fixation of carbon dioxide into organic material.

The mud at the bottom in ponds and lakes where some organic matter is present is a habitat of a variety of anaerobic bacteria, including the sulphate-reducing heterotrophic bacteria (*Desulfovibrio* and *Desulfuromonas*). These bacteria oxidize the end-products (lactate, propionate, butyrate, formate, etc.) produced by other anaerobes as electron donor to reduce sulphate (a common anion in mud and water) and obtain energy, for example:

$$\underset{\text{Lactic acid}}{2CH_3.CHOH.COOH} + \underset{\text{Sodium sulphate}}{Na_2SO_4}$$

$$\underset{\text{Acetic acid}}{2CH_3.COOH} + \underset{\text{Sodium sulphide}}{Na_2S} + \underset{\text{Carbon dioxide}}{CO_2} + \underset{\text{Water}}{H_2O}$$

Often sulphate reduction in water bodies is apparent from the blackened sand and soil. Sulphate is the normal oxidizing agent, but thiosulphate ($S_2O_3^{=}$) and sulphite ($SO_3^{=}$) can also be used. The probable pathway of sulphate reduction is given below[8]:

Sulphate $\xrightarrow{\text{ATP}}$
Adenosylphosphosulphate $\rightarrow$
Bisulphite $\rightarrow$ Trithionite $\rightarrow$
Thiosulphate $\rightarrow$ Hydrogen sulphide.

Strains of *Desulfovibrio* can be cultivated in completely inorganic media in an atmosphere of hydrogen gas and CO_2. According to Postgate[5], 'sulphate respiration is one of the commonest biological processes on earth, though one is rarely aware of it'. Drastic natural manifestations of their activities may occur; there are periodic eruptions of hydrogen sulphide, arising from sulphate reduction in decaying seaweed on the sea bed, off the coast in Walvis Bay, Southwest Africa. On one occasion the town of Swapokmund was invaded, according to press reports, by 'clouds of sulphurous gas' which 'blackened silverware and the clock face'; dead fish were washed up on the beach in heaps, and 'sharks came to surface gasping on the evening tide'! Venice, one of the most beautiful places, is blemished by the foul smell of hydrogen sulphide in its canals.

A group of bacteria known as sulphur bacteria can oxidize H_2S. Indeed, the concept of chemosynthesis (a process by which energy derived from the oxidation of inorganic compounds is used to fix carbon dioxide for biosynthesis) postulated by the Russian microbiologist Winogradsky[8], was based on sulphur bacteria found in sulphur springs and other natural habitats where H_2S occurs. Winogradsky demonstrated that in the presence of H_2S, the filamentous bacterium *Beggiatoa* accumulates granules of sulphur which disappear when the gas is depleted. The oxidation of H_2S and sulphur by bacteria serves as source of energy, for reduction of CO_2 to microbial cell material, with a concomitant excretion of sulphuric acid into the medium, according to the reaction scheme:

$$2H_2S + CO_2 \rightarrow 2S + (CH_2O) + H_2O,$$
$$2S + 5H_2O + 3CO_2 \rightarrow 2SO_4^{=} + 3(CH_2O) + 4H^+,$$

where (CH_2O) is cell material.

Postgate describes how a polluted, flooded claypit led to a possible remedy for such pollution[6]: 'The water in this pit was black and stinking, local residents were threatening the council with legal action, and the treatments we could suggest – filling or pumping out the pit, pouring in masses of acid – promised to be slow and expensive. Then, one day, the council's medical officer phoned us: overnight the water had turned yellowish and stopped stinking – would we come and look? We came as quickly as we could and were amazed: it was quite true. What had happened was that someone had, stealthily, at night and quite illegally, dumped a lorry-load of soil contaminated with waste from a chromium-plating works, half in, half out of the water. Back at the laboratory we did some tests and found that chromates were powerful specific inhibitors of the reduction process, effective in concentrations of a few parts per million'.

However, chemical control of pollution, such as this, can be hazardous over a period of time. For example, in 1990, skin lesions identified in a population in Bangladesh were traced to arsenic present in water used for domestic purposes and irrigation[9].

Several causes of fish death are possible; identification of the ultimate cause is a challenge. Experiments are desirable to study mixed cultures of sulphide and sulphur oxidizing bacteria that are able to multiply in conditions of a particular lake for evolving practical methods involving the release of hardy strains as a microbiological method of converting sulphide to elemental sulphur with oxygen ($2HS^- + O_2 \rightarrow 2S^o + 2OH^-$; $2S^o + 3O_2 \rightarrow 2SO_4^{2-} + 2H^+$)[10]. Regular monitoring of lakes for zooplankton, anaerobic bacteria, particularly of sulphate reducing bacteria, hydrogen sulphide concentration as indicators of pollution may uncover factors regulating fish population in lakes, despite the presence of both sulphate reducing bacteria and sulphide oxidizing bacteria. This episode of fish mortality reminds us that the basic questions in ecology remain not understood: Why do some species suddenly increase in numbers while others decline? Are these natural cycles, such as those of some insects (e.g. the gypsy moth in the UK) and some plants (e.g. *Parthenium hysterophorus* in many places in India), also applicable to aquatic ecosystems?

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