Aquatic Mammals 1987, **13.3**, 83–92

A biological approach to dolphinarium water purification: I. Theoretical aspects

J. D. van der Toorn

P.O. Box 303, 3860 AH Nijkerk, The Netherlands

(Part of this paper has been presented at the 15th Symposium of the European Association for Aquatic Mammals in Hamburg, West Germany, in March 1987)

Abstract

In this paper an overview is given of several alternative techniques for dolphinarium water purification, that may make water conditions more natural. Discussed are water composition, biological filtration, based on the trickling filter principle, and foam fractionation. Indications are given to their possible applications in dolphinarium water treatment systems.

Introduction

In recent years a lot of attention has been paid, and rightfully so, to ways to improve as much as possible the circumstances under which marine mammals, especially cetaceans, live in captivity. Several attempts have been made to set up some standards for pool dimensions and housing conditions. Much progress has been made in the field of marine mammal medicine and husbandry. And also the importance of training for these animals, as an occupational therapy or as a means towards a better understanding of their biology (Pryor, 1986) has been stressed. A logical extension of this development is the underlying attempt towards making the water conditions for inland dolphinaria more natural. When designing an artificial environment for the maintenance of dolphins, both physiology and ecology of the animals should be taken into account. Especially the ecological aspect is often overlooked. In order to create optimal living conditions for these creatures, their natural habitat has to be taken into account. Contrary to what Wallis (1973) stated, it is, at least now, very well possible to make use of the capability of self purification of natural waters for the purification of water in dolphinaria. Manton (1986) already mentioned that water treatment strategies for dolphinaria are based on processes for swimming pools and potable water, and ignore the effects of salt water. He stated: 'Perhaps initially dolphins should have been kept by marine engineers and not by swimming pool designers.' The strategies outlined in this paper are based on marine aquarium management techniques and on wastewater treatment techniques that make use of natural processes, that occur in both fresh and salt water.

Water composition

It is remarkable that it is very often assumed, that dolphins do not need anything more than a pure NaCl solution to live in. But this remains to be seen. Animals that are as well adapted to living in a marine environment as dolphins are, most likely make optimal use of that environment. Therefore the water in dolphinaria should be as close to natural seawater as possible. Elements that are known not to be essential can be left out. But essential trace elements, such as copper, zinc and manganese, should be included, unless it has been proven that dolphins do not take them up from the water. It is difficult to determine to what extent they take up elements from the water, but it is likely that to some extent at least they do. Dudok van Heel (1975) quoted Dr Redlich, saying that humans can take up enough copper by just rinsing the mouth once a day with water containing traces of copper. This happens through the mucosa of the mouth. Obeck (1978) showed that infant rhesus monkeys, kept in galvanized cages, took up so much zinc by just licking and mouthing their caging, that they developed a zinc-induced copper deficiency. If dolphins can also absorb trace elements through the mucosa of the mouth, then there should be ample opportunity for taking up elements from the water, since dolphins often swim with their mouths open. It has also been demonstrated that dolphins do in fact drink some sea water (Hui, 1981 (cited in Gaskin, 1986)) and therefore they might be able to extract elements from the water in the gastro-intestinal tract. A strong indication for absorption of trace elements from the water is the observation that a raw, dark patch around the blowhole of a dolphin (similar to lesions on the nostrils of zinc-deficient cattle) healed, when a mixture of trace elements, containing among others copper and zinc, was added to the water (Dudok van Heel, 1975). This raw patch reappeared when the addition of trace elements was discontinued. If the elements are absorbed through the mucosa of the mouth, the gastro-intestinal tract or through the skin (or maybe to some extent through all of these routes) needs further investigation.

The often heard remark: 'We do not add any trace elements to the water and our dolphins are doing fine' does not contradict this because this phrase is indeed based on what is added. The fact that high levels of certain metals can be present, for instance from the fresh water supply or from contaminations in the salt (Wickins & Helm, 1981), is completely overlooked in that case. The only thing that is important is not what is added, but what is present in the water. (A very readable overview of the metabolism and interactions of trace elements is given by Robbins, 1983.)

When incorporating a biological water purification, in which bacteria, algae and microscopic invertebrates remove the materials the dolphins add to the water it seems even more logical to use (an artificial) seawater instead of a pure NaCl solution in order to satisfy the needs of the micro-organisms. Also for this reason the trace elements, like iron, copper, zinc and manganese are important.

Of the other elements especially calcium and magnesium are of interest. They play a major role in several processes, such as in the buffering system (Kester *et al*, 1975), floc formation (Johnson *et al*, 1986) and foam formation (Degens & Ittekkot, 1986). These processes are all of importance for the self purification of the water. (It is interesting to note that high concentrations of magnesium in the bottom inhibit the growth of the fungus *Aspergillus sp.* (de Bolster *et al*, 1980). Whether magnesium has the same effect in water is not clear though.) Alkalotolerant bacteria in seawater tend to accumulate calcium, to help protect them against sudden pH rises, caused by the breakdown of organic matter (Maeda & Taga, 1980).

The salinity of ocean water is on average about 3.5% (Spotte, 1979b) and this should therefore also be the target value for artificial sea water for dolphins. However, we should keep in mind that this value is the total salinity. The NaCl salinity is approximately 3.0%. The rest is made up of, in order of importance, magnesium, calcium and potassium salts. Of the other elements only traces are present. It should be noted that the salinity also affects a dolphin's buoyancy. At lower salinities a dolphin has to spend more energy on staying afloat. This may have far reaching consequences especially for sick and newly born dolphins. Extra energy that must be spent on staying afloat may severely upset their energy budgets (W. H. Dudok van Heel, J. Geraci, pers. comm.).

Buffering

Natural sea water is buffered. This means that it resists sudden large changes in pH. This is necessary, because biological activities can alter the pH. Respiration and nitrification tend to lower the pH, while photosynthesis and denitrification tend to raise it (Spotte, 1979b). Sea water can cope with this through the carbon dioxide buffering system.

Carbon dioxide (CO_2) in water can form carbonic acid (H_2CO_3). This acid can dissociate to form the bicarbonate ion (HCO_3^-), which in turn can form the carbonate ion (CO_3^{2-}). All these reactions are reversible and by shifting back and forth through this chain of reactions, sea water has a means of removing excess acid or hydroxide (see Spotte, 1979b for a more in depth treatment of the subject). At the normal pH of sea water, 8.2, the bicarbonate ion is the dominant form.

A frequently used measure for the buffering capacity is the carbonate alkalinity, which is defined as the sum of the concentrations of the carbonate and bicarbonate ions in m.eq/l. For details on the methods of determination of the alkalinity, the reader is referred to Almgren *et al* (1983).

Total alkalinity includes also boric acid, but its contribution to the alkalinity is so small that it is frequently left out. The buffering system of sea water is very flexible and several cations play a part in it by forming complexes with carbonate and bicarbonate ions. In seawater the bicarbonate ion HCO_3^- is present as a free ion for 63–81%, 11–20% is present as $NaHCO_3$, 6–14% as $MgHCO_3^+$ and 1.5–3% as $CaHCO_3^+$. Of the carbonate ion CO_3^{2-}, 6–8% is present as free ion, 3–16% as $NaCO_3^-$, 44–50% as $MgCO_3$, 7% as $Mg_2CO_3^{2+}$, 21–38% as $CaCO_3$and 4% as $MgCaCO_3^{2+}$ (Kester *et al*, 1975).

Chlorination

Until now chlorination has been the most widely used technique for the removal of organic matter from marine mammal pools (Dudok van Heel, 1983). The method of chlorination has been described in considerable detail by Andersen (1973) and Manton (1986), both referring to White (1972), and will not be discussed further in this paper. The chlorination of marine mammal pools still gives rise to a lot of problems, despite a vast amount of literature on the subject of chlorination. As Geraci (1986) put it: 'Chlorine as sodium hypochlorite is perhaps the most commonly misused chemical treatment.' Although chlorine is used to remove organic matter from the system, it will not do that completely. In chlorinated marine mammal pools the TOC (total organic carbon) concentration will increase: about 7% of the carbon added to the system (as food) will remain (Spotte and Adams, 1979). Even occasional superchlorination will not remove all the organic carbon from the system (Adams & Spotte, 1980). Among the more persistent organic molecules there could very well be potentially toxic or carcinogenic chlorinated carbon compounds, that could accumulate if a considerable amount of water is not replaced regularly. Duursma & Parsi (1976) pointed to the possible formation of persistent chlorophenols, which are hard to detect. These substances form in water in which phenols are present. Spotte (1979b) noted that phenols are present in aquaria. These phenols probably come from algal pigments and since algae can grow in chlorinated pools, the presence of chlorophenols is likely. (The presence of algae is more likely in marginally chlorinated systems, since phytoplankton, which mainly consists of algae, has been shown to be more sensitive to free chlorine than to monochloramine (Duursma & Parsi, 1976). Monochloramine is the main disinfecting agent in marginally chlorinated systems, whereas systems employing breakpoint chlorination rely more on free chlorine.)

Another disadvantage of chlorination is that it works better against bacteria than against fungi (Dudok van Heel, 1983). This could increase the chance of fungal infections. It seems more likely, that the renewed infection of a dolphin with Lobomycosis when it was placed in a so-called enriched medium (Dudok van Heel, 1975) was not the result of the enriched medium itself, as Manton (1986) suggested, but rather of the absence of competition by bacteria in that medium. Under normal biological growth conditions bacteria will always overgrow fungi: fungi cannot be cultured if the growth of bacteria is not inhibited by the use of antibiotics (McKinney, 1962). Other skin infections, encountered in dolphins kept in chlorinated (or ozonated) water can be the result of the destruction of beneficial microflora and of inactivation of antimicrobial substances secreted by the skin (Geraci *et al*, 1986).

The chlorination of (artificial) sea water is far more difficult than that of fresh water or of a pure NaCl solution. The presence of magnesium makes breakpoint chlorination impossible (Manton, 1986) and also iron and manganese interfere with proper chlorination (Dudok van Heel, 1983).

Chlorination may also affect a certain aspect of dolphin communication. We now know that dolphins have a sense of taste and there is evidence that dolphins secrete signal substances, or pheromones, into the water, that are detectable by taste. These substances can play a role in social and sexual behaviour (Herman & Tavolga, 1980). Chlorine will almost certainly destroy these substances as soon as they are released. The continuous presence of chlorine and chloramines might also interfere in a more general sense with taste.

Ozonation

In recent years, ozone is used more and more for disinfection of water. One of the reasons for this could be that ozone can nowadays be produced more safely and economically photomechanically (McGregor, 1986). The principles of the photochemical generation of ozone (using mercury lamps) have been clearly outlined by Dohan & Masschelein (1986).

Ozone is mainly used for two purposes: disinfection and discolouration, but it can also have some effect in removing turbidity (Ramos & Ring, 1980). It has been claimed that ozone can reduce the TOC (total organic carbon) concentration in water by converting low molecular weight organic substances, such as methanol or ethanol to carbon dioxide (but also to acetic acid or acetone) (Elia *et al*, 1978). However, it does not have any effect on TOC levels in aquaria and marine mammal pools (Spotte, 1979b; Adams & Spotte, 1980). When reacting with organic matter, ozone will first attack carbon double bonds (Kinne, 1976). It is probably this reaction that explains the ability of ozone to remove colour: pigments contain carbon double bonds and can also contain phenol rings (Spotte, 1979b). It means that ozone is active primarily against colour from organic sources.

As a disinfectant it is more active against *E. Coli*, but also against plankton, insect larvae and possibly viruses, than chlorine. Its disinfection powers decrease at higher densities of organisms (Farooq *et al*, 1977) and at higher turbidities (Ramos & Ring, 1980).

Its effect on turbidity can be explained by the conversion of POC (particulate organic carbon) into DOC (dissolved organic carbon). At higher DOC levels this conversion will not take place, because ozone will react with the DOC first (Spotte, 1979b). Ozonation of water containing dissolved organic matter can even increase turbidity. When ozone reacts with organic matter, polar, negatively charged groups (like carboxyl and hydroxyl groups) are formed. Complexing with polyvalent cations can then result in precipitation of the organic matter (microflocculation) (Rice, 1986). Whether turbidity increases or decreases following ozonation depends on the total composition of the water.

In sea water the reactions of ozone are more complicated, because all kinds of side reactions may occur. It can for instance react with trace metals Fe^{2+} and Mn^{2+} and oxidize them to $Fe(OH)_3$ and MnO_2 respectively, both of which can precipitate in this form and may be removed from the solution (Rice, 1986). It can also react with the chloride ion Cl^- and convert that to hypochlorite ClO^- (Keenan & Hegemann, 1978).

Ozone does not leave any, possibly toxic, byproducts in the water and is therefore a convenient disinfectant for animal pools. On the other hand it does not leave a residual disinfecting agent either, so it does not have a long lasting effect. This makes the demands on the hydraulics of a water recirculation system higher.

Disinfection

The most common disinfecting agents are chlorine and ozone. For aquaria in particular ultra-violet (UV) irradiation is also often used for disinfection (Spotte, 1979b). Gewalt (1977) described the use of UV on a small pool for Amazon river dolphins (*Inia geoffrensis*). He mentioned an improvement in water quality after the installation of the unit. This improvement could however also be attributed to the simultaneous increase in water replacement from 12.5% to 20% of the volume per day. Also, his data suggest very high bacterial levels in the pool. He mentioned a coliform count of water before the filter of 23 500 per 100 ml. This confirms the findings of Spotte & Buck (1981), who state that UV can effectively disinfect the water going through the unit, but that it has no effect in more remote parts of the system. Spotte & Adams (1981) suggested that UV should be used only for single pass applications and not for recirculation systems.

On the other hand, the need for powerful disinfection in a biological sea water recirculation system may not be very high, since a bloom of micro-organisms in such systems is very rare (Wickins & Helm, 1981). Aside from extensive grazing of ciliates on bacteria in a biological filter, there are substances excreted by diatoms that inhibit the growth of *Staphylococcus aureus* (Aubert & Pesando, 1969) and also certain lipids, that occur in sea water can inhibit the growth of bacteria (Sieburth, 1971).

The biology of water treatment*

The biological treatment of water is based on the self-purification capacity of natural waters. When looking at the organisms responsible for this, we can distinguish two different groups: surface attached organisms and suspended (free floating) organisms. This is reflected into two different possibilities for water treatment.

The first type is a so-called fixed bed bioreactor, usually referred to as a trickling filter. The aim of the construction of such a filter is to get the largest possible internal surface area, combined with the smallest possible external volume. To this end such a filter is filled up with irregularly shaped bodies. In waste water treatment very often stones are used as filling material. Small scale trickling filters have also been successfully used for aquarium water treatment (Wolff, 1981), often with poly-ethylene filling bodies, which are, because of their shape, sometimes referred to as 'hedgehogs'.

Also slow sand filters can act as fixed-bed bioreactors (Wickins & Helm, 1981). On the top a thick, biologically active, mud layer will form, that will have to be scraped off every now and then (slow sand filters cannot be backwashed). Due to their size, these filters are not very practical for use in dolphinaria. Rapid and high rate sand filters do not sustain a stable microbial population for two reasons. First of all the water flow through the filterbed is usually too fast for the micro-organisms to properly break down dissolved substances. And when the filter is backwashed (nearly) all the micro-organisms are washed out and a new population has to be established (Kinne, 1976). It is therefore better to separate biological and mechanical filtration (Wickins & Helm, 1981).

In the other process use is made of the suspended organisms. In order for this to work, it is essential that the organisms make suspended colonies, or flocs. These flocs are kept in suspension by constant aeration of the water. In a second stage these flocs are allowed to settle and the clear water is removed while the settled flocs, usually referred to as sludge, are recycled. This is called the activated sludge process. This process is probably less suitable for dolphinarium or aquarium water purification for two reasons. First of all it needs a lot of space and secondly it works best at relatively high loads (at least, activated sludge reactors are currently used only for the treatment of waters with high organic loads) (Kinne, 1976). It is quite possible that in an aquarium or dolphinarium type situation the amount of sludge formed will be too low for proper operation of the system. In the following, only the trickling filter process will therefore be considered.

In a trickling filter, water is divided over the top and is allowed to flow slowly over the filter bed. In the course of that flow organic matter, both dissolved and suspended, is attacked by micro-organisms. Because some reactions proceed faster than others there is a stratification in the filter. In the first part of the filter (the top) mainly carbon decomposition will take place. The depth of that carbon decomposition zone depends on the load: if the water contains much organic carbon the zone will be very deep. In heavily loaded filters carbon decomposition may be the only process taking place. In the lower part of the trickling filter nitrification can occur. It will take rather long before a population of nitrifying bacteria has been established, since they grow slowly. It will take several weeks before a filter is properly conditioned (Spotte, 1979b; Kinne, 1976).

If a trickling filter is just put into operation the first organisms that will grow in it will be bacteria and fungi. These will coat the filling material: a biofilm is formed. On this film the secundary colonizers will start growing. To this group belong among others protozoa and nematodes. The growth of organisms

---

*See Mudrack and Kunst (1986) for more information.

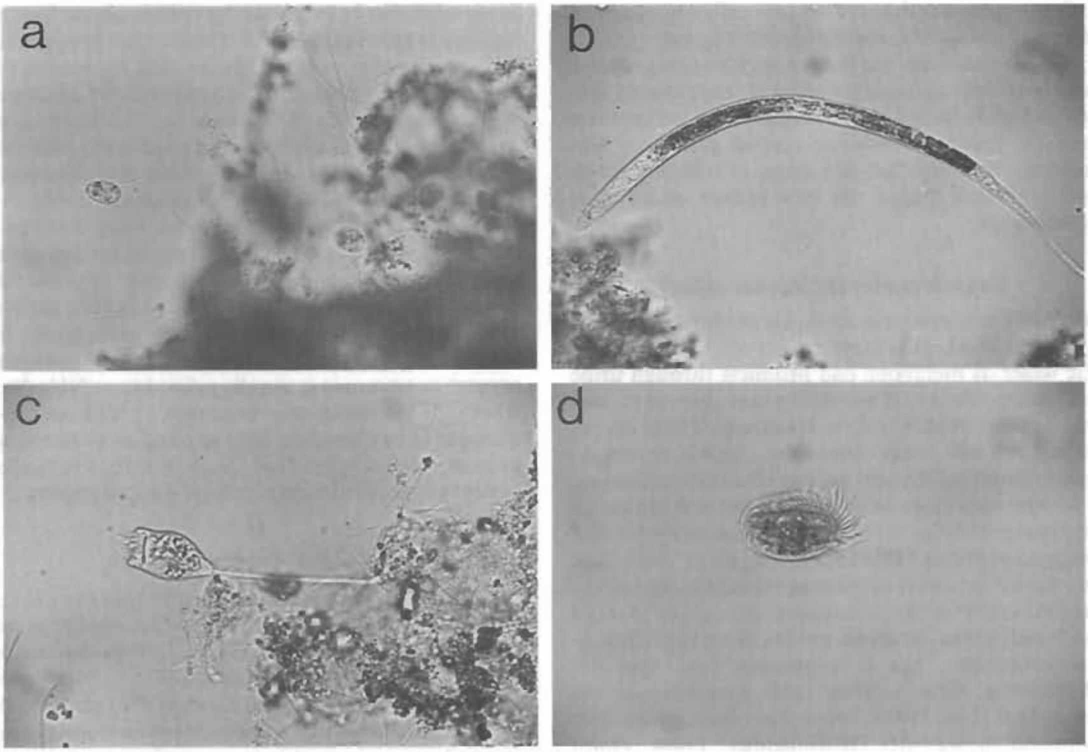

Figure 1: Some organisms, commonly encountered in trickling filters and activated sludge. (Original magnification 400 ×)
a–Zooflagellates: *Bodo sp.* (Note the filamentous bacteria in and around the sludge.)
b–A nematode
c–A stalked ciliate: *Vorticella sp.*
d–A free swimming ciliate: *Euplotes sp.*

in and on the biofilm will increase its thickness. If a film becomes too thick it will start sloughing. This sloughed film will appear in the water as flocs. These flocs settle easily, so if water from the trickling filter is led through a settling area first, nearly all organic matter can be removed.

In short what is happening in a trickling filter is the following:

— dissolved organic matter is mineralized

— particulate organic matter and colloids are either converted into dissolved organic matter and then mineralized, or are adsorbed onto the biofilm and removed from the water as settleable flocs.

— inorganic, particulate, colloidal or dissolved matter can react with or be adsorbed onto the biofilm and can thus be removed with the flocs.

Organisms in a trickling filter

The organisms that can be found in a trickling filter do not differ much from the ones found in activated sludge. No differences have been found between the bacterial flora of activated sludge and that of trickling filters (Lin, 1984). In activated sludge most are attached to suspended flocs, whereas in a trickling filter they will be attached to the filter bed. Apart from heterotrophic and nitrifying bacteria, the following organisms take part in the purification process (see Mudrack & Kunst, 1986; Tri, 1975; Fair *et al*, 1968):

— *Zooflagellates (Mastigophora)* (Fig. 1a); especially in highly loaded systems.

— *Amoebae;* different species appear in differently loaded systems.

— *Ciliates* (Fig. 1c,d); they are very common and they graze on bacteria. There are attached species, like representatives of the genus *Vorticella,* and free swimming species, belonging to genera like *Aspidisca, Paramecium and Euplotes.*

— *Nematoda* (Fig. 1b).

— *Diatoms*; they usually are present in lightly loaded systems.

Other organisms that are occasionally encountered include crustaceans, mites and insect larvae.

The so-called sewage fungus appears in significant amounts only in highly loaded or overloaded systems. This is in fact not a fungus but a filamentous bacteria species. There are several genera of filamentous bacteria that can occur in trickling filters and activated sludge, the best known of which is *Sphaerotilus*.

Organic matter and its conversion*

Most of the organic matter that is put into an aquatic system as food, will at one time or another end up in the water as metabolic end products through urine and faeces. Most of it will still be in organic form and can be converted by micro-organisms. These micro-organisms will break down the organic matter to inorganic forms. This process is called mineralization.

Large molecules will first be broken down to smaller ones by bacterial exo-enzymes (enzymes that bacteria excrete) (Tri, 1975). The most important organisms involved in these conversions are heterotrophic bacteria. It is extremely difficult to identify bacterial genera, let alone species, from water purification systems, but it is obvious that especially the genera *Flavobacterium* and *Pseudomonas* are important (Lin, 1984). These bacteria are also very common in seawater (Rheinheimer, 1980). *Vibrio* species are common in coastal environments. Both *Vibrio* and *Pseudomonas* species grow well in the normal pH range of seawater (Maeda & Taga, 1980). *Vibrio* species are also commonly present in marine mammal pools. Marine mammals are apparently carriers of these bacteria, usually without any adverse effects (Buck & Spotte, 1986). Most of the heterotrophic bacteria are proteolytic, which means that they can break down proteins. In this process, ammonia is released into the water. *Pseudomonas* species play a key role in proteolysis. Most important in the breakdown of fats are *Pseudomonas* and *Vibrio*, which are also responsible, together with *Flavobacterium*, for most of the conversion of carbohydrates (Rheinheimer, 1980). The resulting smaller molecules can be absorbed and will be mineralized further, thereby providing the organism with energy, or will be converted into macromolecules which the organism needs. In the mineralization process three nutrient elements are of interest: carbon, nitrogen and phosphorus.

The carbon in organic matter is mostly converted into carbon dioxide and will leave the water as carbon dioxide gas. In water it can form carbonic acid and thus play a role in the buffering system (see under that heading for more details).

The phosphorus is released into the water as (reactive) phosphate. This is not converted any further. Some of it can be taken up by certain algae, but in aquarium systems, most of it will either precipitate with calcium or magnesium, or will be bound to organic matter that can be removed for instance by foam fractionation. Phosphates that have been complexed with calcium can be re-released into the water by bacteria from the genera *Pseudomonas*, *Aeromonas* and *Escherichia* (Rheinheimer, 1980).

The fate of nitrogen is somewhat more complex. Some algae can convert urea, the main nitrogen source in mammalian urine, directly into useful products and this may also be true for certain amino acids. But in most cases nitrogen in organic matter is mineralized to ammonia (the amount of nitrogen present as ammonia is usually refered to as NH_4-N). Ammonia is toxic to most organisms in high concentrations. It can however be converted to nitrate in a process called nitrification. Nitrate is not known to have toxic properties even at high concentrations.

Nitrification and denitrification

Nitrification, the conversion of ammonia to nitrate, is a process that takes place under aerobic conditions. This is done by bacteria. The first step in the process is the conversion of ammonia, NH_4^+ to nitrite, NO_2^-. According to Kinne (1976), there are 5 species of bacteria that can do this conversion in sea water: *Nitrosomonas*, *Nitrosocystis* (2 species), *Nitrosospira* and *Nitrosolobus*. Spotte (1979b) mentions *Nitrosomonas* as being the most important ammonia oxidizer. Helder & de Vries (1983) tested the growth of *Nitrosomonas* at different salinities (from 0 to 3.5%) and found that this species grew equally well at all salinities. They noted that it needed some time to adapt to a different salinity, but once adapted, grew well.

The next step in the nitrification process is the conversion of nitrite, NO_2^-, to nitrate, NO_3^-. There are 4 species of nitrite oxidizers: *Nitrobacter* (2 species), *Nitrococcus* and *Nitrospina* (Kinne, 1976). Helder & de Vries (1983) found that *Nitrobacter* spp. adapted fast to different salinities and grew equally well over the whole range from 0 to 3.5%. Under aerobic conditions, nitrate is the end stage of nitrogen metabolism.

For the removal of nitrate there are two possible pathways. There are several large algae, among others *Ulva spp.* (sea lettuce), that can use nitrate nitrogen for the formation of organic nitrogen compounds, provided there is plenty of light available (Wickins & Helm, 1981). If there is not enough light, they will start metabolizing organic nitrogen and in that way increase the nitrate concentration.

Under anaerobic conditions, several bacteria species can use nitrate in respiration instead of oxygen. Among these, bacteria from the *Pseudomonas* group are predominant (Rheinheimer, 1980). In the

---

*See Spotte (1979a,b) and Kinne (1976) for more details.

process elementary nitrogen, N_2, is formed, which will escape as a gas. Since the energy gain from nitrate-induced respiration is about 10% less than that of oxygen-induced respiration, the latter will have preference if oxygen is present (Mudrack & Kunst, 1986).

Flocculation

In many dolphinaria aluminium compounds are used as flocculants (Andersen, 1973; de Block, 1983; Manton, 1986). The aluminium ion, when released into the water, will form aluminium hydroxide, $Al(OH)_3$, flocs, which will partly block the pores of a sand filter and thus enhance filtering action.

A flocculant should in principle also be able to destabilize colloid solutions. Whether aluminium compounds are able to do that in sea water is doubtful. A colloid in water containing a low concentration of some ions, will be surrounded by charged particles, that create an electrical double layer around it. The electrical potential of that layer is called the zeta-potential. The higher this zeta-potential is, the stronger the repulsion between the particles and thus the smaller the chance that two particles will collide and then coaggulate. If aluminium ions are added to such a solution, the zeta-potential of the colloid will be strongly reduced, the colloid solution becomes unstable and flocculation will occur. This process is called salt flocculation.

In sea water however the zeta-potential of colloids is already very low due to the presence of high concentrations of ions, including many bivalent ions (the higher the charge of an ion, the stronger its effect on the zeta-potential), such as calcium and magnesium (Liss *et al,* 1975). In sea water colloids are usually stabilized sterically by organic matter. In this case adding aluminium ions will have no effect. In sea water salt flocculation does not, or at least hardly, occur (Eisma, 1986).

In sea water flocculation does occur when particles are brought together mechanically, either by flow conditions causing particle collisions (Eisma, 1986; Kranck & Milligan, 1980) or by organisms that collect particles in faeces or pseudofaeces (Eisma, 1986). Sticky organic matter causes particles, once brought together, to stay together. Especially the mucopolysaccharides, produced by bacteria and algae, are responsible for this glueing process.

There is also another way to create flocs and that involves air bubbles. Because the processes involved are very similar to those in foam fractionation, this kind of flocculation will be dealt with in the next section.

Foam fractionation

Certain organic molecules tend to collect at the air-water interface. These molecules usually have a hydrophilic and a hydrophobic part. At the interface these molecules orientate themselves in such a fashion that the hydrophilic part is in water, while the hydrophobic part is in air. This situation is the most favourable position energetically for those molecules. Therefore they tend to collect at water surfaces and are often called surfactants. When air bubbles are led through water containing surfactants, these materials will also collect on the surface of the bubbles (because the bubble surface is in fact another air-water interface). Thus a film of organic material is formed around the bubble. If it then reaches the surface, the film will stay intact for a while. If many air bubbles reach the surface, many films will stay there and a foam will build up. (If bubbles collapse before they reach the surface, the films will remain as flocs in the water. This is the other way of flocculation, referred to in the previous section.) In nature this process causes for instance the formation of foam on beaches. The surf will cause a lot of air to be forced through the water. If the water is rich in organic matter, a combination of this matter with, among others, calcium will produce surface active substances (Degens & Ittekkot, 1986). These are responsible for the foam formation.

When an air bubble rises through water it will not rise as a motionless sphere, but the surface of the bubble will be in constant motion. A rising bubble can in that way collect material at its surface, also materials that are not specifically surface active. The motion of the surface will compress the film around it and a matrix of strongly adsorbed organic matter is formed (Johnson *et al,* 1986). This explains why a lot of different materials can be trapped in foam.

This process has already been widely applicated in waste water treatment (see for instance Fair *et al,* 1968, or Conway & Ross, 1980) and in aquarium water treatment (see Spotte, 1979b, or Kinne, 1976) in the form of foam fractionation, sometimes also called protein skimming. In a foam fractionator air is thoroughly mixed with water and the foam that is formed is removed in one way or another and with it the materials absorbed onto the foam film. How well a certain fractionator works depends on a combination of the following factors: organic matter concentration of the water, air bubble size, amount of air with respect to the amount of water and the efficiency of foam removal. If the foam is not removed properly the foam will collapse and the skins will appear as flocs in the water (Johnson *et al,* 1986). These flocs can act as substrates for further aggregation of suspended matter. This explains the increase in filterable material that is sometimes observed in systems that use foam fractionation (Wickins & Helm, 1981).

The materials that can be removed by foam fractionation include of course many organic substances such as proteins, fatty acids, polysaccharides and phospholipids (Kinne, 1976; Conway & Ross, 1980). Also larger biological material can be removed that way: flocs, bacteria and algae (Conway & Ross, 1980). Although the paper does not deal with foam fractionation as such, but with the behaviour of air bubbles in water, it can be derived from Johnson *et al* (1986) that also viruses can be removed with foam, since they can be trapped in the film surrounding air bubbles. Also inorganic material can be removed by foam fractionation, if it forms some kind of bond with organic matter. This can happen in two different ways. Calcium carbonate (Degens & Ittekkot, 1986) and calcium phosphate complexes (Tri, 1975) can collect organic material around them, while also other materials can do so. So-called microflocs are often a combination of organic and mineral matter (Eisma, 1986). The flocs thus formed can be removed as already mentioned above. The other way in which inorganic matter can be removed is by the formation of ligands of organic molecules with metal ions. Certain surfactants, especially glycoproteins, have a high affinity for trace metals (Liss *et al*, 1975) so that several metal ion species can be removed by foam fractionation. (See Eichhorn (1975) for an overview of the organic molecules that can act as ligands for trace metals.)

All this indicates that foam fractionation can have a marked effect on both dissolved and particulate organic matter concentrations. The fact that flocs and colloids can be removed indicates that foam fractionation can help reduce turbidity as well. This is confirmed by Spotte (1979b), who stated that foam fractionation helps reduce DOC (dissolved organic carbon) and POC (particulate organic carbon) levels and also that turbidity can be lowered.

Conclusion

In a design for a facility for the keeping of dolphins, as many aspects of their biology as possible should be taken into account. Already in the design of the pools this should be the case. Bottlenosed dolphins usually live in coastal waters, which vary in depth from less than 2 metres to over 10 metres (Wells *et al*, 1980). It could therefore very well be that having access to areas of different depths is better for them than living in a pool of uniform depth. Some dolphins actually seek shallow areas (less than 1.5 metres) in their pool system to rest or sleep (J. D. van der Toorn, unpublished data). The amount of variation in pool design is however limited by hydraulic demands.

The composition of the water should be as close to natural seawater as possible. However, chlorination of seawater is very difficult (Dudok van Heel, 1983; Manton, 1986). Also persistent toxic substances are formed when seawater is chlorinated (Duursma & Parsi, 1976). Therefore large amounts of water have to be replaced regularly. This may make the costs of maintaining an artificial seawater in a chlorinated system prohibitive. It is therefore necessary to look into alternative ways of water purification. As indicated in this paper, biological water treatment can be an alternative.

In a biological water treatment system, the need for water replacement is considerably less. The only substances accumulating in such a system will be nitrate and to some extent phosphates (Spotte, 1979b), both of which are not known to have any toxic properties. One of the main advantages of a biological filter is its flexibility. When the load is high, there is food available for many organisms and consequently more organisms will grow in the filter, thus increasing its efficiency. When the load decreases, part of the microbial population will starve and will be broken down by the surviving organisms. So a biological filter is in fact a self regulating system: there is no need to change the dosage of chemicals when the load changes, either manually or with the aid of expensive equipment.

As already indicated a trickling filter seems to be the best choice for a dolphinarium. In a trickling filter, large amounts of flocs are formed (Mudrack & Kunst, 1986). Therefore the trickling filter should be followed by a sedimentation (or settling) tank, in which the water flow slowly enough to allow the flocs to settle. The clarified water will leave such a tank through an overflow. The sludge collected at the bottom should be removed. This can be done continuously by pumping water with sludge from the bottom and leading it over sand filters. It can also be done intermittently in several ways. An in-depth discussion of all the possible ways of sludge removal is outside the scope of this paper.

Since a biological filter acts primarily on dissolved organic matter, there should be a mechanical treatment parallel to it. Filtration is probably not very effective without the help of flocculants. As indicated already above, the most commonly used flocculants, based on aluminium, might not be as effective in seawater as they are in fresh water and in pure NaCl solutions. And, unless you have a fairly open system in which considerable amounts of water are replaced regularly, there is a chance of accumulation of aluminium, which is potentially toxic (Andersen, 1973). However, foam fractionation can be very effective in removing dissolved, colloidal and particulate matter, both organic and inorganic (Conway & Ross, 1980). Foam fractionation works well without any need for addition of chemicals.

As already indicated, the need for disinfection in a biological system is very small (Wickins & Helm, 1981). It is however desirable to have at least the possibility for disinfection available. Chlorine is not suitable because it leaves a residual disinfectant in the water which may harm the biological filter. A good disinfectant for a biological system should react quickly, does not form any toxic byproducts and leaves no residual disinfecting agent in the water. Ozone meets those demands. If it is used in the clean water, coming from the biological and the mechanical treatment systems, the amount of ozone needed will be small (Farooq *et al*, 1977; Ramos & Ring, 1980). Apart from disinfection, ozone can be active in colour removal and it reacts with dissolved organic matter (Spotte, 1979b).

In short a possible biological water treatment system includes a trickling filter followed by a settling tank. Next to that there should be some kind of mechanical treatment. Foam fractionation is a good candidate for that. Final treatment of the water with low concentrations of ozone can help keep the amount of micro-organisms in the pools low, although there is no real need for that. If the system is closed, provisions should be made for denitrification to prevent the nitrate levels from increasing continuously. Alternatively regular partial replacements of the water could keep the nitrate levels down.

The practical application of a biological water treatment as described here will be outlined in a separate paper (Dudok van Heel & van der Toorn, 1987).

Acknowledgements

The inspiration for this paper comes from lengthy discussions about water treatment with Dr W. H. Dudok van Heel, who stimulated me to dig deeper into the theory of water purification. Dr P. Vuoriranta of the Technical University of Tampere helped me find the relevant literature on microbiology and Dr P. Keskitalo of Air-Ix, Tampere, supplied me with literature on ozonation. Their help is gratefully acknowledged. And finally I would like to thank my wife Jolanda for her help in improving the manuscript.

References

Adams, G. & Spotte, S. (1980). Effects of tertiary methods on total organic carbon removal in saline, closed-system marine mammal pools. *Am. J. Vet. Res.* **41:** 1470–1474.

Almgren, T., Dryssen, D. & Fonselius, S. (1983). Determination of alkalinity and total carbonate. In, Grasshoff, K., Ehrhardt, M. and Kremling, K. (eds.): *Methods of seawater analysis, 2nd revised and extended edition*, pp. 99–123. Verlag Chemie, Weinheim, West Germany.

Andersen, S. H. (1973). Treatment of water in dolphinaria. Aq. Mamm. **1(3):** 1–8.

Aubert, M. & Pesando, D. (1969). Variations de l'action antibiotique des souches phytoplanctoniques en function des rhythmes biologiques marins. *Revue int. Oceanogr. med.* **15–16:** 29–37.

Block, P. de (1983). Floculation by means of aluminium hydroxychloride ('Locron L') $Al_2(OH)_5Cl_2.3H_2O$. *Aq. mamm.* **10(1):** 31–32.

Bolster, M. W. G. de, Goot, H. van der, Kralingen, C. G. van & Reedijk, J. (1980). Metaalverbindingen en farmacotherapie. *Chemisch Magazine*, April 1980: M239–M242.

Buck, J. D. & Spotte, S. (1986). The occurrence of potentially pathogenic vibrios in marine mammals. *Mar Mamm. Sci.* **2(4):** 319–324.

Conway, R. A. & Ross, R. D. (1980). Handbook of industrial waste disposal. Van Nostrand Reinhold Comp., New York.

Degens, E. T. & Ittekkot, V. (1986). Ca^{2+}-stress, biological response and particle aggregation in the aquatic habitat. *Neth. J. Sea Res.* 20(2/3): 109–116.

Dohan, J. M. & Masschelein, W. J. (1986). The photochemical generation of ozone: present state of art of technology. In Masschelein, W. J. (ed.): Ozone and ultraviolet water treatment, pp. B.4.1–B.4.18. Proceedings Aquatech 1986, Amsterdam.

Dudok van Heel, W. H. (1975). Remarks on the composition of water for dolphins in inland dolphinariums. *Aq. Mamm.* **3(2):** 4–9.

Dudok van Heel, W. H. (1983). Chlorination in marine mammal pools: a reappraisal. *Aq. Mamm.* **10(1):** 23–30.

Dudok van Heel, W. H. & Toorn, J. D. van der (1987). A biological approach to dolphinarium water purification: II. A practical application: the Delfinaario in Tampere, Finland. (in preparation).

Duursma, E. K. & Parsi, P. (1976). Persistance of total and combined chlorine in sea water. *Neth. J. Sea Res.* **10(2):** 192–214.

Eichhorn, G. L. (1975). Organic ligands in natural systems. In, Goldberg, E. D. (ed.): The nature of sea water, pp. 245–262. *Phys. Chem. Sci. Res. Rep.* 1, Dahlem Konferenzen, Berlin.

Eisma, D. (1986). Flocculation and de-flocculation of suspended matter in estuaries. *Neth. J. Sea. Res.* **20(2/3):** 183–199.

Elia, V. J., Clark, C., McGinnis, K. T., Cody, T. E. & Kinman, R. N. (1978). Ozonation in a waste water reuse system: examination of products formed. *J. Wat. Pollut. Contr. Fed.* **50:** 1727–1732.

Fair, G. M., Geyer, J. Ch. & Okun, D. A. (1968). Water and wastewater engineering, vol. 2: Water purification and waste-water treatment and disposal. John Wiley & Sons Inc., New York.

Farooq, S., Chian, E. S. K. & Engelbrecht, R. S. (1977). Basic concepts in disinfection with ozone. *J. Wat. Pollut. Contr. Fed.* **49:** 1818–1831.

Gaskin, D. E. (1986). Kidney and water metabolism. In, Bryden, M. M. and Harrison, R. J. (eds.): Research on dolphins, pp. 129–148. Clarendon Press, Oxford.

Geraci, J. R. (1986). Husbandry. In, Fowler, M. E. (ed.): Zoo & wild animal medicine, 2nd edition, chapter 47: Marine mammals (Cetacea, Pinnipedia and Sirenia), pp. 757–760. W. B. Saunders Comp., *Philadelphia*.

Geraci, J. R., St. Aubin, D. J. & Hicks, B. D. (1986). The epidermis of odontocetes: a view from within. In, Bryden M. M. and Harrison, R. J. (eds.): Research on dolphins, pp. 3–21, Clarendon Press, Oxford.

Gewalt, W. (1977). Ultraviolet sterilization of water in a pool for tonina (*Inia geoffrensis*). A preliminary report. *Aq. Mamm.* **5(3):** 69–71.

Helder, W. & Vries, R. T. P. de (1983). Estuarine nitrite maxima and nitrifying bacteria (Ems-Dollard estuary). *Neth. J. Sea Res.* 17(1): 1–18.

Herman, L. M. & Tavolga, W. N. (1980). The communication systems of cetaceans. In, Herman, L. M. (ed.): Cetacean behavior: mechanisms and functions, pp. 149–209, John Wiley & Sons, New York.

Hui, C. A. (1981). Seawater consumption and water flux in the common dolphin *Delphinus delphis*. *Physiol. Zool.* **54:** 430–440.

Johnson, B.D., Xianliang Zhou and Wangersky, P. J. (1986). Surface coaggulation in sea water. *Neth. J. Sea Res.* **20(2/3):** 201–210.

Keenan, J. D. & Hegemann, D. A. (1978). Chlorination and ozonation in water and wastewater treatment. *Chemosphere* **1:** 9–28.

Kester, D. R., Ahrland, S., Beasley, T. M., Bernard, M., Branica, M., Campbell, I. D., Eichhorn, G. L., Kraus, K. A., Kremling, K., Millero, F. J., Nuernberg, H. W., Piro, A., Pytkowicz, R. M., Steffan, I. & Stumm, W. (1975). Chemical speciation in seawater—Group report. In, Goldberg, E. D. (ed.): The nature of seawater, pp. 17–41. *Phys. Chem. Sci. Res. Rep. 1*, Dahlem Konferenzen, Berlin.

Kinne, O. (1976). Cultivation of marine organisms: water quality management and technology. In, Kinne, O.: Marine ecology, vol III, part 1, pp. 19–300. John Wiley & Sons, London.

Kranck, K. & Milligan, T. (1980). Macroflocs: production of marine snow in the laboratory. *Mar. Ecol. Prog. Ser. vol. 3:* 19–24.

Lin, K. Tsung-Ju (1984). Bacterial community structure in secondary wastewater treatment. *Thesis Rutgers Univ.*, New Brunswick, N.J.

Liss, P. S., Bertine, K., Clearfield, A., Kratohvil, J., Lyklema, J., MacIntyre, F., Marcus, R. A., Martin, J. M., Morrison, G. H., Nancollas, G. H., Ottewill, R. H., Parsons, R., Pravdic, V. & Sherry, H. S. (1975). The colloidal state and surface phenomena—group report. In, Goldberg, E. D. (ed.): The nature of seawater, pp. 453–475. *Phys. Chem. Sci. Res. Rep. 1*, Dahlem Konferenzen, Berlin.

Maeda, M. & Taga, N. (1980). Alkalotolerant and alkalophilic bacteria in seawater. *Mar. Ecol. Prog. Ser.* vol 2: 105–108.

Manton, V. J. A. (1986). Water management. In, Bryden, M. M. and Harrison, R. J. (eds.): Research on dolphins, pp. 189–208. Clarendon Press, Oxford.

McGregor, F. R. (1986). Photochemical generation of ozone and other oxidants for drinking water and cooling water treatment. In, Masschelein, W. J. (ed.): Ozone and ultraviolet water treatment, pp. B.5.1–B.5.12. Proceedings Aquatech 1986, Amsterdam.

McKinney, R. E. (1962). Microbiology for sanitary engineers. McGraw-Hill Book Comp., Inc., New York.

Mudrack, K. & Kunst, S. (1986). Biology of sewage treatment and water pollution control. Ellis Harwood Ltd., Chichester.

Obeck, D. K. (1978). Galvanized caging as a potential factor in the development of the 'fading infant' or 'white monkey' syndrome. *Lab. Anim. Sci.* **28:** 698–704.

Pryor, K. (1986). Reinforcement training as interspecies communication. In, Schusterman, R. J., Thomas, J. A. and Wood, F. G. (eds.): Dolphin cognition and behaviour: a comparative approach, pp. 253–266. Lawrence Erlbaum Associates, Hillsdale, N.J.

Ramos, N. G. & Ring, J. F. (1980). The practical use of ozone in large marine aquaria. In: Ozone: science and engineering, vol 2, pp. 225–228. Pergamon Press.

Rheinheimer, G. (1980). Aquatic microbiology, 2nd ed., John Wiley & Sons, Chichester.

Rice, R. G. (1986). Applications of ozone in water and wastewater treatment. In, Rice, R. G., Bollyky, L. J. and Lacy, W. J. (eds.): Analytical aspects of ozone treatment of water and wastewater, pp. 7–26. Lewis Publishers, Inc., Chelsea, Mich.

Robbins, Ch. T. (1983). Wildlife feeding and nutrition. Academic Press, Inc., Orlando.

Sieburth, J. McN. (1971). An instance of bacterial inhibition in oceanic surface water. *Mar. Biol.* **11:** 98–100.

Spotte, S. (1979a). Fish and invertebrate culture: water management in closed systems, 2nd ed. John Wiley & Sons, N. Y.

Spotte, S. (1979b). Seawater aquariums: the captive environment. John Wiley & Sons, N. Y.

Spotte, S. & Adams, G. (1979). Increase of total organic carbon (TOC) in saline, closed-system marine mammal pools. *Cetology* **33:** 1–6.

Spotte, S. & Adams, G. (1981). Pathogen reduction in closed aquaculture systems by UV radiation: fact or artifact? *Mar. Ecol. Prog. Ser.* **6:** 295–298.

Spotte, S. & Buck, J. D. (1981). The efficacy of UV irradiation in the microbial disinfection of marine mammal water. *J. Wildlife Diseases* **17(1):** 11–16.

Tri, W. (1975). Lehr- und Handbuch der Abwassertechnik, Band II, zweite Auflage. Verlag von Wilhelm Ernst & Sohn, Berlin.

Wallis, A. P. L. (1973). The maintenance of satisfactory water conditions in dolphinaria. *Aq. Mamm.* **1(3):** 19–25.

Wells, R. S., Irvine, A. B. & Scott, M. D. (1980). The social ecology of inshore odontocetes. In, Herman, L. M. (ed.): Cetacean behavior: mechanisms and functions, pp. 263–317, John Wiley & Sons, New York.

White, G. C. (1972). Handbook of chlorination. Van Nostrand Reinhold Comp., New York.

Wickins, J. F. & Helm, M. M. (1981). Seawater treatment. In, Hawkins, A. D. (ed.): *Aquarium systems*, pp. 63–128. Academic Press, London.

Wolff, E. (1981). 'Der Tropfkörper', eine Alternative zum herkömmlichen Filter für grosse Aquarien. *Zeitsch. Kölner Zoo* **24(1):** 31–35.