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# Marine Mammal Water Quality: Proceedings of a Symposium

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# Marine Mammal Water Quality: Proceedings of a Symposium

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# Letter of Transmittal

Twenty-six years ago, in December 1972, the Marine Mammal Protection Act was passed, specifying that captive marine mammals would be provided with adequate care. Since September 1979, the Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) has been responsible for inspections of facilities maintaining marine mammals for compliance with both the Marine Mammal Protection Act, under Memorandums of Understanding with the National Marine Fisheries Service (NMFS) and the U.S. Department of the Interior's U.S. Fish and Wildlife Service (FWS), and the Animal Welfare Act.

Throughout the 13-year period from 1979 to 1992, many discussions among NMFS, FWS, the Marine Mammal Commission, and APHIS identified concerns about the lack of information available on water quality for marine mammals. In October 1991, APHIS published Sterilization of Marine Mammal Pool Waters: Theoretical and Health Considerations, by Stephen Spotte (USDA Tech. Bull. 1797). In June 1992, APHIS sponsored a symposium with the John G. Shedd Aquarium in Chicago and the Chicago Zoological Park (Brookfield Zoo) in Brookfield, IL, on water quality for marine mammals.

The present volume captures the major presentations from that symposium and documents APHIS' commitment to ensuring adequate care of marine mammals by training our personnel and providing information to other agencies involved in regulating marine mammal care in captivity, to institutions and individuals maintaining marine mammals, and to the concerned public.

W. Ron DeHaven, D.V.M.
Deputy Administrator
Animal Care
Animal and Plant Health Inspection Service
U.S. Department of Agriculture

# Acknowledgments

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Thanks to all of the speakers who presented papers at the symposium: Gary Adams, Brad Andrews, Patrick Case, Greg Early, Lou Garibaldi, Joseph Geraci, Randy Hamilton, Jeff Keaffaber, Ed Krajniak, Michael Kreger, David LaBonne, William Medway, David Merritt, Brad Miller, Jim Robinett, Paul Sieswerda, Edwin Skoch, and William Watts.

We thank all our hosts in Chicago: Bill Braker and the staff of the John G. Shedd Aquarium, especially Jim Robinett, Carol Fitzhugh, Jeff Boehm, Blair Duff and Kent Semmen; and George Rabb and the staff of the Brookfield Zoo, especially Ed Krajniak, Mary Burke, Ed Hauesknecht, Tim Sullivan, Janine Darmofaz, Karen Herman, Tara Gifford, Jan Susman, Raquel Luna, Keith Morris, and Rita Stacey.

Within the APHIS family, we acknowledge the contributions of Monica Lorenzano and Michele Nicol for assisting with the symposium preparations and typing the proceedings. We also wish to thank Janet Wintermute for editing this volume and to Mary Ann Hines for typesetting it. Several APHIS facilitators helped during the course: Kay Carter–Corker, JoAnne Garbe, Peter Kirsten, Lisa Rothe, and David Sabala. And Joan M. Arnoldi, D.V.M., who was Deputy Administrator of Regulatory Enforcement and Animal Care at the time of the symposium and is now the Associate Administrator of APHIS, demonstrated continuing interest and support of this project.

Finally, we appreciate the support and comments of William Medway, representing the Marine Mammal Commission, and Art Jeffers, representing the National Marine Fisheries Service.

John Coakley

Richard L. Crawford, DVM

Richard L. Crawford

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# Introduction

**By John Coakley and Richard Crawford**

Keeping marine mammals in captivity requires all of the husbandry entailed in doing the same for terrestrial mammals (e.g., dietary management, provision of space and shelter, veterinary care, etc.) but in addition requires that proper water quality be maintained. Just what proper water quality is for each species of marine mammal, and how to achieve that, are subjects constantly discussed but rarely agreed upon. In June 1992, APHIS, in conjunction with the John G. Shedd Aquarium in Chicago and the Chicago Zoological Park (Brookfield Zoo) in Brookfield, IL, sponsored a symposium on water quality for marine mammals. The goal: to begin to define the parameters of good water quality for marine mammals in captivity.

The intent of this effort was to stimulate discussion about what constitutes good water quality; to provide a symposium proceedings containing research and philosophies about water quality for marine mammals, which will be of use not only to APHIS personnel but to all persons interested in captive marine mammal husbandry; to obtain information on water quality for possible future review or revision of standards for marine mammal care under the Animal Welfare Act; to identify areas about water quality for marine mammals on which there is a general consensus and also areas where further research is needed; and to train APHIS marine mammal veterinarians in the concepts of water quality and methodologies to determine if proper water quality is being provided for marine mammals.

We hope that the information in this book will be of interest and assistance to all those involved in the maintenance and husbandry of marine mammals and that it will represent a beginning point for discussion of proper water-quality parameters for these animals, not the final word on the subject. The points on which there are agreement are general in nature. What specific water-quality parameters are good or bad for the animals are still not agreed upon by the experts. But we must start somewhere in consolidating the theories and facts about what constitutes good water quality.

We also sincerely hope that not only will additional seminars be held concerning water quality but that additional seminars addressing other areas of marine mammal care, such as nutrition, training, transportation, and veterinary care, will also be held. The sharing and collection of such information would be of value to all concerned with marine mammal care. USDA, APHIS will do what it can in this regard, and we encourage other interested persons or agencies to participate and share in this effort to provide better care and conditions for the animals maintained in captivity.

We thank all of the speakers and participants who, by sharing their ideas and experiences, made the symposium a true learning experience for all. But most of all, we hope that the symposium results help the animals because all the presenters and organizers agree that this is what our efforts are all about.

# Saltwater

**By Gary Adams**

**Abstract**—Keeping marine mammals in captivity requires careful attention to the water in which they live. The ionic content is best characterized by stating the temperature and the salinity. Salinity is normally determined by first measuring a property which varies with salinity, then calculating the salinity through known mathematical relationships. The most common of these properties are chlorinity, density, specific gravity, refractive index, and electrical conductivity.

> According to Dr. Murray, 10,000 parts of water from the Firth of Forth contains 220.01 parts of common salt, 33.16 of sulfate of soda, 42.08 of muriate of magnesia, and 7.84 of muriate of lime.
>
> Elements of Chemistry, 1841
> J. L. Comstock, M.D.

Water is the medium in which marine mammals live. In the wild, cetaceans, pinnipeds, sirenians, and others live in waters of varying properties. During their lives, many experience wide ranges of temperature, salinity, and turbidity, seemingly with no ill effects. In captive populations, these conditions are often nearly constant.

Marine mammals in captivity live in fresh water, brackish water, sea water, diluted brine (sodium chloride solutions), or artificial sea water. For husbandry or regulatory reasons, managers of marine mammal facilities must characterize the strength of these solutions. Sea water, brackish water, and artificial sea waters all contain nearly the same ratios of the major dissolved ions (fig. 1, data from Spotte [1992]). Salinity, the term describing the total concentration of these ions, was initially measured by weighing the residue from a mass of sea water, evaporated

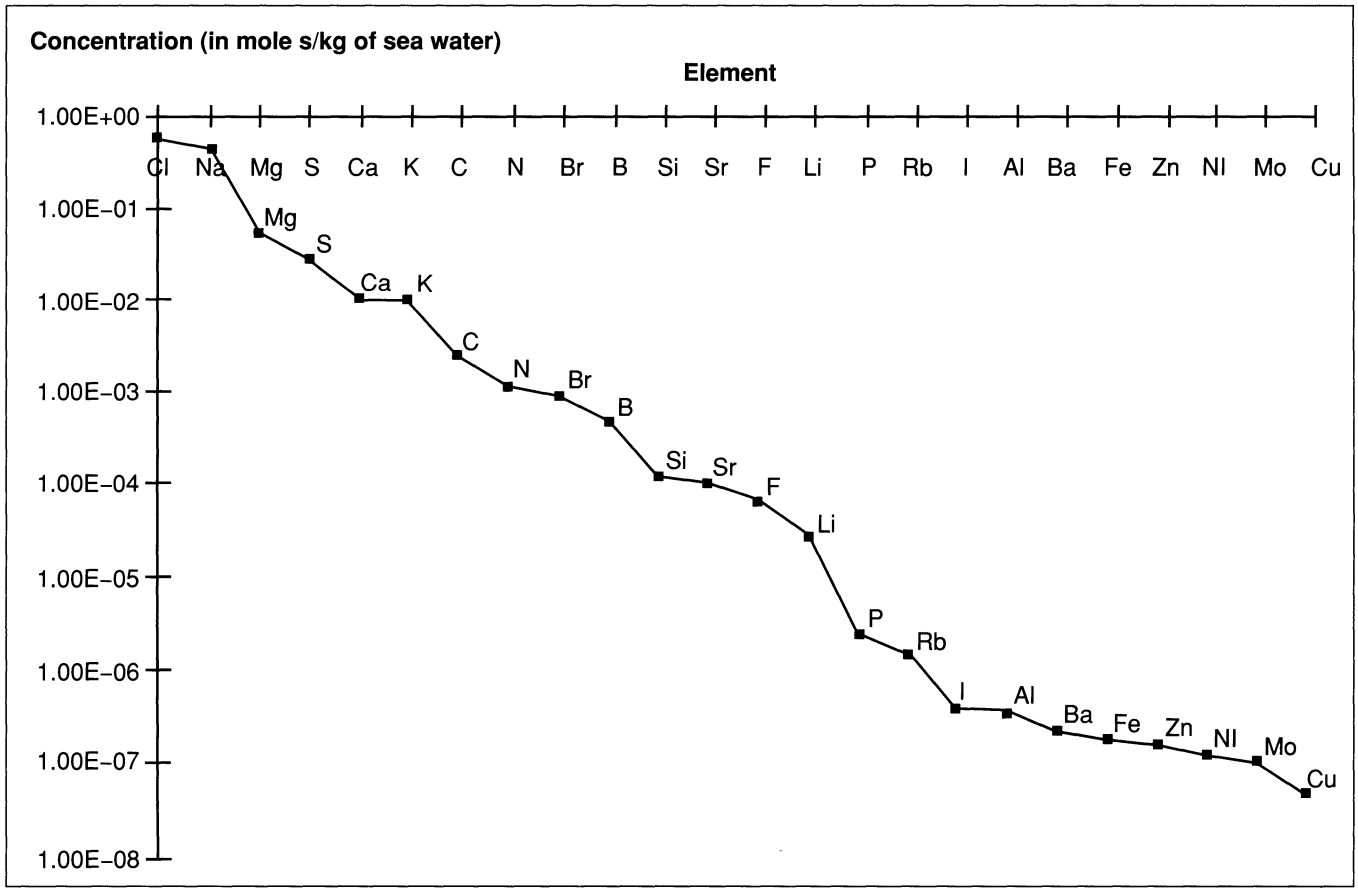


**Figure 1.**

to dryness under specified conditions. The results are reported in parts per thousand or grams of residue per 1,000 grams of sample water. Direct measurement of salinity by evaporation and weighing is difficult and rarely done. Salinity is a mass ratio—as long as no water evaporates and no ions are removed, the salinity remains unchanged. Temperature and salinity, the two critical properties of sea water, control various physical properties. Density, specific gravity, electrical conductivity, and refractive index each vary enough with salinity to enable them to serve as indirect methods of measuring salinity. These methods follow.

## Titration of Chlorinity

Several techniques for titration of the chloride present in sea water are discussed in Standard Methods for the Examination of Water and Wastes (American Public Health Association 1985) and other sources. The salinity can then be directly calculated from Knudsen's equation (Wheaton 1997, p. 36) because of the fixed ratio of ions in sea water:

$$S = 1.803 \times Cl + 0.3$$

where Cl is the chlorinity in grams of chloride per kilogram of sea water. This method is accurate and does not depend on temperature.

## Density

The relationship between density, salinity, and temperature is the international 1 atmosphere equation of state of sea water. According to Millero and Poisson (1981), density is approximated by:

$$\rho = \rho_0 + AS + BS^{3/2} + CS^2$$

where

$$A = 8.24493 \times 10^{-1} - 4.0899 \times 10^{-3}t + 7.6438 \times 10^{-5}t^2 - 8.2467 \times 10^{-7}t^3 + 5.3875 \times 10^{-9}t^4$$

$$B = 5.72466 \times 10^{-3} + 1.0227 \times 10^{-4}t - 1.6546 \times 10^{-6}t^2$$

and $C = 4.8314 \times 10^{-4}$

$\rho_0$ is the density of water approximated by:

$$\rho_0 = 999.84259 + 6.793952 \times 10^{-2}t - 9.095290 \times 10^{-3}t^2 + 1.001685 \times 10^{-4}t^3 - 1.120083 \times 10^{-6}t^4 + 6.536336 \times 10^{-9}t^5$$

(t °C, $\rho_0$ kg/m$^3$, for 0 to 40 °C, and S = 0.5 to 40 o/oo)

Unfortunately, this complex equation calculates density! We want to know the salinity. However, if we measure density and temperature, tables exist to allow estimation of salinity to sufficient accuracy. We measure density directly by determining the mass of a known volume of water at a specific temperature. This is not particularly difficult in a laboratory with an analytical balance, but it is not fast or convenient to carry samples back to the laboratory. For this reason, density is often estimated from specific gravity, electrical conductivity, or refractive index.

## Specific Gravity

The ratio of the density of sea water at a particular temperature (called the standard temperature) to the density of pure water at 15.5 °C (or reference temperature) is the usual definition of specific gravity. A hydrometer measures specific gravity using the principle that objects float higher in denser fluids. Hydrometers are convenient and sufficiently accurate for most husbandry practice; however, they are fragile. If the sample is at other than 15.5 °C, we can easily correct the reading to that temperature.

To determine salinity do the following:

- Read the hydrometer to three decimal places (e.g., 1.024). Also record the temperature.
- Find the temperature correction for the temperature and observed specific gravity in figure 2.

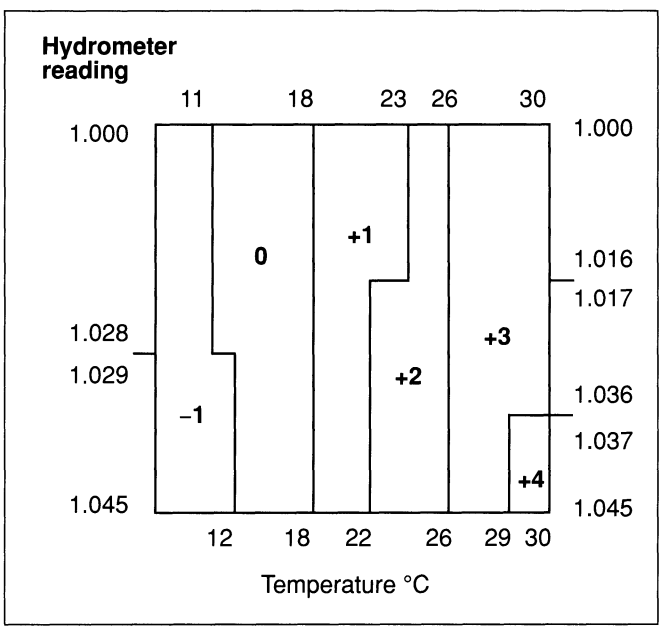


**Figure 2.**

- Add (or subtract) the temperature correction to the last place in the hydrometer reading.
- Enter the corrected specific gravity (SG) into the following equation to get the salinity in o/oo (parts per thousand)

$$S = 1.1 + 1{,}300(SG - 0.999)$$

**Example:** A hydrometer reads 1.019 in a sea water at 24 °C. From figure 2, the correction factor is +2. Thus,
SG = 1.019 + 0.002 = 1.02 and
S = 1.1 + 1,300 (1.021 – 0.999) = 28.6 o/oo.

## Refractive Index

The ratio of the speed of light in a sea water sample to the speed of light in a vacuum is the refractive index of the sea water. We measure refractive index by determining the angle at which all light reflects internally at the water surface. This is the critical angle, measured by the refractometer. The refractive index at 25 °C is related linearly to the salinity of sea water at (based on Wheaton 1977, pp. 36 and 60) by the equation

$$N_{25} = 1.85041 \times 10^{-4} \cdot S + 1.33248.$$

A portable direct-reading instrument is available with a built-in thermometer and temperature adjustment. This device is a good choice for aquaculture and marine mammal husbandry.

## Electrical Conductivity

The electrical conductivity of sea waters varies with salinity because pure water is a very poor conductor, while the addition of ions causes a large increase in conductivity. Conductivity is strongly dependent on temperature. Two equations summarize the entire relationship (Wheaton 1977).

$$S = 0.08966 + 28.29720\ C_{15} + 12.8083\ C_{15} - 10.67869\ C_{15} + 5.9862\ C_{15} - 1.32311\ C_{15}$$

where $C_{15}$ is the conductivity at 15 °C in μmho. $C_{15}$ can be corrected to temperatures other than 15 °C by adding a correction term given by:

$$C_T(C_T - 1)(T - 15)[96.7 - 72C_T + 37.3C_T^2 - (0.63 + 0.21C_T^2)(T - 15)]$$

where $C_T$ is the conductivity at the measurement temperature and T is the measurement temperature in °C.

This method of measurement and computation is useful for continuous monitoring and recording using current recording and data analysis techniques. Portable meters use a platinum conductivity cell and incorporate temperature correction in all of their instruments. Like refractive index, conductivity measurement is an appropriate means of salinity measurement in marine mammal pools.

## Brines

Brines are solutions of technical grade salts, such as sodium chloride, diluted to an ionic strength similar to the sea water salinity desired. Brines are low-cost substitutes for artificial or natural sea water and have the benefit that sterilization of marine mammal pools by chlorine is simpler. Natural sea water contains large amounts of bromide that reacts with chlorine or ozone disinfectants to form OBr (hypobromite) with much less disinfecting power. In addition, ozone ultimately converts bromide ($Br^-$) to stable bromate ($BrO_3^-$), an ion with unknown health effects. Brines do not have this disadvantage. Because the ion ratios of brines differ from those of sea water, salinity does not have the same meaning for brine solutions. We should state brine concentration as parts per thousand of NaCl (o/oo NaCl). Salt manufacturers provide tables relating specific gravity to o/oo NaCl at various temperatures.

## Summary

- For sea water, salinity in parts per thousand is the preferred measure; for brines, p/m NaCl.
- The hydrometer, with temperature correction, is a rapid, low-cost instrument suitable for marine mammal husbandry.
- A portable refractometer graduated in salinity units is a quick and accurate means of direct measurement of salinity.
- Conductivity with temperature correction is best for continuous monitoring and recording of salinity.
- Titration for chloride requires careful laboratory technique and can work for sea waters or brines. It is not recommended for routine work.

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# Ozonation of Marine Mammal Pool Waters

By David L. LaBonne

Ozone has been applied to marine mammal life support systems for more than 2 decades. Its success as an effective oxidant and disinfectant continues to be documented. In recent years, the chemistry of ozone has been more closely studied and become more clearly understood. In many marine mammal facilities, it has superseded chlorine as the primary chemical treatment, resulting in promising gains in water quality and improvements in animal health.

Ozone may be described as triatomic oxygen or as an allotrope of molecular oxygen. However defined, it is a powerful oxidant with a potential greater than that of chlorine or bromine, two elements also used in the treatment of marine mammal systems. The resonance structure of ozone in water is shown in figure 1.

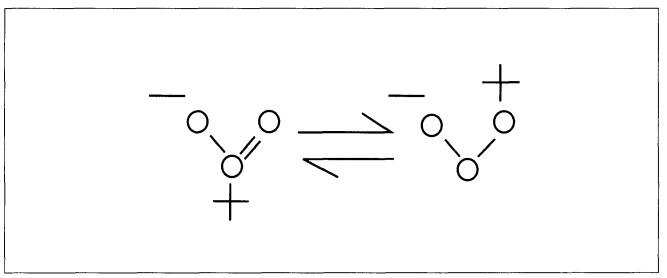

**Figure 1**—The resonance structure of ozone in water.

Ozone will react in water as a strong dipolar molecule and as a powerful electrophile. These characteristics make ozone a practical option in effective water treatment. Disinfection, odor control, oxidation of organic and inorganic contamination, removal of organics in solution, color removal, turbidity reduction, and decreased chlorine demand are all achievable results when ozone is used properly in a life-support system (American Water Works Association Research Foundation 1991, Rice et al. 1986).

Traditionally, in marine mammal pools, chlorine has been used as the primary disinfectant and oxidant of choice (Spotte 1992). In recent years, however, greater emphasis has been placed on ozone. Chlorine treatment requires that a residual of free and combined chlorine be maintained at all times throughout the pool water. The free and total chlorine content vary widely from facility to facility. There is no industry standard as to what constitutes "too much" chlorine. Total chlorine in closed or semiclosed marine mammal pools ranges from 0.5 p/m to more than 2 p/m. It is generally agreed amongst husbandry personnel that the higher levels are not desirable and can be harmful to the skin and eyes of the animals. When used properly, ozone can eliminate the need for chlorine. Where chlorine cannot be eliminated entirely, such as in heavily loaded or outdoor exhibits, chlorine can be reduced to less than 1 p/m. At any target concentration, total chlorine demand can be significantly reduced when ozone is used in conjunction with foam fractionation (see Control of Total Organic Carbon, below). These changes can reduce or eliminate the numerous disinfectant byproducts associated with chlorination (Trussel 1992).

Ozone treatment is different from chlorination in that no ozone residual is left in the pool with the animals. The gas is usually applied to a sidestream of the water recirculating in the pool's life support system.

## Ozone Generation

Ozone is formed endothermically by the following reaction:

$$3O_2 \leftrightarrow 2O_3$$

The oxygen needed to produce ozone gas may come from a prepared dry air source or a pure oxygen supply. Since 1994, clean dry (low dewpoint) air has predominated as the source gas for ozone production when used in marine mammal pools.

Because the ozone molecule is unstable, the gas must be generated onsite. There are two methods of ozone generation, photochemical and corona discharge. Photochemically produced ozone is not effective for marine mammal waters because the concentration of the gas produced is usually much less than 0.5 percent. This concentration affects its solubility in the water to be treated. The higher concentrations of 1–2.5 percent produced by the corona

discharge method ensure enough ozone for proper mass-transfer of the gas to liquid phase. Corona discharge ozone generators are, therefore, the most commonly used in the aquarium industry (Aiken 1995, LaBonne 1993).

A schematic diagram of how ozone is produced in a corona discharge dielectric cell is shown in figure 2. The basic ozone delivery system used in most aquariums follows the format shown in figure 3.

Ozone production of these units is usually measured in pounds per day. The largest units in aquarium use can generate up to 70 lb/day. However, the average generator for marine mammal facilities generates somewhere between 10 and 30 lb/day.

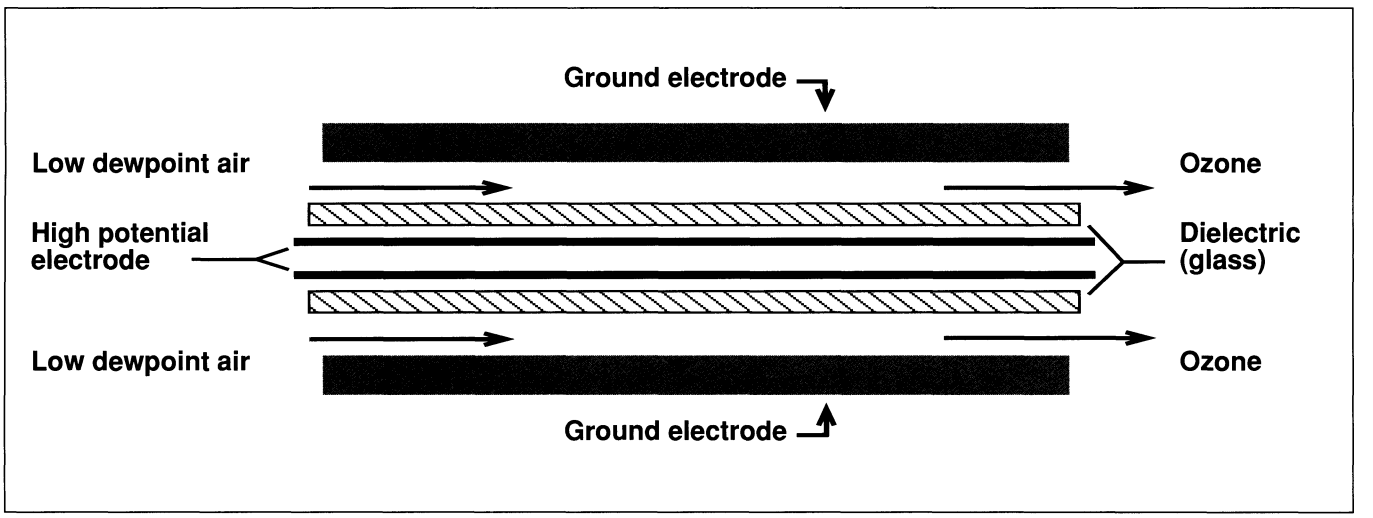


**Figure 2**—Schematic diagram of how ozone is produced in a corona discharge dielectric cell.

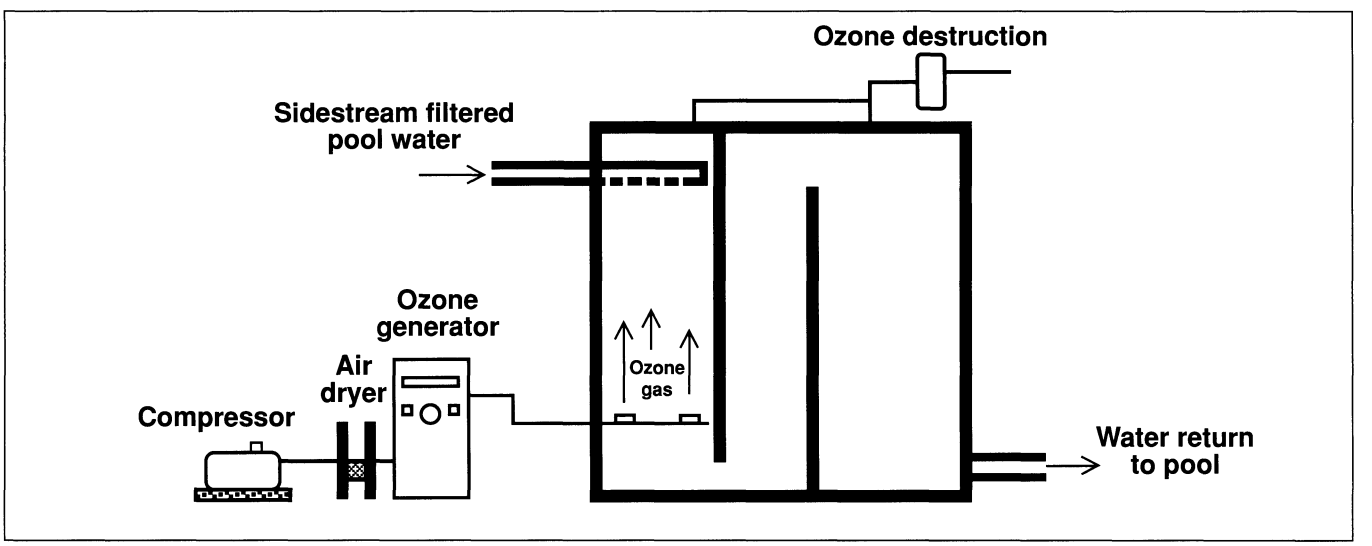


**Figure 3**—The ozone delivery system used in most aquariums.

Figure 4 shows the ozone generator at the National Aquarium in Baltimore (NAIB), MD. The size (in gallons) of the exhibit, the number and species of animals, the salinity of the water supply (natural/ artificial sea water), and its temperature most affect the quantity of ozone needed to treat a system properly.

## Application of Ozone in Marine Mammal Systems

The health of the animals in any facility is of the utmost concern. Unless proper concentrations and doses of ozone can be achieved, all the benefits of good water quality outlined at the beginning of this chapter cannot be consistently realized. Proper design is therefore essential in order to:

(1) safely and efficiently deliver the gas to the treatment reactor chamber,

(2) establish an effective ozone residual in the treated water for a specified contact time,

(3) strip any residual gas from the treated water after the contact times are complete and destroy the ozone off-gas (not necessary for foam fractionators), and

(4) return the treated water at a sufficient turnover rate to maintain the water quality standards of the facility.

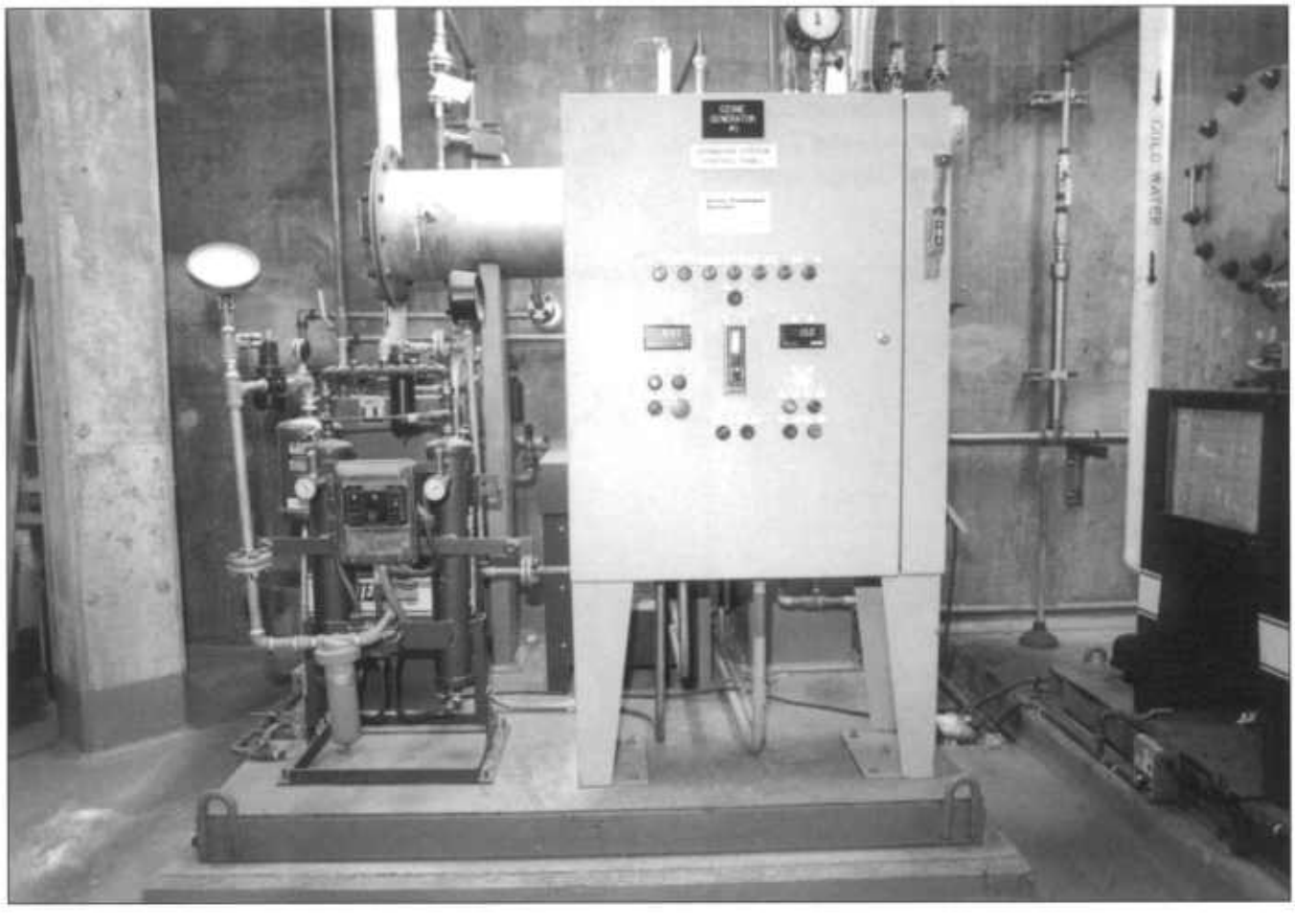

**Figure 4**—The ozone generator at the National Aquarium in Baltimore. (All photos in this chapter were taken by APHIS photographer Laurie Smith at the NAIB.)

To assure safe ozone gas production and delivery, proper maintenance of the generating equipment is essential. Stainless steel tubing should be used for transport of the gas to the reactor chamber. Employee and animal safety is further enhanced if the facility uses ambient ozone gas monitors to detect leaks in the system (Aiken 1995).

It is the chemistry of ozone in marine mammal water (brine and natural or artificial sea water) that has generated the greatest debate and study in the past 5 years. Important questions must be asked:

1. What is the facility's goal for water quality in using ozone?

2. What should the applied dose of ozone be?

3. How much ozone should be put into solution, and what should the residual contact time be?

If the answer to the first question is that the facility wants a healthy, natural environment for its collection, it may choose, as NAIB has (fig. 5), to utilize the greatest potential of ozone and design treatment systems that have the capability of delivering the following results.

1. Sterilization of water passing through a reactor chamber.

2. Reduction of chlorine use initially to a secondary role with the future possibility of eliminating it.

3. With lowered chlorine use, the establishment of biological filtration for ammonia removal.

4. Dosing ozone to oxidize color, odors, and organics.

5. Encouraging ozone-enhanced biological filtration (removal of biodegradable organic carbon) in the active filters.

6. Use of ozone to generate microflocculation (coagulation) of suspended solids and organics for water with extremely low turbidity.

7. Creation of as natural an environment as possible (i.e., change from a traditional brine water system to artificial sea water).

**Figure 5**—Recirculating pumps and filters for the 1.3-million-gal marine mammal life-support system at the NAIB.

NAIB has accomplished most of these goals in the existing life-support systems of the 1.5-million-gal Marine Mammal Pavilion (housing whales) and the 70,000-gal harbor and gray seal exhibit.

## Measuring Ozone

When employing these systems, it is necessary to have the ability to measure ozone in the gas and liquid phases. This ability also allows an ozone system to operate safely and efficiently. Gas phase measurement is commonly done with high- and low-concentration monitors that use ultraviolet light (Aiken 1995, LaBonne 1993).

To measure ozone in solution, there is one widely accepted method that is approved by the American Water Works Association and the American Society for Testing and Materials—the indigo dye method. It is specific to ozone and is not significantly interfered with by other oxidants, such as chlorine. The indigo dye method performs well in brine and artificial sea water (LaBonne 1989). Materials to conduct this test are now available commercially.

Oxidation reduction potential (ORP) probes are widely used and poorly understood. They are not specific to ozone and are susceptible to interference from other oxidants, such as the chlorine commonly used in marine mammal pools. ORP probes can be used effectively as a "front line" monitor of the general oxidation state of pool water or as a measure of the higher oxidation state within the ozone reactor chamber when monitoring disinfection. When used in conjunction with indigo tests, ORP millivolt readings become a more valuable tool in determining ozone effectiveness. Figure 6 shows data collected using ORP and indigo tests and their relationship to disinfection of artifical sea-water systems. With sufficiently high millivoltage in the reaction chamber (650–850 mV), complete sterilization of a sidestream of pool water can be achieved (fig. 7).

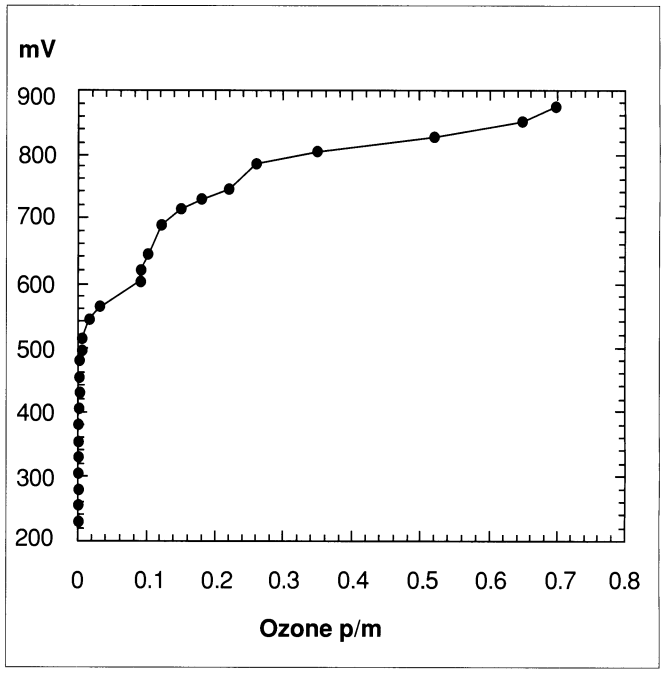


**Figure 6**—Oxidation reduction potential (ORP), measured in millivolts, plotted against ozone concentration in artificial sea water as determined by the indigo dye test. As readings from ORP probes exceed the 650–850 mV range, sterilization is achieved.

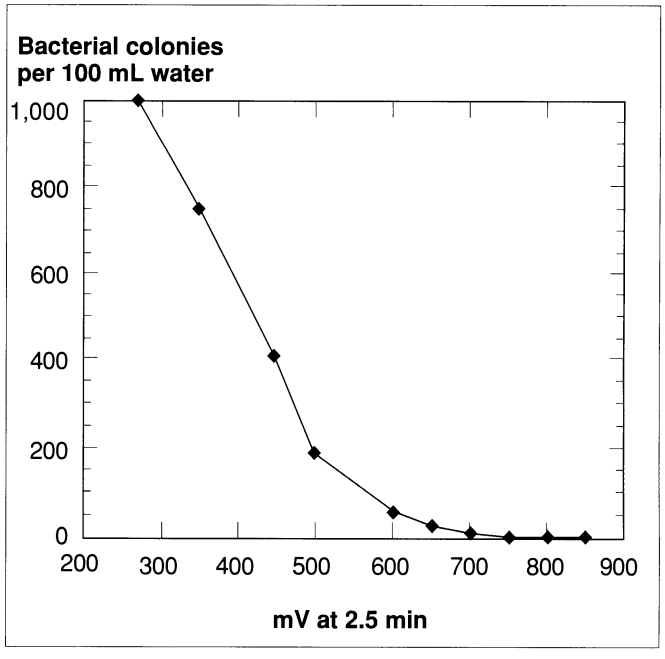


**Figure 7**—A sidestream containing 1,000 colonies of bacteria per 100 mL of pool water can be sterilized rapidly when enough ozone is added to bring ORP probe readings up to the 650–850 mV range.

The effects of ozone in nonchlorinated artificial sea water on bacterial and fungal agents are shown in figure 8. This system was operated with a 10-percent sidestream that was dosed to create an ozone residual of 0.3–0.5 p/m for 3 minutes' contact time. Complete sterilization was achieved and confirmed with continuous readings of 800 mV in the reactor chamber and heterotrophic plate counts. The exhibit pool millivolts rose close to 400 during this period. This value has been shown to be free of ozone residual.

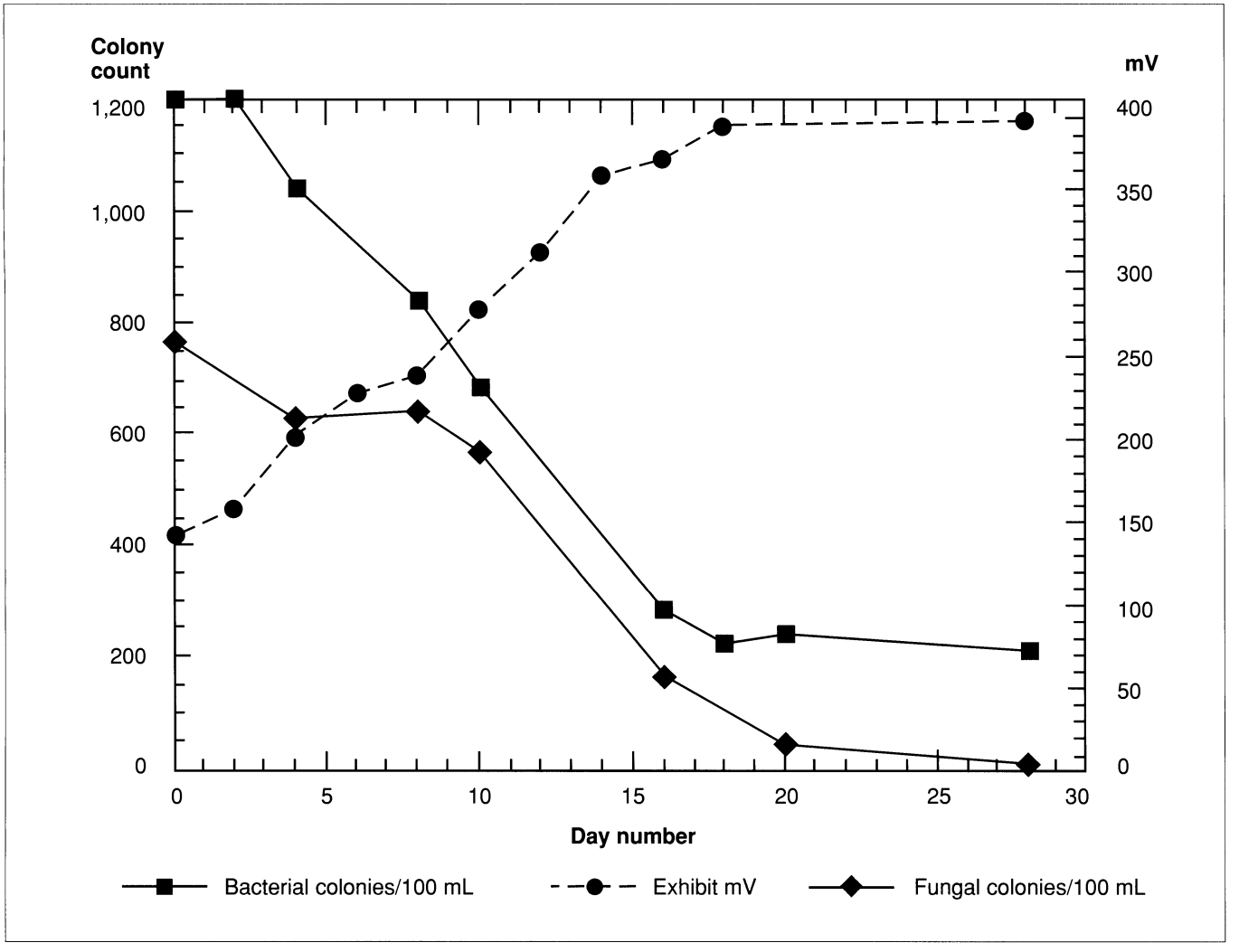


**Figure 8**—The continuous use of ozone in nonchlorinated artificial sea water in NAIB's marine mammal exhibit over a 4-week period led to complete sterilization.

## Sterilization of Marine Mammal Pool Water Using Ozone

Using heterotropic plate counts and the indigo method of analyzing ozone in solution as an indicator to sterilize the sidestream water, it was necessary to establish specific ozone residuals with associated contact times.

The applied doses of ozone needed to achieve the 0.3–0.8 p/m residual solutions ranged from 1.2 to 1.5 p/m. These values are comparable to those of the drinking and wastewater treatment industries (American Water Works Association Research Foundation 1991). The gas was applied in each ozone tower by means of fused glass diffuser stones. This method is the most common in the aquarium industry.

With a 24-h-per-day inflow of clean water, the pollutant load in the system was reduced. The sidesteam volume varied in the seal pool and whale pool from 10 to 25 percent of the total flow. In the case of the whale system, the ozone tower treated between 2,000 and 2,800 gal/min. The seal pool system treated 50–100 gal/min. This flexible approach allowed for greater or lesser treatment of the pool water if the number of animals changed in the pool or the temperature increased or decreased.

After operating both the mammal pools in this way for 18 months, NAIB personnel compared water quality and treatment needs (table 1).

In the seal pool, water changes of 35,000 to 70,000 gal were done on a weekly basis. With the colony of 6 to 10 harbor seals and 2 gray seals in the

**Table 1—Comparison of water quality in the whale and seal pools, 1991 v. 1993**

| | Whale pool | | Seal pool | |
|---|---|---|---|---|
| | Fall 1991 | Spring 1993 | Summer/fall 1991 | Spring 1993 |
| Applied ozone dose (mg/L) | <0.7 | 1.2 | Not applicable | 1.5–2 |
| Residual in solution during contact (mg/L) | <0.2 | >0.5 | Not applicable | 0.7–1.2 |
| Sidestream flow (gal/min) | 2,500 | 2,000–2,800 | Not applicable | 60–100 |
| Contact time (min) | 4 | 4–6 | Not applicable | 3–6 |
| Water color | Green/yellow | None | Green/yellow | None |
| Chlorine—total (p/m)<br>Chlorine—free (p/m) | 0.8–1.2<br>0.1–0.2 | 0.4–0.5<br>0.2 | 0.8–1.6<br>0.1–0.2 | 0.5–0.8<br>0.1–0.3 |
| pH | 7–7.8 | 7.5–7.7 | 7.2–7.8 | 7.5–7.8 |
| Buffers added (lb/wk) | 100–200 | Discontinued | 25 | Discontinued |
| Coliform MPN | <3 | <3 | <3–200 | <3–10 |
| Water changes (gal/yr) | 1 million | Discontinued | 3 million | 100,000–150,000 |
| Water type | Brine (NaCl) | Artificial sea water | Brine | Artificial sea water |
| Salinity (p/t) | 24–34 | 30–32 | 24–34 | 24–34 |
| Turbidity (NTU) | 0.10–0.20 | 0.03–0.08 | Not taken (high) | 0.1–0.2 |
| Flocculant use | Daily | Discontinued | Infrequent | Only during molts |
| Filter backwash and water recovery | Poor/none | Good/routine | — | — |
| Ammonia (p/m) | 0–2 | 0 | *0–3 | 0 |
| Chlorine odor | Light–moderate–heavy | Light to none | Light–moderate–heavy | Light to none |
| Dissolved organic carbon (p/m) | 8–18 | <4 | 8–20 | 4–8 |
| Shock chlorination of entire system | 3 times/yr | Discontinued | — | — |

*Note: These figures are kept this low due to frequent water changes.

pool, it was not possible to maintain good water quality for more than 7 days. Since installing an ozone contact chamber, the pool water has been maintained to standards for up to 6 months. Longer duration is possible. However, there is little reservoir capacity to hold the pool water during medical procedures or cleaning and maintenance and other events that require routine draining of the pool.

## Control of Total Organic Carbon

Organic material from pool makeup water, animal wastes, and unused food can accumulate in a system. Modern life-support systems with water-processing turnover times of 1–3 h can adequately remove the bulk of the suspended wastes. This suspended organic material and the dissolved organic carbon (DOC), which are less effectively removed by sand filtration, are commonly referred to as total organic carbon (TOC). Without the ability to reduce or remove TOC, water quality can deteriorate rapidly and require continuous flowthrough of new water (open systems) or numerous routine water changes (semiclosed systems). Closed systems that change or add water infrequently are candidates for TOC problems. Shock-chlorination of these systems has been widely used to reduce organics and save on water-change costs. This process requires removing the animals to a safe area, and it does not prevent the eventual return of organic buildup.

Alternatively, ozone can be used to reduce TOC by three chemical mechanisms: direct oxidation, coagulation/microflocculation, and ozone-enhanced biofiltration (Janssens 1989, American Water Works Association Research Foundation 1991). Direct oxidation of organic material can take place at a double carbon bond of any size molecule. The net result is two or more organic molecules of lower molecular weight. If the water sustaining this process were tested, the TOC might not show a reduction. Removal of TOC by direct oxidation requires that all organics be oxidized to $CO_2$. Direct oxidation of organics most likely occurs in marine mammal pools using ozone. Recirculating closed systems continuously return oxidized organic material to ozone reactors and aeration towers for retreatment. However, doses commonly used in aquariums are not high enough for direct oxidation to be the primary mechanism for organic control.

Microflocculation and coagulation (Rice et al. 1986, American Water Works Association Research Foundation 1991) are mechanisms that can reduce dissolved and suspended organics, respectively. Microflocculation occurs when DOC is oxidized by ozone, creating carboxyl and carbonyl radicals. These are polar molecules with negative charges. These molecules may combine with one another through hydrogen bonding, or they may combine with divalent cations, such as calcium and magnesium, which are abundant in sea water. This process creates molecules of higher molecular weight that are insoluble. These materials can eventually be filtered from the pool. Backwashing system filters removes the flocculated organics from the recirculating loop.

Coagulation destablilizes suspended organics when small doses of ozone reverse some of the like surface charges keeping particles in suspension (Rice et al. 1986, American Water Works Association Research Foundation 1991, Sander 1991). These negative species will combine with still positively charged suspended material so that flocculation can occur. The processes of coagulation and microflocculation probably account for the bulk of organic removal from mammal pools. Backwashing filters removes some of this organic material from the system. Foam fractionators, however, are designed specifically to combine air floatation, adsorption, coagulation, and microflocculation to destablize, concentrate, and remove organic contaminants from the water column, and are therefore a more efficient means of reducing TOC. Both air and ozone are bubbled into the fractionator, counter-current to the water flow, to create a thick foam of coagulated organic material that is then continuously removed from the top of the unit. While air effectively adsorbs surfactants, it will not facilitate the removal of nonsurfacting solutes. Using air and ozone together to create only a small dose of ozone, coagulation can be utilized to cause nonsurfacting particles to coalesce or combine with

divalent cations, making them less soluble in water. A more detailed explanation of the mechanisms and impacts of foam fractionation can be found in LaBonne and Rozenblum (1995) and Aiken (1995).

Reduction and control of DOC in marine mammal pools is the key to the reduction and control of chlorine as a secondary disinfectant and oxidant. In the NAIB seal pool, chlorine concentrations were susceptible to great fluctation due to environmental changes encountered in the small, heavily loaded outdoor exhibit. The target residual of 0.6 p/m was therefore difficult to maintain. In December 1995, a foam fractionator was retrofitted to the life-support system. Because foam fractionation provides constant removal of organic contaminants from the water column, chlorine demand was cut in half. The peaks and valleys previously seen in maintaining 0.6 p/m were dramatically leveled out. Also important to note was a 40-percent reduction from the number of water changes previously necessary to reduce DOC. In the whale pool, continuous operation at the 1993 ozone doses shown in table 1 has eliminated the need for water changes and allowed the reduction of chlorine to levels approaching 0 p/m.

Less studied and less understood is the process of ozone-enhanced biofiltration. Research in this area has grown in the past 10 years in the drinking-water treatment industry (Janssens 1989; American Water Works Association Research Foundation 1991; Huck 1990, 1991; VanDer Kooij 1989). The process involves the oxidation of DOC to a biologically assimilable molecular fraction. Assimilable organic carbon (AOC) is generated in marine mammal pools that are ozonated. However, the benefit of its being removed cannot be realized unless chlorine levels are kept low enough in pool water so that the filters and other substrates (surfaces) can become biologically active. For example, bacteria colonize biofilms in pipes and plumbing as well as the media in filters. Shock-chlorinating mammal pools will destroy these biofilms. If chlorine levels are kept below 1 p/m, AOC removal can occur at an effective rate. Chlorine can be kept at these low levels only if ozone is the primary oxidant in the life-support system. Closed recirculating marine mammal systems can potentially control AOC more effectively than a drinking-water plant, where only one pass through a treatment system is probable on the way to the consumer. Figures 9 and 10 show the level

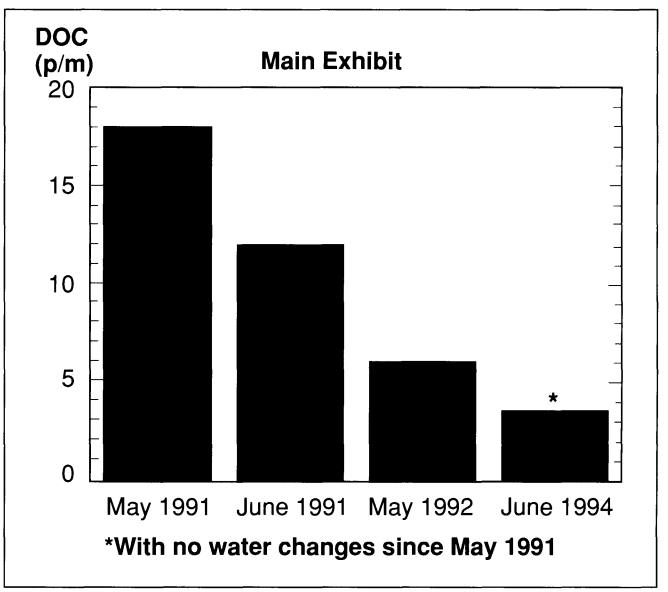


**Figure 9**—In NAIB's main marine mammal exhibit, readings for dissolved organic carbon (DOC) went from 18 to 12 p/m between May and June 1991 and reached 6 p/m a year later.

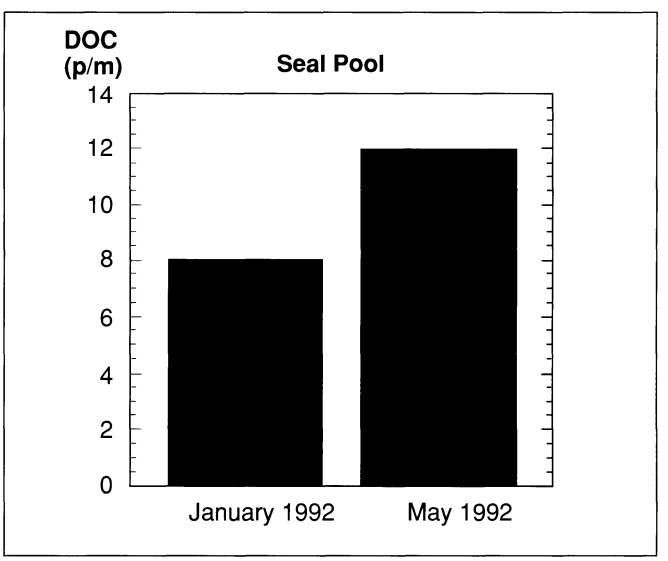


**Figure 10**—In the seal pool, DOC readings went up by 50 percent between January and May 1992.

of organic removal in the NAIB whale and seal pools in 1991 and 1992. These are fully closed sys-tems with minimal water replacement (figs. 11 and 12).

Buildup of DOC in the seal pool has been slow. After almost 6 months, the level had gone up only 4 p/m. This is a heavily loaded system with 9 animals in 70,000 gal. Its bioload is 15 times as great as that of the whale pool. In contrast, Spotte (1992, p. 25) described a seal pool with sand filtration in which organic content increased more than 10 p/m in just 30 days. That pool had no ozone capability.

**Figure 11**—The NAIB's marine mammal isolation pool.

**Figure 12**—It's showtime at the main marine mammal pool at the NAIB.

## Summary

1. Ozone is a more powerful oxidant than chlorine or bromine. If used effectively, it can become the primary disinfectant and oxidizer for water treatment.

2. Ozone is generated onsite by corona discharge generators, and gas mass transfer takes place in ozone reactor chambers and towers.

3. Sidestream ozonation is most commonly used in marine mammal life-support systems.

4. Residual ozone can be measured in sea water using the indigo dye test method. Oxidation reduction probes can also be an aid.

5. Specific ozone residuals must be maintained for specified times to achieve sterilization of water.

6. No ozone residual is maintained in marine mmmal pools. Ozone is stripped from the water after treatment in the reactor.

7. Chlorine demand in pool water can be greatly reduced with the use of ozone.

8. Water treatment of closed life-support systems can be improved by the use of ozone. More stable environmentas can be established, and artificial sea water can become a practical and economic alternative to brine systems.

9. Ozone removes organic material by direct oxidation, coagulation, and microflocculation.

10. If residual chlorine is kept below 1 p/m, AOC produced by ozone can be removed in biologically active filters.

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# Ultraviolet Sterilization

By Randy Hamilton and Chuck Farwell

The Monterey Bay Aquarium (MBA) uses ultraviolet (UV) sterilization on the water system in its sea otter exhibit. A major remodeling of this exhibit has prompted numerous questions. What is UV sterilization? Why did the MBA initially choose UV? Does it work? What are the advantages and disadvantages of using UV? Why would the MBA continue to use such a system?

The original goal of the MBA was to have a sea otter exhibit that closely resembles a central-California subtidal rocky-shore habitat, complete with temperate fishes, invertebrates, and algae. To date, the exhibit has not achieved this goal. Why does the sea otter tank look so barren? How can the MBA hope to achieve its goals? This paper will focus on these questions.

## What Is UV Sterilization?

Ultraviolet sterilization is a process using UV radiation to kill micro-organisms. This radiation includes the wavelengths between x-rays (0.4 nm) and visible light (400 nm). The wavelengths that can kill bacteria and viruses range from 190 nm to 300 nm, with the greatest lethal effect occurring at 260 nm (Koller 1965).

In a typical UV sterilizer, UV energy is produced by low-pressure mercury lamps. Inside the lamp, mercury gas is vaporized by an electric current, and as the excited mercury atoms drop back to their normal energy level, they radiate UV light at 254 nm. The UV rays penetrate the outer membrane of the micro-organism and destroy the deoxyribonucleic acid (DNA).

The dosage level necessary to kill is dependent on time and intensity, and dosage differs with the type and size of organisms, water turbidity, and water depth. Dosage is measured as microwatt-seconds per square centimeter ($\mu Ws/cm^2$). Generally, a dosage of 3,000 to 30,000 $\mu Ws/cm^2$ is required to kill bacteria, viruses, and yeast. Larger organisms usually require higher dosages. Turbidity greatly diminishes UV effectiveness by shielding micro-organisms from lethal UV rays. Filtration prior to UV treatment can increase UV effectiveness significantly. Water depth also greatly lessens UV rays' ability to disinfect by absorbing the radiation before it can penetrate the micro-organisms.

Typical commercial UV sterilizers produce a dosage level of 40,000 $\mu Ws/cm^2$, which is five times the dosage required to kill coliform bacteria. After 1 year of continuous use, the equipment can produce energy levels as much as 30 percent lower, and lamps should be replaced to ensure lethal dosage levels.

UV lamps are housed in sterilizers but never contact the water directly. Quartz or Teflon™ tubes separate the water from the lamps but permit the transmittance of UV radiation. Water is generally 2 to 4 inches away from the UV lamps. Quartz tubes require periodic cleaning as they can become fouled by substances in the water stream. Teflon tubing naturally avoids fouling and thus requires much less maintenance.

Further information on all aspects of UV radiation has been published. Koller (1965) presents an extensive review of the subject. See also the excellent reviews of UV light by Wheaton (1976), Spotte (1979), and Dupree (1981).

## Why Did the MBA Initially Choose UV Sterilization?

USDA sets and regulates water-quality standards for the maintenance of marine mammals in captivity. Coliform bacteria are used as an indicator of water quality. The maximum allowable level of coliforms in marine mammal pools is 1,000 MPN/100 mL. Coliform bacteria are typically controlled by chlorine (sodium hypochlorite), ozone ($O_3$), or UV disinfection systems.

Chlorinating of the entire water system is commonly used and highly effective at controlling coliforms in dolphin and whale pools. Chlorinating MBA's entire water system was not used because chlorine disinfectant levels are toxic to the fishes, invertebrates, and algae the Aquarium planned to include in the exhibit. Furthermore, chlorine is damaging to sea otter fur, which insulates the animal from the cold.

Ozone has also been successfully used for disinfection of marine-mammal water. As with chlorine, residual levels of ozone and ozone-generated byproducts can kill fishes and algae and damage sea otter fur. The use of ozone in multispecies systems was not well understood and was considered problematic in 1980, when the exhibit was originally designed.

MBA chose UV disinfection because UV rays can kill coliform bacteria without harming algae, invertebrates, fishes, or sea otters. Unlike chlorine and ozone, UV rays do not produce any residual oxidants.

## Does It Work?

Although coliform samples taken just after UV sterilization have consistently measured less than 2 MPN/100 mL, coliform levels within the display have occasionally exceeded 2,400 MPN/100 mL. When that happened, the rate of coliform production within the tank was obviously greater than the rate at which the bacteria were then being destroyed. This phenomenon is consistent with theoretical (Spotte and Adams 1981) and empirical (Spotte and Buck 1981) predictions.

A review of coliform levels in the sea otter tank at MBA from the fall of 1984 to the summer of 1988 was reported by Farwell and Hymer (1988). Increased frequency of high coliforms was noted over time. Increased biological load and possible retention of coliforms on algae and rock surfaces were suggested as potential contributing factors. Draining the tank biweekly and power-hosing it with sea water to remove organic debris failed to maintain coliform levels below 1,000 MPN/100 Ml. The life-support system as currently used in the sea otter tank was determined to be ineffective at reducing and maintaining low coliform levels. An additional disinfectant would be necessary to control coliforms.

The current cleaning routine involves weekly removal of the sea otters from display and either (1) circulating chlorine throughout the entire system at a dosage of 50 p/m for 7 hours or (2) draining the tank and spraying 12.5-percent chlorine directly onto rock surfaces for half an hour. After each treatment, the chlorine is neutralized with sodium thiosulfate and the tank is rinsed, flushed, and then refilled. The sea otters are returned to the exhibit after tests confirm that no residual chlorine is present. While this procedure has significantly reduced tank coliform levels, the multispecies exhibit goal has been reduced to a "moonscape."

## What Are the Advantages and Disadvantages of Using UV?

Ultraviolet sterilizers are simple to operate and maintain. There are no moving parts to break, no complex control systems to monitor, and no probes to oversee. UV units are compact and require little space. Typical units are designed so they produce no residual byproducts that might adversely affect sea otter fur or fish. When used in natural sea water or artificial waters containing bromides, UV rays produce no hypohalides such as hypobromous acid. Large contact and de-gas towers are unnecessary with UV units.

The main disadvantage of using UV radiation in marine-mammal systems is that a large amount of side-stream flow must pass through the sterilizer in order to maintain tank coliforms at acceptable levels under APHIS' standards. Filtration is usually required before UV sterilizers can efficiently kill micro-organisms. UV rays do not remove dissolved organics from water and cannot improve water clarity. If UV radiation is applied at dosages that are too low, bacteria can repair damaged DNA, survive, and reproduce.

## Why Would MBA Continue To Use UV?

Factors other than the type of disinfection influence water quality. In the existing sea otter exhibit, poor circulation and the accumulation of suspended solids are believed responsible for the high coliform counts. We think that removing suspended solids is the key requirement for reaching the Aquarium's disinfection objectives in the absence of a disinfectant residual in the pool. Because initial remodeling efforts will concentrate on the removal of micro-organisms rather than their destruction, the reuse of existing UV equipment is prudent.

Micro-organisms are removed along with suspended solids by improved water circulation, placement of water jets to resuspend settled particulates, and redesign of decorative rocks to prevent accumulation of solids. Removal of suspended solids will be further enhanced by changing the media within the existing sand filters.

In the event that initial remodeling fails to remove coliforms from the system, MBA will undertake further modification of the sea otters' tank. The next phase would install additional sand filters to the system. The MBA will continue to modify this system until tank coliform levels are within acceptable standards.

## Summary

Ultraviolet sterilization is used on the sea otter tank at the Monterey Bay Aquarium to kill coliform bacteria but, to date, has been ineffective at maintaining tank levels below 1,000 MPN/100 mL. UV sterilization will be used in the first phase of MBA's remodeling of the otter tank because, in coliform control, the emphasis is on removing coliforms from the system rather than destroying them. UV radiation has advantages over ozone and chlorine disinfection in that UV produces no harmful residual oxidants that might negatively affect algae, fish, invertebrates, or sea otters.

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# Chlorination in Marine Mammal Systems

By Edwin J. Skoch

## Perspectives

Managers of marine mammal exhibits need to consider each system as an ecosystem. No two systems are the same; no two marine mammal systems can have the same or identical data sets. Likewise, managers must look at each park as a group of ecosystems or a biome. Clearly, no two parks can be identical. Each must be viewed as a separate entity, and the only similarities will be the type of animals in each system.

In addition, park systems are not the same as nature. They are totally artificial ecosystems, managed for animal health and welfare. Animal health and welfare are the prime concerns of every park, public or private. Chemical parameters for exhibit facilities can be established only in the most general sense because of the above, and because each system has its own chemical system of reactions. Each system should be viewed as a separate "chemical soup" of water and wastewater. The chemical reactions in each system follow the rules of "dilute" organic chemistry, not "concentrated" chemistry. This means that reactions are almost instantaneous and not always governed by the product rule. Because of these facts, trends in data accumulated over time, are more important than single analytical measurements.

## Chlorine Utilization

For disinfecting marine mammal pools, chlorine is available as a liquid (bleach), a gas, in dry chlorine compounds, and as chlorine dioxide. Chlorine can be added to the systems via injection of gas or liquid or mechanical or hand application of dry chemicals.

To calculate the expected or preferred chlorine level in marine mammal pools, divide the concentration of chlorine expected by the volume of water to get the concentration of chlorine to add. For example, if you want a level of 1 p/m (part per million) chlorine in a 10,000-gal tank, you would need to add 10,000 p/m of chlorine. If your chlorine additive is at a concentration of 1,000 p/m per gallon, you would need to add 10 gal of the solution. Similar calculations can be made for dry chemicals or gas injection.

What does chlorine do? Because of the strong polar nature of the chlorine atom, it has the ability to oxidize organic material, or break down complex organic compounds into smaller units. Thus, chlorine can kill bacteria. During oxidation, the chlorine added to the system is used up. The higher the amount of organic material in the system, the more chlorine is necessary to achieve the desired result.

## Chlorine Reactions

The following chlorine reactions are pH dependent. Thus the end product will be determined by the acidity or alkalinity of the marine mammal water.

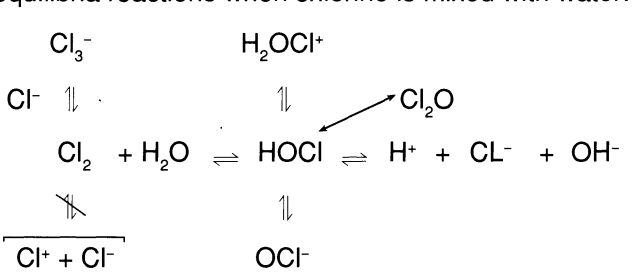
$$Cl_2 + H_2O \rightleftharpoons HCl + HOCl$$

$$Cl_2 + H_2O \rightleftharpoons 2\,HCl + (O^{=})$$

$$NaOCl + H_2O \rightleftharpoons HOCl + Na^{+} + (OH^{-})$$

$$Ca(OCl)_2 + 2H_2O \rightleftharpoons 2HOCl + Ca^{++}(OH^{-})_2$$

The following chart illustrates the chemical equilibria reactions when chlorine is mixed with water:




The direction of the arrows will be determined by the amount of chlorine and the pH of the water.

## Chloramines

Chloramines are combinations of chlorine and ammonia (Cl and $NH_3$). Monochloramine ($NH_2Cl$), dichloramine ($NHCl_2$), and nitrogen trichloride ($NCL_3$) are readily formed from amino acids and free ammonia if there is an excess amount of chlorine in relation to the organic load and free ammonia levels.

Chloramines are formed if the ratio of chlorine to ammonia N increases from 5:1 to 10:1 or higher.

$$HOCl + NH_3 \rightleftharpoons NH_2Cl + H_2O$$

$$HOCl + NH_2Cl \rightleftharpoons NHCl_2 + H_2O$$

$$HOCl + NHCl_2 \rightleftharpoons NCl_3 + H_2O$$

## Important Considerations on Chloride Residuals

High-level chlorine residuals (>0.4 p/m) maintain a better quality water than low-level residuals (<0.05 p/m). Water quality decreases when chlorine residuals are lost. There is a definite relation between water temperature and the persistence of chlorine residuals. Residual chlorine persistence is decreased at temperatures above 60 °F. Residuals are combinations of free chlorine and combined chlorine (chloramines). But free chlorine is much more of a disinfectant than combined chlorine.

## Problems With the "Facts" of Chlorine Utilization

Chlorine residuals, their persistence, and their reactions apply more to pathogenic bacterial control in municipal water supplies, not necessarily to water which is high in organic wastes, such as ammonia nitrogen, uric acid, and fecal material. Further, the reactions deal with fresh water, not saline water. The above reactions apply to relatively simple, clean water chemistry, not the "soup" in marine mammal systems.

Chloramines are intentionally formed in the purification of drinking water because, although they are weaker disinfectants, they are much more persistent and thus, useful.

In marine mammal systems or, for that matter, in swimming pools, the presence of persistent chloramines is detrimental. Persistent high levels of chloramines are known to cause corneal damage in humans and might affect marine mammals. However, one must keep in mind that marine mammals are adapted to high chloride (i.e., salt) in their natural environment and thus may well be less affected by residual chlorine than terrestrial mammals such as people. To put this in perspective, consider that, for people, swimming in the ocean causes more eye irritation than swimming in fresh water. Further, swimming in a warm indoor chlorinated pool is much more irritating than swimming in a cold indoor pool.

## Understanding Chlorine in Marine Mammal Systems

1. Does the presence of NaCl and other "salts" modify the reactions of chlorine? Yes and Yes

2. Does the temperature of the water modify the reactions of chlorine on the organic material? Yes

3. Since all chlorine reactions are pH sensitive, does pH affect the activity and effectiveness of added chlorine? Yes

4. Does the presence of chloride affect the analytical methods used to determine free chlorine and residual chlorine? Yes and No

5. What is the difference, you should ask, between chlorine and chloride? Salinity is a measure of the chloride ion ($Cl^-$). The result of the dissociation of salts such as NaCl, $CaCl_2$, or $MgCl_2$ in water.

$$NaCl + H_2O \rightleftharpoons Na^+ + Cl^- + [H_2O]$$

$$CaCl_2 + H_2O \rightleftharpoons Ca^{++} + 2Cl^- + [H_2O]$$

Chlorine is measured by the presence of hypochloride ($OCl^-$), a much larger and more polar molecule than the chloride molecule. Thus, chlorine is a better disinfectant and oxidizer than chloride.

6. How then does one apply the rules of chlorination, which were developed for drinking water distribution and swimming pools, to marine mammal systems?

A. With great care! The standards and techniques were developed for the ingestion of water, not immersion in it.

The standards and techniques were developed for the disinfection of drinking water and have been applied to swimming pools because of the serious health threat posed by ingestion of pool water that contains human pathogens.

The standards for chlorine and its residual levels in drinking water were also developed to keep the water delivery system flowing.

The standards and techniques were developed for fresh water, not saline waters.

B. Can we set arbitrary standards for free and residual chlorine levels? **No.** However, one might use ranges and accumulated data on a single system as a point of reference for that one system only. Why? As with any ecosystem, animal loading (number, size, and age), environmental temperatures, light, season of the year, and behaviors such as feeding or breeding will cause normal fluctuations in the organic loading of the system and thus normal fluctuations in analytical data.

## In My Opinion

As an environmental chemist and ecotoxicologist, I suggest that several weeks of total chlorines (free and residual) continuously above 3 p/m might indicate a system problem. However, animal health records must be part of the interpretation. If the animals are healthy and showing no problems, then there is probably no water-quality problem.

Under the above scenario, I would suspect that the analytical method is at fault. I would suspect the same problem if the chlorine was being used but the data never showed a residual.

One might also consider the possibility of a Type II error, otherwise known as human error. For instance, the chemical technician can't record the data correctly.

Systems using both chlorination and ozonation should have fewer problems when both systems are functioning properly because the ozonation can greatly assist in the oxidation of organic materials and decrease chloramines. The incorporation of both chemicals would add stability to the control of the chemical process of water-quality management.

Finally, in a system that is under good control, managers may be able to add less chlorine as bacterial counts decrease and the system stabilizes. The addition of chlorine should be determined by bacterial counts and health of the animals, not by some arbitrary, steady parts-per-million of chlorine at all times.

## Heavy Metals in Systems

Data accumulating over the past 8 years indicate some potential of hazard to marine mammals in the wild from exposure to pollutants. This threat can be broadened to cover all vertebrates, including people. Of prime concern are pesticides, herbicides, and heavy metals. The most common route of exposure is ingestion of contaminated water and food materials. Because most of the marine mammals now in captivity are carnivores (piscivores) and are fed fish, the mammals may be accumulating toxins in their tissues. Data accumulated over the past 10 years at John Carroll University indicate that market fish (for human consumption) have varying levels of poisonous metals in their tissues, possibly at levels toxic for humans.

Fish, probably due to their being cold blooded, can concentrate higher levels of toxins than the warmblooded species without harming themselves.

Fish also accumulate toxic chemicals in the mucus covering their bodies. In one case we investigated at John Carroll, there was more of a toxic metal load in the ice that fish were packed in than in the fish tissues themselves. The source of this metal could have been the water the fish were frozen in or the water they had been exposed to before capture. Preliminary data suggest that some coastal areas have a higher risk factor for metal exposure than others. And often the supplier has no way of knowing just where the fish were taken.

Keep in mind that the marine mammal eats the whole fish, including the digestive tract contents, bone, nervous system tissues, etc., not just the meat. Most of the accumulated data in the literature deal with metals in specific tissues, not the whole fish. Total metal load in fish has been recorded at 200 times the level in muscle tissue. It is well documented that specific metals accumulate at different concentrations in different tissues; the highest concentrations are often found in liver, bone, and nervous system tissue.

Further, we do not even know how much heavy-metal contamination a marine mammal can handle. There is little information as to what constitutes normal levels of these metals in blood or tissue in noncaptive marine mammals. Likewise, one cannot compare marine mammals with terrestrial animals because their excretory and detoxification systems are quite different.

Larger parks have been accumulating metal data on their animals and also on food stocks. This practice follows my recommended policy in chapter 7 of the Handbook of Marine Mammal Medicine (Skoch 1990). I would object strongly to the establishment or the enforcement of any arbitrary standards for these animals or for the parks. Heavy-metal contamination of marine mammals is definitely an area that needs more scientific investigation.

## Final Problem of the Metals

1. Metals can come from the makeup water and salt used.

2. Metals might also accumulate in the water because the animals are not able to absorb them from their food.

3. Water treatment does not remove these contaminants.

4. Presence of metals can interfere with the standard analytical procedures for water quality.

5. Metals can interfere with maintenance of residual chlorine levels by reacting with the chlorine to form metal–organochlorine species or metal–chlorine complex similar to chloramines. Metals chemistry is an area of ongoing research.

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# Copper and Other Algicides in Marine Mammal Pools

By Paul Sieswerda

*Abstract:* Copper sulfate has been utilized in the aquarium field as an algicide and antibacterial agent for many years. The standards for bacterial limits as put forth by APHIS have required water treatment with oxidants such as chlorine and ozone. The use of copper in systems that preclude strong oxidation products is discussed.

The use of algicides in marine mammal systems has been often overshadowed by the new technologies of water treatment that combat bacterial, viral, and clarity problems. While new techniques for sterilization and filtration have been developed, algae control remains quite primitive. Fortunately, the processes designed to control the chemistry of water disinfection also effectively reduce algae in marine mammal pools.

The presence of algae is a natural condition in any body of water from the open ocean to very small aquariums. Algae growth ranges from microscopic single cells to the giant kelp *(Macrocystis pyrifera),* which can extend 60 feet as virtual trees towering from the sea floor to the surface. The well-known phenomenon of "red tides" is the population explosion of microscopic algae or "bloom" covering hundreds of square miles in the open ocean. In a marine mammal pool, a bloom would indicate little or no water treatment. A bloom is the exponential growth caused by optimum conditions of temperature, light, and nutrients. At a critical point, the unimpeded growth changes the requisite conditions by depletion of the nutrients or restriction of light by the sheer numbers of organisms in the water column.

In nature, a bloom is self-limiting. It is an opportunistic event in the wild and is probably even less regular in a closed environment. However, the elements that affect the natural growth of algae in the wild are the same within the captive environment. Algae will grow in all aquatic environments that contain nutrients and receive light. The control of this fact is another requirement for responsible maintenance of marine mammals.

The uncontrolled growth of algae is not necessarily deleterious to the health of marine mammals in or by itself but could indicate conditions that are unhealthful, e.g., improper treatment of waste products that provide excessive nutrients or unclean conditions that promote bacterial growth. Traditionally, a clean and algae-free pool has been viewed as a healthy pool. This situation is usually referred to as "swimming pool" conditions.

For the most part, algae control is a simultaneous coproduct of the sterilization procedures regularly used in marine mammal pools. Chlorine and ozone are effective oxidants of organic materials and can even kill algae cells directly. Filtration techniques reduce the nutrient load as well. So that while the marine mammal pool is a literal culture of light, water, and nutrients, the techniques in place to control bacteria and other pathogens also control algae. In some circumstances, however, special measures are needed. For instance, exceptional sunlight will promote algae growth even in the presence of strong oxidants. Once established, strains of algae resistant to oxidation are difficult to remove. The use of ozone may disinfect the water remote from the pool containing the animals. Without an effective residual level of oxidant at the site of potential algae growth, nothing impedes its proliferation. In these cases, and as a general assist to the total process, the use of an algicide can be recommended.

Copper is a widely used and effective algicide. Ponds and ornamental pools have long been treated with "bluestone," elemental copper ore which destroys algae growth. Refined copper sulfate is the base of numerous commercial algicides and treatments. Formulas have been developed to expedite the application and handling of its use, but most algicides have some component of copper sulfate. Copper precipitates rapidly in sea water, and many formulas use agents such as citric acid to chelate the copper to extend its time in solution.

To be effective, copper levels must be kept constant. The experience of using copper against fish diseases has shown that the continued maintenance of a lower level of copper is more effective than periodic levels of greater concentration. Copper is toxic to

fishes at 0.3 p/m but is regularly used at lower levels to protect fish against parasitic infestations. Marine mammals can tolerate copper concentrations in the water up to 1.0 p/m. At that level, signs of irritation to dolphins (squinty eyes) have been noticed. A safe dosage at (0.75 p/m) will control algae and is actually effective against bacteria as well.

At the Aquarium for Wildlife Conservation in New York, we had a situation that illustrates the circumstances where the use of copper can replace chlorination as a treatment process. Prior to 1989, the source of sea water for the Aquarium was a deep-water well that drew from an ancient aquifer containing water with both desirable and problematic characteristics. That water is a constant temperature (55 °F) at a salinity of 32 parts per thousand. It is completely sterile and devoid of oxygen. It is also high in ammonia, iron, and manganese. Marine mammal systems could be maintained with good results using this water in a semiopen system. The use of chlorine precipitated the iron and manganese, turning the water an unsightly brown. Substituting copper for chlorine did not discolor the water. This method served the Aquarium well for a number of years. In 1989, the Aquarium installed a sea-water intake system that is compatible with chlorine, and copper has not been used as a disinfectant since then.

It is clear from the other topics presented in this forum that water treatment is a changing science. The direction at the Aquarium is toward a greater use of ozonation in water treatment, both for fishes and mammals.

Copper treatments as algicides can be found most widely used in ornamental pools, aquaculture, and outdoor fountains in circumstances where chlorine is incompatible. The following is a representative list taken from "A Guide to Approved Chemicals in Fish Production and Fishery Resource Management," by Rosalie A. Schnick (1986). Copper is the active ingredient in numerous formulations. Diquat dibromide is another common algicide used in commercial preparations. A listing is given to familiarize inspectors with the kinds of products available; however, their use in marine mammal systems is not widespread.

Copper (elemental, Cutrine® algicide, Weed-A-Way®)

Copper sulfate

Algimycin®

Aqua-Clear

Aquashade (acid blue and acid yellow)

Diquat dibromide (Aquaquat, Weedtrine-D®)

MCPB (PDQ®)

Simazine (Aquazine®)

In our experience, simazine has been found useful. This is a commercial preparation that inhibits the photosynthetic process of algae. Dosage is 5 p/m. The chemical can be used in conjunction with chlorine treatments as an aid to control stubborn or resistant algae. Application is best done as a preventative. Treating a clear pool with Aquazine retards new growth, but this product is not as effective on established growth. Another product, Aquashade, is an interesting concept for the control of algae in shallow pools. Aquashade is a dye that simply blocks out light penetration through the water. While nontoxic to both fishes and mammals, Aquashade is unlikely to be used in marine mammal pools.

It is clear that the presence of algae is natural in aquatic systems. The waters of the world abound with prolific species of algae as common as the plant life in terrestrial settings. Algae are not harmful or detrimental to marine mammals. Most marine mammals live in close proximity to either the seaweeds and macroalgae of shoreline resting areas or the planktonic microalgae of the open ocean. The trend toward natural settings for marine mammals is clearly the next step in their public display. The exhibit presentation of natural settings—and the move away from chlorination or the "swimming pool" approach to marine mammal enclosures—is taking place rapidly in oceanariums and aquariums.

The Monterey Bay Aquarium and others that maintain sea otters are forced to face the problem of water treatment without chlorine. Sea otters (fig. 1) maintain their body heat by means of air that is trapped in the fine hairs of their fur undercoat. The fur consists of up to 1 million hairs per square inch, the most dense pelage of any animal. Because sea otters do not have the thick blubber layers of most other marine mammals that inhabit cold waters, it is imperative to maintain their fur in perfect condition. Any contamination or fouling of the fur would create matting, and infiltration of cold water could cause critical heat loss to the otter. The harsh oxidation process of chlorine on their fur has been shown to be detrimental to the maintenance of sea otters. Some institutions have used a continual supply of fresh sea water to provide an otter environment that is chlorine free but with acceptable coliform counts. Others are looking to ozonation as the means to achieve the necessary water treatment. Discussion of those treatment methods is presented elsewhere at this workshop. However, further discussion is relevant for consideration of algae in the exhibit pools.

**Figure 1**—Sea otters spend most of their time in the water. The majority of their waking hours is spent grooming their fur to keep it clean and fluffed with air, which they sometimes blow into the hair. This practice increases their buoyancy and helps keep them warm at the same time. Soiled fur can be a death sentence to a sea otter due to exposure resulting in hypothermia. (Photograph supplied by chapter author Lou Garibaldi. This image is copyrighted by the New York Zoological Society/The Wildlife Conservation Society and is used by permission.)

As noted before, algae are natural to marine mammal environments. It is also true that algae are not harmful to marine mammals. Sea otters are so closely adapted to life in the kelp beds that they wrap themselves in the fronds of the giant kelp, using it as an anchor while they sleep. They love to play in it and even nibble the leaves. As alternative methods are developed, chlorine-free sea-water systems will allow algae to grow in marine mammal exhibits. Selection and control of algae will be the desired end rather than total eradication. The Monterey Bay Aquarium is looking to establish natural algae in its sea otter pool.

The Shedd Aquarium uses ozonation. In April 1993, the Aquarium for Wildlife Conservation opened an exhibit—Sea Cliffs—which presents penguins, sea otters, fur seals, and walruses in naturalized settings that include fish, invertebrates, and seaweeds (which are desirable macroalgae). Ozonation will maintain bacterial levels that are acceptable. The growth of seaweeds will be encouraged to provide the natural environment. But algae growth must not be allowed to provide areas of protection for unrestricted bacterial growth or fouling of the mechanics that run the filters. APHIS inspectors will require a new perspective as the traditional look of the pristine swimming pool changes. The objective of both the Service and the exhibit will be to provide clean water in a natural and healthy setting. It is encouraging that the technology of water treatment is beginning to allow this progress.

While chlorine has allowed the maintenance of marine mammals in captivity for many years, the desire to achieve more natural conditions is an improvement wished for by most in the field. Even though the presence of algae is not restricted per se, APHIS guidelines may have to change somewhat.

Algae growth as an indicator of poor management conditions will still be a trigger for inspectors' comments and the facility's corrections. Bacterial levels will be monitored, and animal health will remain the same utmost concern. But in some cases, algae growth will be a measure of success rather than an indicator of substandard conditions. The conditions that promote the growth of macroalgae (seaweeds) are as demanding as those to house marine mammals properly. Low nutrient levels, good clarity and circulation, Ph, and no chemical residual of oxidants are all desirable. The growth of seaweeds will indicate water quality that is required for marine mammals and also can contribute to the removal of some organics, a natural filtration process by itself.

The presence of algae in the marine mammal environment, therefore, is likely to increase as more exhibits are naturalized. It is encouraging that APHIS is ready to work with aquariums in determining acceptable standards which indicate healthful conditions (bacterial counts seem the best standard available). It is also promising that just as a clean and healthful environment in the wild supports a broad range of marine life, a clean and healthful aquarium display will support a similarly broad range from algae to elephant seals.

## Summary

The presentation of marine mammals in aquariums is changing from sterile swimming pools to naturalized exhibits that house mixed species or animals that are sensitive to strong oxidative products. The need to maintain healthful conditions and the standards of APHIS will require the use of products and applications that are alternatives to the swimming pool technology. Copper sulfate is one chemical that can be considered. The control of algae growth is possible with copper-based additives. Algae, particularly macroalgae, may in fact be encouraged as a natural display component of new exhibits.

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# Coliform Testing in Marine Mammal Holding Pools

By Greg Early

At first glance, the question of fecal contamination in marine-mammal water systems seems straightforward enough. Marine mammals spend most, if not all, of their lives in the water, eating, sleeping, and eliminating in it. It seems, then, a bit absurd to use a microbiological test to quantify individual bacterial colonies from an environment that is receiving them by the pound.

What, then, is the value of total coliform (TC) tests, and why perform them? In his Technical Bulletin "Sterilization of Marine Mammal Pool Waters," Dr. Steve Spotte gives two reasons for monitoring and attempting to control the numbers of bacteria in marine mammal enclosures. He first states that "Superior captive environments are defined, partly, by low numbers of suspended microorganisms." In other words, good environments are, by definition, low in bacteria. And so they are! The Animal Welfare Act, as revised in 1979, sets limits for coliform bacteria in marine mammal holding systems. The regulations state that the MPN for coliform bacteria shall not exceed 1,000 colonies per 100 ML of sample. If this limit is exceeded for one sample, two subsequent samples may be tested over a 48-hour period and the results of the three tests be averaged. What MPN is and what biological significance the test may have is not so clearly explained, however.

Spotte's second reason for keeping track of bacteria in marine mammal systems is his contention that "[t]he advantages of sterile water outweigh most attendant disadvantages associated with sterilization." In other words, sterilizing pool water won't hurt, usually . . . so why not? There is at least one problem, however, that may be summed up as Early's corollary: "Water can be made as clean as you are willing to pay for." The biological appropriateness of a sterile environment, and the technical risks and problems notwithstanding, one of the greatest balancing forces for controlling bacteria in a water system is expense. It seems that sterile water systems may not be biologically, technically, or financially feasible; and, therefore, we are left to keep track of the micro-organisms that remain.

Yet the questions "Why use a microbiological test?" and "What is the significance of the data derived from the tests?" remain. It is probably best to look at the history of the coliform test and its uses for some clue to its function in this context.

## History

There is a long history of the association of contaminated potable (drinking) water and disease. One of the most famous incidents was the Broad Street Pump Epidemic of 1854, when cholera swept several sections of London. A physician named John Snow traced a connection between the occurrence of the disease and the use of a single water pump in the city. Dr. Snow removed the handle from the pump, and the disease outbreak ended. His observations were made without microbiological evidence, which was popularized a decade or so later as the germ theory of disease transmission, through the work of Pasteur and Koch. Soon the connection between epidemic disease (such as cholera, typhoid fever, and dysentery) and enteric pathogens in contaminated drinking water was clearly established.

In 1885, Escherich identified and named *"Bacillus coli,"* a bacteria characteristic in feces of warmblooded animals that was later renamed (probably to the chagrin of his family) *Escherichia coli*. This bacteria is often thought of as the definitive indicator of the presence of fecal contamination in water. In actual practice, however, several groups of organisms may be used as indicators, each yielding somewhat different information about the level of contamination and the potential risk to drinking waters.

Ideally, to be an effective indicator, an organism or group of organisms should meet several well-defined criteria (Millipore Corp. 1973):

(1) An indicator organism should be present in much greater numbers than the suspected pathogens;

(2) An indicator should not proliferate to a greater extent than the pathogens;

(3) An indicator should be more resistant to disinfectants and aquatic environments than pathogens;

(4) Indicators should yield characteristic and unambiguous reactions, enabling clear identification.

*E. coli* itself does not meet these criteria and is generally not used alone as an indicator organism. Unfortunately, it is doubtful that TC, the most widely used indicator for potable water, meets all of these criteria either. Many examples from the records of the Public Health Service support this notion. In one well-documented case, from 1963 through 1965 more than 18,000 people in Riverside, CA, were affected by an outbreak of *Salmonella* poisoning caused by drinking water that contained less than 2.2 colonies of TC's per 100 mL of sample. The apparant weakness of this indicator prompted the Public Health Activities Committee of the American Society of Civil Engineers to state, "There is little, if any, proof that disease hazards are directly associated with large numbers of coliform." The use of TC as an indicator for the presence of enteric pathogens in the complex environment of a marine mammal life-support system seems even more dubious.

## Indicator Organisms

Several groups of organisms are commonly used as indicators of water quality. TC counts are generally the standard indicators of potable water acceptability. Fecal coliform (FC) and fecal *Streptococcus* (FS) counts are used as indicators of nonpotable (bathing or other recreational) water quality. A ratio of FC counts to FS counts may be used to indicate the possible source of fecal contamination.

Total coliform bacteria are members of the Enterobacteriaceae, including the genera *Escherichia, Klebsiella, Enterobacter,* and *Citobacter,* which are aerobic gram-negative, nonspore-producing, rod-shaped bacteria. They ferment lactose to carbonic acid, producing gas (hydrogen and carbon dioxide), and grow well at 35 °C.

There are contradictions in the literature regarding the survival of TC's in an aquatic environment. Although sea water and low temperatures are generally reported to inhibit or injure bacteria, "nutrient-rich" water (usually found in marine mammal enclosures) is also reported to promote survival and growth of both pathogens and indicators. Generally, increased nutrients are reported to depress *E. coli* but increase *Klebsiella, Pseudomonas,* and *Aeromonas*. This picture may be further clouded by differences in the ability of each species to grow, in culture, following injury.

Fecal coliform bacteria are *Escherichia* and *Klebsiella* species that may be separated from other TC's by the ability of FC's to ferment lactose at a higher temperature (45 °C). They are, therefore, more definitive indicators of recent fecal pollution. FC's generally are more fragile in an aquatic environment and require more stringent culture methods. As such, FC's would seem to be a better indicator organism. In practice, however, because FC's are difficult to grow, methods for their growth and recovery are subject to a greater technical error.

Fecal *Streptococcus* is a group of bacteria made up of Lancefield's group D and O *Streptococcus.* FS's are incubated for 48 hours at 35 °C. Because FS bacteria generally survive better than TC's in water sources, streptococci are not as good an indicator of recent contamination. They may, however, be used in comparison to FC counts to pinpoint a source of contamination.

Table 1 shows generally acceptable levels for these indicator organisms as set by the Public Health Service for potable (drinking) water, primary contact water (swimming pools, etc.), and secondary contact waters (boating, etc.). (APHIS' acceptable limits in marine mammal enclosures: <1,000 colonies per 100 mL MPN.)

**Table 1**—Maximum allowable limits of fecal and total coliform in water

| | Total coliform | | Fecal coliform | |
|---|---|---|---|---|
| | Optimal | Permissible | Optimal | Permissible |
| | *(Colonies per 100 mL of water sampled)* | | | |
| **Potable** | 0 | 1 | 0 | 0 |
| **Nonpotable** | | | | |
| Primary contact | <1,000 | <2,400 | <200 | <1,000 |
| Secondary contact | <5,000 | <10,000 | <1,000 | <5,000 |

Source: Millipore (1973).

The ratio of FC to FS may be used to help pinpoint a source of contamination. Generally, a ratio equal to or greater than 4 indicates contamination with human waste. A ratio equal to or less than 0.7 indicates contamination with poultry or livestock waste. Ratios between 2 and 4 indicate mixed human and animal waste, and a ratio between 0.7 and 1 indicates mixed contamination by poultry and livestock. Although they may be of little use in completely closed systems, these ratios can be of great use in determining the quality of source or discharge waters.

Table 2 lists the FC/FS ratio for various animals.

Another approach to the problem of determining the potential for hazard in a water source is to test for selected pathogens, themselves. In general, there are several problems with this approach. The most notable is the likelihood that testing for one pathogen does not exclude the possible presence of another pathogen unless the two organisms have very similar growth requirements. Testing for specific pathogens also has the technical problems associated with safety and handling of cultures. Not all water-quality labs will be suited for handling potential pathogens. Testing for some selected potential pathogens, however, may be useful in identifying specific problems in a water system. Some pathogens may be more resilient to cold sea water and disinfectants than TC's (particularly fungi and yeasts), and some pathogenic bacteria (e.g., *Vibrio* sp.) may be resident in sea water systems. As such, these organisms may better indicate the effectiveness of disinfection.

**Table 2**—FC:FS ratios for selected animals

| | |
|---|---|
| Man | 4.4:1 |
| Sheep | 0.4:1 |
| Cow | 0.2:1 |
| Turkey | 0.1:1 |

Source: Millipore Corp. (1977).

*Pseudomonas* sp. bacteria are easily grown on specific restriction media and may be recovered from waters that are cold enough to seriously injure TC and FC (less than 70 °F). Gram-negative bacteria of the *Vibrio* species are ubiquitous in fresh and salt waters. Among this group are potent pathogens responsible for cholera and other life-threatening infections, as well as other more benign bacteria species. *Vibrio* sp. may also be grown on a specific indicator media and may be further classified by testing for lactose fermenting ability. Because of public health concerns, the ecology of *Vibrio* bacteria in natural waters has been studied extensively. The distribution of this bacteria has been found to vary with temperature and salinity. In waters warmer than 70 °F, an abundance of *Vibrio* bacteria can be found in the water column, but at temperatures below 70 °F, they are usually found in bottom mud. During warm months, vibrios are known to cause various diseases and infections. At lower temperatures, vibrios appear to be associated with the decomposition of chitin. Most marine vibrios require a salt-rich medium to grow; however, their nutritional requirements are simple, and they are among the fastest

reproducing bacteria known. Vibrios appear to be quite sensitive to disinfectants in aquatic life-support systems. Because of this, and the fact that they grow quickly and relentlessly in marine environments, their presence in a holding system may indicate a problem with sterilization procedures.

## Methods

### *Sampling*

There are many methods for growing (culturing) and counting coliform bacteria. Improper sample collection and handling, however, can seriously affect the results of any culture method. Using collection bottles that have not been properly cleaned can result in high counts (if bottles are dirty) or low counts (if bottles are not properly rinsed of disinfectants or detergents). Contaminating material may be suspended on the surface of water, so samples should be taken from below the surface. Because most marine mammal holding pools have some level of disinfecting chemical, it is essential to remove disinfectants (usually with sodium thiosulfate) as soon as possible after sampling (and certainly before culturing) to avoid erroneously low counts.

### *Culturing and Counting*

There are four general types of methodology approved by EPA for demonstrating coliform bacteria in water samples: MPN, most probable number method; MF, membrane filtration method; P/A, presence/absence method; and HPC, heterotrophic plate counts. Only the two methods that provide counts adequate for meeting reporting requirements (MPN and MF) will be described in further detail below. (A fifth method, not EPA approved as yet, allows bacteria to be counted directly, without culturing. Bacteria counts may be done by filtering samples through special [nucleopore] membrane filters. The bacteria are stained (usually with fluorescent stains) and counted directly with a microscope. Specific bacteria may be treated with special stains, or total bacteria may be counted. This method has the advantages that it is quick [bacterial colonies do not have to grow] and does not involve extensive handling of bacteria and media [bacterial colonies do not have to grow]. The disadvantages of this method are that it requires expensive equipment, and if used to count TC's, it would, at best, be an indirect measurement of an indirect indicator.)

There are advantages to both of the approved methods used to count TC's, though the counts do not mean the same, nor are the results directly comparable. The MPN method is a statistical estimate of the number of bacterial colonies producing growth in media. The MF method provides counts of individual colonies grown from isolated bacteria. The MF method generally provides results in 24 hours (48 for FS counts) as opposed to the 24- to 96-hour incubation time required for most MPN tests. The MF method is better suited to water with lower bacterial loads, and larger (perhaps more representative) samples may be used. This method may result in a reporting error for samples with low concentrations of bacteria. If no growth is detected on an MF culture, it may reported as zero colonies per 100 mL. It should be reported, however, as "below detectable limits" (or "less than"). For example, if 10 mL of sample were tested and no growth occurred, this would represent "less than 10 colonies/100 mL," not zero growth. The best quality control method, in this case, would be to repeat the test with a larger sample until some reportable growth occurred. Reports should clearly indicate if counts were based on colony growth or "no growth."

Both methods provide reproducible results, but the MF technique is generally less cumbersome and may be more readily modified to test for a variety of bacteria. EPA recommended the MF technique in its publication Microbial Methods for Monitoring the Environment (Bordner and Winter 1978).

The MPN method is performed by making sequential dilutions of water-inoculated tubes of material, a process that encourages the growth of the desired bacteria (presumptive media). The tubes are incubated. Bacterial growth is demonstrated by cloudiness (turbidity). Bacteria may be further identified by gas production (double-tube method) or by color indicators. Laurel tryptase broth will differentiate

FC from TC as will fluorescent indicators. The number of tubes producing results is generally compared to a chart indicating the appropriate MPN count estimate.

Because it makes a statistical estimate (and most estimates are based on published charts used to interpret results), the MPN method is also subject to some reporting error. For example, if one consults a chart used to interpret results at the 95-percent confidence limit on a 15-tube MPN test, a result will vary by 700 colonies/100 mL based on the interpretation of growth in a single tube.

The MF method is performed by filtering measured amounts of sample through fine filters (0.45 micron for TC and 0.47 micron for FC) that trap bacteria on their surface. The filters are then placed on media-soaked pads or gelatin (agar). Colonies that grow from the individual bacteria are counted after incubation. The MF method may use a variety of media. TC's are generally grown on media containing lactose, digested protein, vitamins, chemicals, and Schiff reagent. Acid aldehyde complex, produced by lactose fermentation, reacts with the Schiff reagent to produce a characteristic "green sheen" on TC colonies when viewed under fluorescent light.

FC's are grown on a medium similar to that for TC's with the substitution of an aniline dye as an indicator. This forms a characteristic blue color when reacted with acid, turning FC colonies blue. FC cultures are incubated at 44.5 °F to inhibit the growth of nonfecal coliforms. The bacteria are also cultured on a membrane with a larger pore diameter to provide more moisture to the colonies at the higher incubation temperature.

FS colonies are filtered in the same way as TC colonies; however, FS's are grown on a medium that contains sodium azide to inhibit growth of nonfecal *Streptococcus* bacteria and an indicator that, when reduced, forms a formazine dye that turns FS colonies a characteristic red. The cultures are incubated for 48 hours.

Regardless of the bacteria to be cultured, technical errors (e.g., failure to dechlorinate samples; use of the wrong filter pore size, wrong incubation time, or temperature; and improper filtration techniques) will compromise culture results.

In using the MF method, colonies must be counted. Generally, membrane filters are marked with a grid with approximately 200 squares to facilitate counting. If a filter has more than 1 colony/square, it should be reported as "overgrown" or "TNTC" (too numerous to count). At this density, bacterial growth may be inhibited, indicator dyes may not react properly, and counts are too unreliable to report. For technical accuracy, the counts should be repeated and accurate counts made. It is important to note, however, that if a sample of greater than 29 mL was filtered, an overgrown filter could represent less than the 1,000 colonies/100 mL maximum allowable limit under APHIS regulations. All counts should be reported in terms of colonies per 100 mL of sample, and the presence of "background" bacteria should be noted. Background bacteria are colonies that have grown on the filter but do not react to indicators in the medium. High background counts may indicate high levels of bacterial activity in sampled water, or may indicate technical problems with the test, particularly if the background counts are high enough to inhibit the indicator bacteria.

## Conclusion

In the 15th edition of Standard Methods for the Examination of Water and Waste Water (American Public Health Association 1980), there is a summary of bacterial water testing strategy. The text states, "Examination of routine bacterial samples cannot be regarded as providing complete or final information concerning water quality." Bacterial results must be considered in the light of information available concerning the sanitary conditions surrounding the source of any particular sample. Precise evaluation of the quality of a water sample can be made only when the results of laboratory examinations are interpreted in the light of sanitary survey data. It is recommended that multiple samples over a known, protracted period of time be analyzed, and interpreted along with information about "sanitary conditions."

The source of contamination in a marine mammal enclosure is generally quite obvious (they stare at you from the pool). TC counts per se are not good indicators of contamination, nor are they good indicators of "disease potential" per se. TC (and other bacteria) counts are, however, good, reliable, and valuable indicators of water treatment inadequacy. Interpreted in light of other water chemistry parameters or observations about life support systems, these tests can demonstrate water treatment deficiencies. As such, the methods, techniques, as well as the results should be carefully checked and monitored.

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# Water Filtration and Sterilization in Maintenance of Marine Mammal Systems

By Louis Garibaldi

The process of achieving optimal water quality in a marine mammal system has gone through a continual evolution in definition and techniques. As our understanding of the water quality needs of marine mammals has grown, the definition of "optimal" has taken on an expanded meaning. However, the diversity of operating strategies, equipment, and marine mammal species makes it difficult to define any one system as optimal.

A water system that achieves (1) a very low concentration of animal waste, (2) a low concentration or absence of toxic chemicals, and (3) low turbidity has traditionally been thought optimal. Of the three, turbidity is the least well defined relative to an optimal standard and can be the least critical factor depending on the cause.

Filtration and sterilization are two processes by which good water quality is achieved. In general, filtration removes from circulation and concentrates undesirable materials in the filter media. Sterilization, the nonselective destruction of all micro-organisms, is applied by various strategies to control potential and known pathogenic organisms, especially in a recirculated water system.

Three basic types of water systems are used in keeping marine mammals: open, semiopen, and closed systems. There are many variations of these three basic types.

Open systems include open-ocean pens like those used by facilities in Florida and by the Navy in San Diego, CA, and Hawaii. "Open" also describes marine mammal pools that use water for only one pass through, filtered or nonfiltered, and then throw it away at the same rate of fill with no recirculation of any portion of the water volume. If properly designed with adequate turnover and flowthrough, an open system can provide many of the water-quality advantages of living in the open ocean. Water quality is maintained primarily by means of diluting or dispersing the animal waste products and any accumulation of micro-organisms. Many early facilities near the ocean used open systems, and some still do for holding cetaceans and pinnipeds. The main disadvantage of an open system is dependency on local water-quality conditions, which can vary due to the influence of weather and uncontrolled human activity resulting in water pollution.

Today most facilities built along the coast also have converted to the so-called semiopen system to take advantage of both the availability of reasonably good water and the added control of recirculation capabilities. Semiopen systems recirculate the majority of the pool water, which is filtered at a 60- to 120-minute turnover rate. The new water being continually added is also filtered prior to addition to remove turbidity and potential pathogens.

"Semiopen" and "semiclosed" are relative terms, sometimes used interchangeably, with no specific definition of the percentage of water continuously changed. Both terms refer to a system that has controlled additions or water changes while the majority of the water in the pool is being recirculated. This kind of a system provides the controls of a closed system (described below) and some benefits of an open system, such as the dilution of the animals' organic waste products, which accumulate in a closed system.

Closed systems are most often used by facilities located inland, away from the ocean or on polluted bodies of water. Such systems typically use manufactured sea water or brine solutions produced onsite. Closed systems require the most sophisticated monitoring and management to control chemical balances, biological contamination, and buildup of organic wastes.

As the word implies, closed systems recirculate 100 percent of the system water and, in the case of salt-water systems, must reclaim their backwash water and recycle it into the system. Most sanitation and sewage authorities will not allow the discharge of salty backwash water into sanitary sewers because the salt negatively affects the bacteria in the sewage treatment process.

## Filter Systems

Filter systems are not all created equal but usually reflect the best available technology at the time of construction to achieve the desired water quality within the parameters of the facility. By various conventions, filter systems are described as being primarily mechanical, biological, or chemical in their activity. Many filters incorporate more than one of these functions.

### *Mechanical Filters*

Mechanical filters are used to remove the lumps and particulates that contribute to turbidity. The mechanical filter technology used in marine mammal pools has been borrowed from drinking-water treatment and swimming-pool filter technology. Such filters are designed to remove suspended solids efficiently in order to achieve "clean" water that is crystal clear.

Two kinds of mechanical filters are in use: permanent media filters (e.g., sand filters) that reuse the filter material, and filters in which the medium (usually diatomaceous earth) is disposed of with the material it has filtered out of the system.

**Sand Filters.**—The three different kinds of sand filters are described by the rate at which the system water passes through the sand. **Slow-rate** or gravity-fed filters process water at less than 3 gallons per square foot of filter surface per minute. These are usually older filter units, built as much as 50 years ago. Slow-rate filters usually are open-topped concrete basins in which the water goes in the top and drains out the bottom through collector pipes under the sand. Although these filters are effective and low energy consumers, they take up a significant amount of space. **Rapid-rate** filters are large pressure vessels, usually of steel, in which there are layered grades of sand with the finest sand on top. The system water is passed through the sand under pressure at 3 to 7 gallons per square foot of filter surface per minute. These filters also require much space and considerable quantities of backwash water. **High-rate** filters are also pressure vessels, usually made of steel but now also available in fiberglass as well. They pass system water at rates from 10 gallons to 22 gallons per square foot of filter area per minute. High-rate sand filters are manufactured both as vertical pressure vessels and the newer horizontal cylinder designs introduced in the last 10 years. Advantages of the high-rate sand filter include increased dirt load capacity, more efficient particle removal down to <20 microns, reduced floor space, and reduced consumption of backwash water. Disadvantages of the high-rate filters include higher energy costs associated with the higher water pressure required to push the water faster through the sand and the liabilities of improper backwashing practices.

Improper backwashing procedures are the most common cause of filter bed failure. Inadequate upflow rates and short backwash cycles during backwash procedures can lead to the formation of "mudballs" in the filter bed and to channeling and other solidification of the sand bed so it becomes less effective. Long running times between backwash cycles with little or no pressure buildup across the filter bed are a symptom of channeling, a condition that allows the water to pass around the sand bed without being filtered.

On occasion, liquid filter-enhancement additives (e.g., alum and polyelectrolytes) are also used in conjunction with sand filters. These additives increase filter efficiency by producing a gellike substance that coagulates microscopic and colloidal particulates so they can be trapped in the filter.

**Diatomaceous Earth Filters.**—Diatomaceous earth filters are very efficient particle filters that use the microscopic sievelike skeletons of diatoms to filter out the smallest particles as water passes through them. Used extensively for filtering drinking water and "polishing" industrial cooling water, diatomaceous filters are not as widely used in marine-mammal systems as they used to be. High maintenance costs and the need to dispose of great quantities of used diatomaceous earth after each backwashing are two disadvantages of such filters. Constant replacement of the filter medium is also expensive.

### *Biological Filters*

Biological filters come in many configurations, but their essential component is a substrate (e.g., sand, gravel, or plastic medium) on which *Nitrosomonas* and *Nitrobacter* bacteria can grow. These two groups of bacteria are essential in detoxifying nitrogenous wastes such as ammonia ($NH_3$) and nitrite ($NO_2^-$) in system water. Any filter, including a high-rate sand filter, becomes biologically active if an oxidizing agent such as chlorine is not used in the system.

The advantages of using biological filters in marine-mammal systems are many. These filters reduce potential irritants to mucous membranes and establish a more natural balance of microbial populations that help to control such pathogens as fungi. Sand filters can function both as mechanical and biological filters in the absence of high circulating levels of disinfectants or oxidizing agents.

### *Chemical Filters*

Chemical filters include activated carbon filters and foam fractionation or protein skimming. Both types of filtration have been used to reduce or remove organic carbon from system waters, especially in closed systems. However, because of their operating costs, chemical filters have not proven useful in marine-mammal systems. Both types are regularly used on fish and invertebrate systems, however.

## Sterilizing Agents

A number of agents are used to kill micro-organisms, such as bacteria, viruses, yeasts (or fungi), and algae.

### *Light*

Ultraviolet (UV) light is a form of electromagnetic radiation that is germicidal at its shorter wavelengths. UV's primary mode of action is to deactivate DNA in living cells upon contact. Effective dosages require exposures at specific energy levels for specific amounts of time. Although quite effective in some applications in aquaculture and in disinfecting drinking water, UV light has not been used effectively on large marine-mammal systems. Also, it is most effective when used on very clean water.

### *Chlorine*

Chlorine is the most widely used oxidizing agent for sterilizing water both for swimming pools and marine-mammal pools. Chlorine comes in four forms: as sodium hypochlorite, a liquid widely used in a low concentration as a laundry bleach but in a more concentrated form (up to 15 percent active ingredients) for pools; as calcium hypochlorite, known most commonly as the dry powder HTH (70 percent active ingredients); as the gaseous $Cl_2$ form that comes in high-pressure cylinders and is sometimes used for swimming pools; and the $Cl_2$ gas generated onsite electrolytically from salt water.

Chlorine chemistry can be quite complicated, but it is important to know that chlorine is most effective in the pH range 7.2 to 7.8, and in warmer water. Chlorine is not fast acting. Chloramines—byproducts produced in the presence of ammonia—are also oxidizers but even more irritating than $Cl_2$ and should be avoided at levels above 0.5 mg/L in marine-mammal pools.

Breakpoint chlorination is the term for chlorine used at concentrations above 20 mg/L to remove or oxidize high levels of nitrogenous waste compounds in system water. During breakpoint chlorination, the animals are removed from the water or isolated in an adjacent pool.

### *Ozone*

Ozone, the unstable $O_3$ oxygen molecule, is being used more commonly, especially in closed systems in conjunction with a low residual level of chlorine. Ozone is a very powerful oxidizer, 1.5 times stronger than chlorine and much faster reacting. Ozone is generated onsite from very dry air at concentrations of 1 to 2 percent of the oxygen that passes

through the ozone generator. Ozone is mixed with system water either in large contact chambers or dynamically inline with venturi injectors to get the $O_3$ molecules into solution, where they react very rapidly. Because of ozone's short half-life and rapid reactions, there is seldom any residual oxidant left circulating in the system. The lack of residual action is compensated for in most systems by using chlorine to provide a low-level circulating oxidizer, resulting in a one–two punch. Sea World employs this approach. In some systems with low organic loads, ozone alone has been shown to be adequate and also allow biological activity to occur in the filter system as well.

### *Bromine*

Bromine is beginning to be used in some marine-mammal pools, especially in conjunction with ozone. Bromine is a more stable oxidizer than chlorine and does not form irritating byproducts at the levels used.

### *Silver*

Silver ions are known for their germicidal effects, and a system using silver and copper electrodes is employed at some facilities. The free silver ions given off at the silver electrode reduce bacteria levels while the copper ions from the copper electrode reduce the viability of algae in the system water.

### *Copper*

Copper has been used for years as an algicide in marine-mammal systems, long before the silver/copper electrode system was developed. Copper ion concentrations as high as 0.6 mg/L have been used, frequently in conjunction with chlorine.

## The Staff

When all is said and done, it is not the kind, style, brand, age, cost, or size of the filtration or water treatment system that is most important, but rather the operator who is able to get the job done by doing what is necessary to maintain that water quality. The best designed system is only as good as the staff responsible for its operation. The filtration plant can be a scientific marvel, but the marine mammals may be suffering from poor water quality as a result of mismanagement of the system.

The bottom line in evaluating any water filtration system is the condition of the animals. The "system" is a combination of the mechanical equipment used to process and pump the water and the operators, who have the responsibility to get the system to accomplish the task it was designed to perform.

# Water Quality for Sirenians

**By Jeffrey J. Keaffaber and Christopher J. Coston**

The term "sirenians" refers to the collective group of manatees and dugong. The Living Seas at Walt Disney World's EPCOT Center is home to two Florida manatees (fig. 1), and the display offers an education perspective of the importance of enhanced protection for sirenians and their habitats.

The Florida manatee has been classified as a subspecies of the West Indian manatee *(Trichechus manatus)*. The latest census of the Florida manatee population was 1,850 in February 1992 (Conrad Litz, pers. commun.). This figure is significantly higher than the 1,400 reported in 1990 (Reynolds and Odell 1991); however, improved counting methods are probably responsible for this "increase." Excessive recreational activities like boating in manatee-populated inland waterways, excessive winter cold, and sluggish reproductive rates are among the factors affecting the survival of these delicate marine mammals.

The Living Seas' manatee exhibit capacity is approximately 124,000 gal, split between a front (show) pool and a back holding area. The manatees are free to swim throughout the entire volume of the system. The animal density in the environments includes a 1,500-pound female and a 600-pound male (measured at 1 year old) born in the exhibit on September 13, 1991. A small collection of miscellaneous fish is also present. The entire system is recirculated every 90 minutes at an average flow rate of 1,400 gal/min.

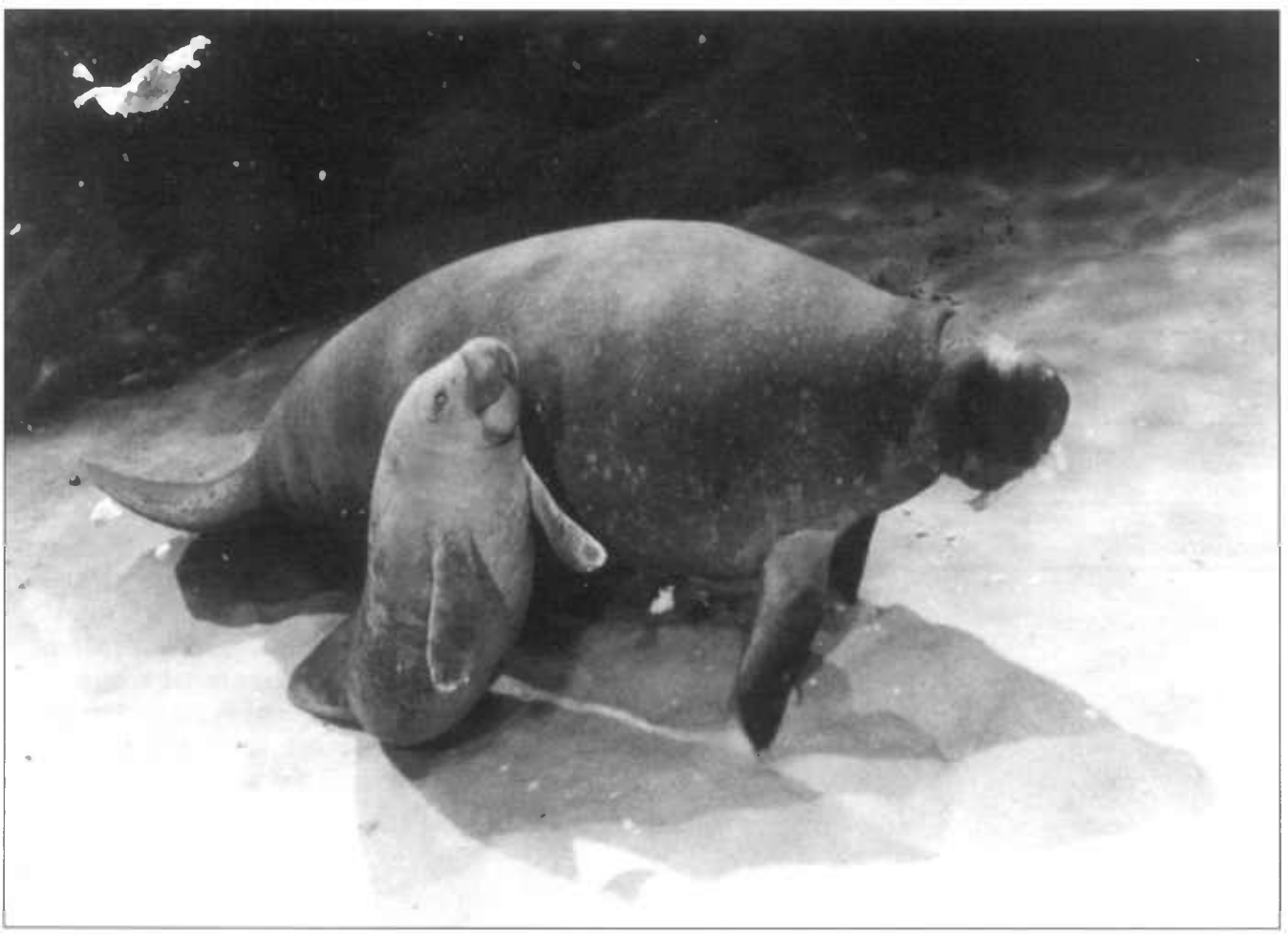

**Figure 1**—A manatee mom munches some lettuce while her baby looks up curiously at another leafy morsel in their pool at the Living Seas exhibit. (Photo ©1991, The Walt Disney Company, and reproduced by permission.)

The daily feed load on the system is about 60–90 lb of lettuce, oat sprouts, and other vegetative matter. The marine-mammal staff regularly removes large food waste while skimmers in each pool remove smaller particulate waste from the system. The wastewater is combined with that of the 5.7-million-gal coral reef aquarium. The combined volume is filtered, ozone-disinfected, and returned to the pool as a sidestream of the all-inclusive aquarium system.

## Results and Discussion

### *Manatee Pool Water Chemistry*

The chemical parameters affecting water quality in the manatee pool include salinity, temperature, pH, turbidity, and nitrogen speciation. A summary of these parameters is provided in table 1 (included in the table are author comments).

**Table 1**—Chemical parameters of Living Seas' manatee system

| Parameter | Value | Comment |
|---|---|---|
| Temperature | 25–29 °C | ~75 °F |
| Salinity | 28 g/kg | 0–35 g/kg |
| pH | 8.1 | 7.8–8.2 |
| Alkalinity as $CaCO_3$ | 250 mg/L | Variable |
| Turbidity | 0.09 ntu | < 0.5 ntu |
| Ammonia (total available nitrogen) | 0.01 mg/L | < 0.2 mg/L |
| Nitrite as $NO_2^-$ | 0.007 mg/L | < 0.1 mg/L |
| Nitrate as $NO_3^-$ | 190 mg/L | Unknown |

The artificial sea water is prepared from a mixture of the five major sea salts—sodium chloride, magnesium sulfate, magnesium chloride, calcium chloride, and potassium chloride. A mix of minor elements was also provided initially when the pavilion opened late in 1985. The proportion of the individual salts is adjusted to provide a salinity of 28–30 g/kg. Because the Living Seas uses ozone for disinfection, incompatible manganese and bromide salts were not included in the artificial sea-water formula. It should be noted that manatees in the wild are found in salinity environments ranging from 0 to 35 g/kg.

The entire Living Seas system that includes the manatee pool is maintained at 75 °F (24 °C) to simulate an Atlantic coral reef environment. The Living Seas is contained within a building in central Florida; therefore, temperature control is not a problem. The life-support system does include a heat-exchange system that can be used to make fine adjustments in temperature. For example, during a period following the birth of the manatee calf, the temperature was raised to 79 °F (26 °C).

The Living Seas aquarium environment is a highly buffered system and is very resistant to fluctuations in pH. The buffering capacity is achieved by maintaining an alkalinity of 250 mg/L as $CaCO_3$. Alkalinity is controlled by the denitrification process, which will be discussed later. The sirenian system pH is 8.1, a value very close to that of the coastal ocean and the intercoastal estuarian environments where manatees are found.

The Living Seas employs nearly 3,000 $ft^2$ of sand filtration that minimizes suspended particulate matter and turbidity. Turbidity in the manatee pool is maintained at < 0.1 national turbidity unit (ntu). An exception to this very low turbidity occurs during sprout-feeding events, where the turbidity in the pool approaches 0.2 ntu. This turbidity is still considered low, and water clarity is restored during one 90-minute recycle period.

## *Nitrification and Denitrification Processes*

Nitrogen balance is achieved at the Living Seas by nitrification and denitrification processes that occur in the filtration system. Because the Living Seas is a marine fish and mammal environment, ammonia (total available nitrogen and $NH_3$+$NH_4^+$) and nitrite ($NO_2^-$) are converted into nitrate ($NO_3^-$) by the nitrifying bacteria *Notrosomonas* and *Nitrobacter*. Ammonia levels are maintained at < 0.01 mg/L, which is far below the level at most marine-mammal facilities. The coexistence of fish in the environment is responsible for this stringent requirement. Nitrite has been observed to measure 0.007 ± 0.001 mg/L. The very low observed nitrite concentration is probably due to a combined effect of biofiltration (i.e., nitrification) and ozone-enhanced chemical oxidation of nitrite to nitrate.

The efficient nitrification process, lack of water changes, and the high cost of artificial sea water were all related to the gradual increase in nitrate over a 6-year period. During that time, the nitrate concentration as $NO_3^-$ was allowed to reach 650 mg/L. Discharge of waste sea water from the Living Seas has not been permitted in recent years because of its massive volume and environmental-impact regulatory requirements.

A series of laboratory and pilot plant experiments led to the 1991 installation of a denitrification system. The 300,000-gal batch system operates under anoxic conditions (dissolved oxygen < 1 mg/L) with methanol ($CH_3OH$) injection. Nitrate is biochemically reduced back to nitrite. As nitrite reaches its steady-state concentration, it is converted smoothly to nitrogen gas ($N_2$), which bubbles out of the top of several fluidized bed bioreactors. Methanol serves as the electron donor or carbon source for the oxidation–reduction (redox) reactions of nitrate and nitrite. As a result, methanol is oxidized to carbon dioxide ($CO_2$), which is retained in the sea-water solution as bicarbonate ($HCO_3^-$) and carbonate ($CO_3^{2-}$)which are responsible for alkalinity and pH control.

Figure 2 shows a history profile of nitrate concentration in the Living Seas manatee pool. After nearly a year of operation, the nitrate concentration had been decreased by nearly 70 percent to < 200 mg/L. Figure 3 shows the batch filtration process, which spans a 9-day period. A decrease in nitrate coupled with an increase and subsequent decrease in nitrite verified complete conversion of the dissolved nitrogen ions to $N_2$ at the end of the period.

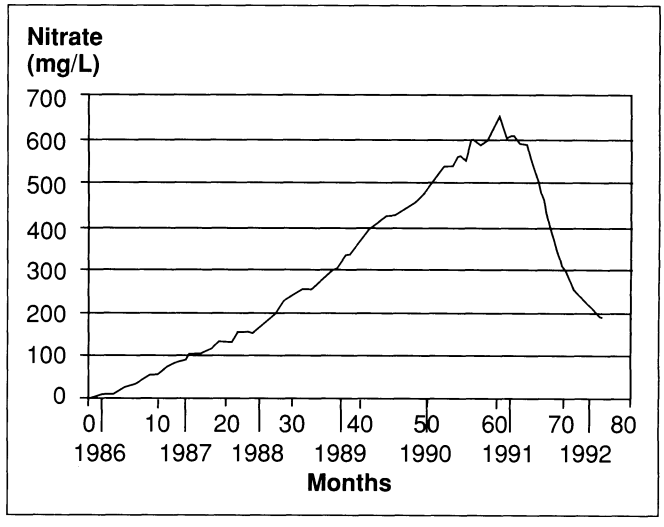


**Figure 2**—Average monthly nitrate over time (1986–92) at the Living Seas exhibit, EPCOT Center, Walt Disney World, Orlando, FL.

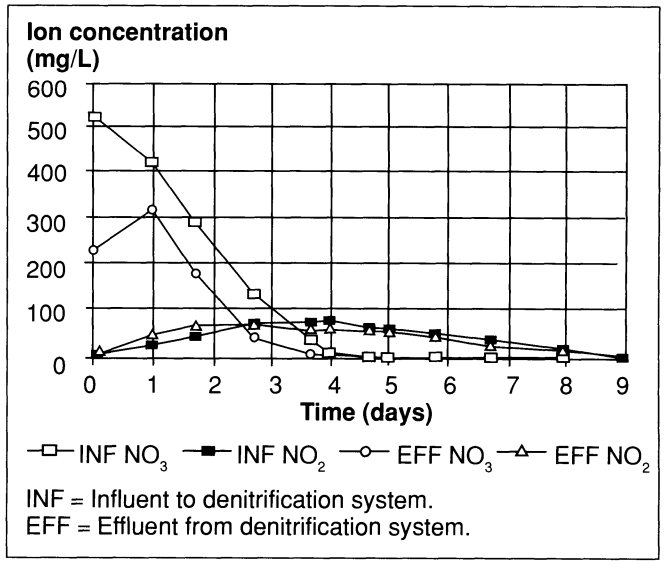


**Figure 3**—Ion concentration of $NO_2$ and $NO_3$ during the 9-day batch denitrification process at the Living Seas exhibit.

Little is known about the chronic effect of nitrate exposure on marine aquarium species. We are not aware of any sirenian nitrate-exposure studies. In humans, the biological conversion of nitrate to high levels of nitrite has been demonstrated to cause methemoglobinemia, resulting in the potential for impaired oxygen transport in the blood (Amdur et al. 1991). Closed recirculating systems, where water changes are minimized, clearly have a nitrate presence. At the Living Seas, the effect of nitrate on marine fish was the primary motivation to develop the large-scale denitrification system described here. Clearly, an unrealized benefit also includes nitrate removal in the manatee pool. The magnitude of this benefit is difficult to quantify.

## *Ozone, Coliform Bacteria, and Redox Modeling*

The redox chemistry of the manatee pool and the Living Seas is controlled by the oxidation conditions created by ozonation. Ozone gas is injected in line at a contact time of 2 minutes. The ozonated return line is then aerated to purge any excess ozone from solution. The filtered, ozone-disinfected sea water is then returned to the manatee pool. Fifteen pounds of ozone is required to disinfect nearly 50 million gal/day (8–9 cycles of the Living Seas volume).

Ozone has been shown to control fecal and coliform bacterial counts in a variety of water treatment systems (Evans 1972). Table 2 shows selected 1992 data in which control of fecal and total coliforms in the manatee pool was confirmed. In all cases, the fecal count was very low, typically < 2 MPN per 100 mL of water. An interesting caveat was observed in the total coliform data. It was determined that high (> 1,000 MPN) total coliform counts correspond with the feeding of hydroponically grown oat-sprout plant matter. The relationship between sprout feeding and sample time is currently under study. It has been shown that coliform samples taken several hours following a sprout feed return to lower levels while samples taken before a feed event are never elevated. Subsequent samples taken at 48-h intervals while avoiding feed events resulted in average total coliform counts well below the 1,000 MPN limit (Code of Federal Regulations 199X).

**Table 2**—Coliform counts in the manatee pool, March–May 1992

| Date | Fecal count | Total count | Sample comment |
|---|---|---|---|
| 3/17 | < 2 | > 1,600 | During sprout feed |
| 3/24 | < 2 | 2 | Before sprout feed |
| 3/31 | < 2 | 6 | Before sprout feed |
| 4/14 | < 2 | 17 | Before sprout feed |
| 4/21 | < 2 | > 2 | Before sprout feed |
| 5/5 | < 2 | 7 | Before sprout feed |
| 5/12 | < 2 | > 1,600 | 1 hour after feed |
| 5/19 | < 2 | 11 | Before sprout feed |
| 5/26 | < 2 | < 2 | Sprouts NOT added |

Finally, a redox balance model was designed for sirenian and other marine-mammal and fish aquarium systems. It was found that three distinct redox environments are required to achieve nitrogen management, disinfection, and overall animal health in recirculating systems. The redox potential (Eh) values at the Living Seas were observed to be +850, +500, and +200 mV in the aeration tower (after ozone aeration), the manatee pool, and the denitrification basin, respectively (fig. 4). Note: Approximately 200 mV must be subtracted from these values to determine the redox potential observed in the field using standard state Pt redox electrodes. These three values differ by approximately 300 mV, and they represent three very different water-quality environments. They range from a highly oxidizing, disinfecting environment to a nitrate-reducing, anoxic environment.

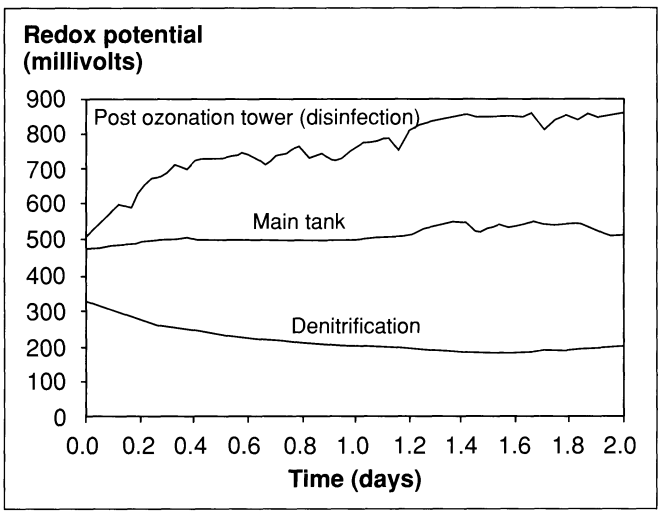


**Figure 4**—The Living Seas redox model showing three redox potential regimes for disinfection, aquarium, and denitrification environments.

## Conclusions

1. Sirenians require water quality similar to that of other marine mammals. If possible, systems may be developed to provide temperature and pH conditions like sirenians' native environment.

2. Nitrogen may also be controlled by adequately sized nitrification and denitrification systems. Ammonia and nitrite can be maintained near 0 mg/L while nitrate as $NO_3^-$ may be kept low, < 100 mg/L.

3. Ozone works well to control coliform bacteria counts and act as a clarifier to control dissolved organic matter (DOM) and other turbidity-causing materials. Filtration must be adequate to handle the manatee load.

4. Redox potential environments may be established to balance out water quality. One redox regime each for disinfection, nitrification, and denitrification may be established corresponding to Eh values +800, +500, and +200, respectively.

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# Water Quality for Polar Bears

By David B. Merritt

The polar bear *(Ursus maritimus)* is unlike any other marine mammal in both its physiology and natural history. The needs this animal has for water in a captive setting, and the impact it has upon the water it accesses, are different from those of other marine mammals. With the first 2 years of operation of the polar bear exhibit at the Indianapolis Zoo serving as example, I will share some observations and opinions about these needs and impacts. Additionally, I will talk about how management of staff and animals can play as vital a role to the success of an aquatic exhibit as architecture, mechanical engineering, and chemistry.

In the early 1970's, a gentleman who had made his living through the display of several species of bears inquired as to why the polar bear was soon to be protected under the Marine Mammal Protection Act (MMPA). "Because they eat fish and swim in the sea," was the response he received. The gentleman replied that he, too, ate fish and swam in the sea, yet he was afforded no protection under the Act.

Like other marine mammals, the polar bear has evolved to make its living from marine food chains. Unlike most other marine mammals, given another viable option, such as the town dump in Churchill, Manitoba, the polar bear can survive totally apart from the marine environment.

Regardless of where they live in the United States, polar bears are protected under the MMPA of 1972. To keep this species on public display today in this country, one has to provide a pool of water of specified minimum dimensions which must be tested daily for levels of any chemical additives and weekly for total coliform bacterial count.

In comparison with other marine mammals, there is a long history in the captive maintenance of polar bears. In the United States, they have been kept since before 1800 in the early animal menageries that were the precursors to the modern zoo. Typically, the animals have been maintained by means of the same routines, and with the same relative successes, as the various brown bears. Indeed, from an evolutionary standpoint, polar bears are more closely related to the brown bears than they are to any other marine mammal.

For survival and general well-being, the one need that captive polar bears have exhibited for water is that of fresh water to drink. Other than the fact that a polar bear in the wild spends a great portion of its life associated with the sea and sea ice, and subsists almost entirely on arctic marine organisms, there appear to be no data to indicate that these animals have a physical dependence on a marine environment. But because their need for fresh water is documented, they should be housed in an exhibit where fresh water is available.

In exhibitry design for the modern zoo, there are considerations other than those dictated by strict interpretation of current regulations and knowledge of a species' most basic physiological needs. One trend is to create a space in which the animal can make use of, and display, its natural abilities and adaptations. In light of this, a pool of larger dimensions than those required by APHIS regulations would be desirable to exhibit the swimming abilities of the polar bear. Although unnecessary for survival in captivity, swimming skill is a big part of the polar bear story.

As with human medicine, there is a growing sensitivity in animal care to the correlation between psychological and physiological well-being. This sensitivity further justifies an area of water of sufficient volume to allow the animal to exercise in as natural a way as possible (fig. 1). This thinking also inspires the creation of exhibit features that provide potential for variety of activity, as well as features that will alleviate predictable social stresses. It is with thoughts such as these that the water feature in the polar bear exhibit at the Indianapolis Zoo was designed.

Although it has little to do with the actual mechanics of the exhibit, the public perception of the water feature is as follows: A 15-foot waterfall ends in a small pool of water (approximately 7 feet diameter and 3.5 feet deep), which spills over into a 5-foot-wide stream that meanders for 20 feet before spilling an additional foot into a large pool. This organically shaped pool features an island area. Heights of banks from water surface and water depths at edges of banks vary greatly. All vertical walls are made from gunnite and are stained to look like real rock. With the excep-

tion of the pool bottom, all horizontal surfaces in the exhibit that are accessed by the animals are constructed in this same manner. The pool bottom is flat, smooth concrete that is spatter-painted to give it a textured appearance.

The entire exhibit has three distinct public viewing areas. The first allows viewing of a 2,300-$ft^2$ dry land area, as well as the waterfall and the first pool previously mentioned. Containment here is afforded by means of a dry moat. The second view is directly above water level at one end of the pool through two 1.25-inch-thick glass panels. The third viewing area is a cutaway view of the pool through five similar glass panels, which allow viewing from pool bottom to above the water surface. Public exit surveys indicate that this is the most popular animal viewing area in our zoo.

With the advent and subsequent popularity of underwater viewing of polar bears, the criteria for water quality have become more stringent. In addition to the bacterial counts required by APHIS, exhibitors are now interested in attaining water clarity. Although it may seem logical that one would include the other, in reality, this is not the case.

Figure 1—Polar bears use their pools for recreation as opposed to life support. (Photo taken by the Indianapolis Zoo and reproduced by permission.)

When water clarity becomes an integral part of the exhibit criteria, in a closed water system adding mechanical filtration becomes necessary. To maintain this water in compliance with APHIS regulations for longer than 1 week, some method of chemical sterilization must be applied. Systems to accomplish these tasks are initially costly but pay for themselves quickly in light of the fact that water itself is an increasingly costly resource. Additionally, it is not unusual that sending water to a municipal plant for treatment costs twice as much as getting it to a facility originally.

The water feature in the polar bear exhibit at the Indianapolis Zoo is a 76,000-gal fresh water system that is filtered through two large, rapid sand and gravel filters at a rate of 1,250+ gal/min. Water temperature is controlled automatically by means of plate heat exchangers, and is kept between 50 and 60 °F. Sodium hypochlorite (12.5-percent solution) is injected into the lines returning from the filters to the pool. Between the filters and the chlorine injection site, there is a sidestream that creates a waterfall and stream effect in the exhibit. About 7 percent of the water changes weekly, due to backwash, evaporation, and animal activity.

Pool water is tested weekly for total coliform count and ammonia. Each morning, pH is ascertained. Free and total chlorines are determined at the beginning and end of each day, at which time adjustments are made to the chlorine injector system. Temperatures are constantly monitored by computer.

Armed with all of this modern gadgetry and input from rigorous monitoring, operators should have an easy time managing this exhibit. In fact, it took well over a year to find the proper balance necessary to run the exhibit. Contributing to our problems was the assumption that we should maintain chlorine concentrations identical to those used in pools housing our other species of marine mammals.

A closed water system has many similarities to a living organism. It is comprised of several systems, each affecting the next and all affecting the whole. For optimal operation, a balance must be sought and maintained. A wide array of both internal and external influences dictates an individuality for each exhibit; only by identifying these influences can one begin to prescribe the correct regimes for that particular system.

Some of the external influences of note are management philosophies, climate, and local water. The specific components of what runs out of the tap can vary greatly both geographically and seasonally. Because water is a major component of an aquatic exhibit, subtle variations in it can have a major impact on how that exhibit needs to be maintained.

Internal influences include the physical design and materials, mechanical components, chemical additives, and the animals themselves. Certain assumptions can be made about the animals' influence upon the water system based on what they eat, how much of it they eat, and how they eat it. Individual animals' specific behavioral quirks may alter these assumptions, and the polar bears at the Indianapolis Zoo serve as a good example of this.

During the first 2 years of operation of the polar bear exhibit at the zoo, two pairs of bears (2.2) accessed the pool at different times each day. We are blessed with a large, airy, and very flexible off-exhibit holding area, into which the animals will shift with ease. This layout allows us to display different pairs of animals at different times each day. Of the four individual bears we had at the time, two defecated exclusively on dry land, and two exclusively in the water. We cannot pretend to explain this behavior, as it correlated to nothing obvious, such as gender, age, time of day, pair bonding, or lineage. This situation certainly does illustrate how the habits of individual

animals can have dramatic impact on water quality in a aquatic exhibit. It also adds insight into the age-old question of a bear's behavior in the woods.

With such a variety of factors exerting an impact on an exhibit, it is almost impossible for one person to make informed decisions about water quality. For this reason, we use a team approach to water-quality decisionmaking. Decisions relevant to water-quality routines in any of our four marine mammal exhibits are made by a team comprised of the curator, the water chemist, the senior keeper in charge of the relevant species, and a facilities mechanic. Only by way of input from all these individuals we can begin to understand and resolve some of the puzzles our aquatic exhibits have presented to us.

To assist this team in decisionmaking, we maintain records of all things that might affect the water-quality. We record results of all the water-quality tests conducted previously, daily weather information, work routines, and schedules. We have records of diet, behavior, and animal health. Mechanical inspections, breakdowns, and repairs are recorded. There are times when keeping these records seems ponderous beyond any reason. Often, however, they become the keys to problem solving.

We first introduced polar bears to our exhibit in June 1988. The first 3 months of operation did not include enough consistency in routines to begin to see trends emerge. During this period, the exhibit was drained and refilled several times to facilitate leaching of the rockwork. Animals were introduced to the exhibit at various times and in different combinations. A number of design flaws were discovered and addressed. These included moving the chlorine injection site from the bottom to the top of the return line, where it was less likely to become clogged by fine sediments. A timer added to the injector pump has proven much more reliable than the stroke and rate settings on the pump itself. (We currently set the pump to run 1.5 minutes out of every 10, injecting 2–2.5 gal/day.) Shrouding was installed around the underwater viewing windows to prevent the bears from digging out the caulking. The drain grating was reconstructed of a stronger material and installed with several additional bolts. The skimmer cleanout basket was redesigned to prevent the bears from cleaning it out themselves.

By September 1988, we had routines established pertinent to animal care, recordkeeping, and maintenance of the exhibit and support mechanicals. It was at this point that we identified a problem of unacceptably elevated coliform counts.

APHIS regulations require a weekly testing for total coliform bacteria in any pool housing marine mammals. The coliform count cannot exceed 1,000 MPN (Most Probable Number) per 100 mL of water without the institution taking remedial action. At the Indianapolis Zoo, we do this testing onsite, making use of the multiple tube fermentation technique. We use the term "spike" to refer to a coliform test that results in a total count greater than 1,000 MPN per 100 mL of water.

During the last 4 months of 1988, we logged eight spikes in total coliforms in the polar bear exhibit (50 percent of the period's total tests). When testing showed high coliform counts, our reaction was to "super-chlorinate" the system. Once the bears had been secured in the backup areas for the evening, we would add 7 to 12 gal of sodium hypochlorite (12.5-percent solution) directly to the pool. The pool system would run overnight. In the morning, sodium thiosulfate would be added by hand, and the water would be tested for chlorine levels. Once proper chlorine levels were attained, a sample of water was taken for a followup coliform test, and the bears were allowed back onto the exhibit.

This routine always proved successful in that results of our followup coliform tests were consistently below 1,000 MPN per 100 mL of water. This routine was not, however, the intended mode of operation for this exhibit, and we recognized that we needed to make changes.

One perplexing observation made during this time period was that the water in the exhibit was always esthetically pleasing and the chlorine levels were very stable. Other marine mammal pools at our zoo would typically show signs of imminent coliform spikes through a reduction in either water clarity or prescribed chlorine levels.

Until this point, we had been maintaining the same chlorine levels in the polar bear pools as in our other marine mammal pools. We began to suspect that the polar bears' water might require a higher chlorine level. Due to the number of high coliform counts, we decided to raise the targeted free chlorine level in the water from 0.2 p/m to 0.6 p/m in the morning sample. We felt that because the bears used their pool for recreation, as opposed to life support, the increased chlorine levels were acceptable.

During calendar 1989, we experienced 11 spikes in the coliform tests (approximately 20 percent of the year's total) run on our polar bears' pool water. Although this was an improvement over the previous time period, we still felt the rate was unacceptable. Three facts extrapolated from different records led us to a hypothesis that eventually solved our coliform problem.

First, we noted that comparison of morning and afternoon chlorine levels in all four of our marine mammals' water systems showed a substantially larger chlorine demand in the polar bear pool than in the others. Free chlorine in the polar bear exhibit decreased an average of 0.28 p/m between morning and afternoon samples. Our other exhibits showed an average decrease of 0.01 p/m. Behavioral notes indicated that this demand was not created by the direct introduction of fecal material. What other animal activity would create chlorine demand?

Secondly, and even more confusing, there were no coliform spikes in June, July, or August. Five occurred between January 1 and May 31, and six occurred between September 1 and December 31. We thought this situation odd because between Memorial Day and Labor Day, water and air temperatures are highest, and the bears are on exhibit longer each day and are at the height of their seasonal appetite. (Our bears eat 150–200 percent more in August than they do in January.) Wouldn't logic dictate that the summer months would be those during which our polar bear pools would experience high coliform counts?

The third piece of information that led us to a hypothesis that eventually solved this problem was found not in animal or water records but in records of work routines. Due to the seasonality of our business, operating hours are expanded during the summer months. To accommodate this, some work routines are shifted to different times of the day. Indeed, our records indicated that during the summer, samples of coliform tests were being taken in the early morning while during the rest of the year they were taken at midday. (It is advisable to take water samples from a polar bear pool only when the animals are not on the exhibit. Because our animals are put in holding overnight and shifted midday, we are afforded three potential collection times each day.) None of our other marine mammal systems showed dramatically different readings of coliform levels resulting from the change in time of day for sampling.

We could now say that our polar bear water system displayed a greater demand for chlorine than our other marine mammal pools, and that despite the maintenance of higher free-chlorine levels, as well as the observation that minimal amounts of fecal matter were being introduced directly to the water, there was an unacceptably high occurrence of high coliform counts in the pool water. From this combination of facts, we developed the following hypothesis:

> The obvious difference in density of a polar bear's fecal material from that of other marine mammals we maintain prevents it from easily entering solution to react with the chlorine in the water. This material instead settles to the bottom of the pool. Because the pool bottom is flat and interrupted with rockwork, fecal material could accumulate, rather than being pulled to the drain and filters. Thus a layer of coliform-laden water was formed near the pool's bottom, and this layer would affect the entire body of water once that volume was mixed by way of animal activity.

To test this hypothesis, in February and March 1990, we collected three distinct samples each time we ran total coliform tests from the polar bear exhibit. As a further step, we allowed only those animals whose habit it is to defecate on dry land to have access to the pool between the water sampling times.

The first sample was taken early in the morning, before the introduction of animals to the exhibit, and from our usual sample site (approximately 18 inches off the face of a particular rock, and approximately 12 inches below the surface). Pool depth at this spot is 9 feet. The second sample was taken at the same time by a scuba diver, from a spot 3–6 inches from the bottom of the pool, directly below the site of the first sample. The third sample was taken at midday from the same location as the first sample.

The results of this sampling lent credence to our hypothesis. Test results from the first samples ranged from 36 to 43 MPN; the second samples ranged from 1,000 to 2,400 MPN; the third sample group, from 240 to 460 MPN. During this period at least, we can say that a higher concentration of coliform bacteria was present at the pool's bottom than at its surface in the morning, after no animals had been present overnight. Furthermore, coliform levels increased at the pool's surface after animals had been active in the pool for several hours.

Armed with this information, we increased the efficiency of our vacuuming and other bottom-cleaning routines. We also increased targeted chlorine levels in the pool until our morning samples reached 0.6 to 0.9 p/m free chlorine. These changes in routine were initiated in April 1991, and between then and June 1992, we have experienced only two spikes in our coliform count (less than 2 percent of the total), both of which were directly attributable to mechanical failure. By all indications, our bears are healthy and robust. The underwater viewing area of this exhibit remains the single most popular viewing area in the zoo, and aside from labor in cleaning, the exhibit is relatively inexpensive to maintain.

## Conclusions

- Polar bears are different from other marine mammals.
- Polar bears in a captive setting have a demonstrated need for fresh water but no such need for salt water.
- Management techniques and recordkeeping play an important role in the success of aquatic animal exhibits.
- Proper levels of chemicals, such as chlorine, cannot be dictated for all marine mammal pool systems. Each system is unique.
- The density of polar bear fecal material, as well as the animals' recreational use of water, may dictate that higher levels of chlorine are required in their pool systems than in those for other marine mammals.
- Sloping bottoms and/or strong currents toward drains should be considered in design of water features for polar bears.

# Marine Mammal Water Quality Questions

By John Coakley and Richard L. Crawford

We believe these eight areas of research warrant funding to resolve questions about what constitutes adequate water quality for marine mammals.

—Should saltwater pinnipeds be kept in fresh water or only in saltwater?

—What are acceptable temperature ranges for various species?

—Should coliforms or some other organism(s) be used for indicators of acceptable water quality?

—What are the best methods of testing various water quality parameters (e.g., coliforms)?

—What types of coliform testing are acceptable?

—What effects do sound and/or shock waves through water have on various species?

—What other water quality tests should be performed on marine mammal pools?

—What effects does turbidity have on the various species?

# Marine Mammal Habitat Design

**By William H. Watts, Jr.**

*Abstract*—This paper provides recommendations and insights that will aid inspectors in their evaluation of existing and new domestic facilities and overseas facilities and determine their fitness to hold marine mammals in the best physical and behavioral health reasonably possible. The basic considerations for designing the best habitat for a particular marine animal are outlined.

Marine mammal habitat design at best is very empirical. As designers and curators learn more about how animals adapt to captivity, the design of their habitat also progresses. Because there are many variables, it cannot be assumed that a habitat is a panacea for all the animal health problems that may be encountered in an artificial environment. Creating what we consider an ideal physical enclosure does not necessarily assure we will be creating the ideal animal environment. While the physical habitat is important to the well-being of the animal, it is not the only factor that contributes to its wellness. The quality of the animal's environment can be affected by other conditions, such as the quality of the water that it lives in, the social structure of other animals within the pool (both species and sexes), and variations of husbandry and training procedures.

While it is recognized that there are legal minimums established in the Animal Welfare Act for volume, water quality, depth, and horizontal dimension, there is also much left to subjective interpretation in the evaluation of a facility. Such terms as "sound construction," "strength as appropriate," "adequate potable water," and "adequately ventilated" are representative of the clauses in the USDA standards (subchapter F, paragraph 3.125) that put inspectors in a more subjective position in their evaluations. Since the standards for dimension are specific, this report will focus on the more nebulous recommendations of the USDA Standards from Sections 3.101A through 3.104A, as well as other related topics.

## Quality of Construction

A fundamental consideration of development of a marine mammal habitat is to create an enclosure that has good, durable construction requiring minimal repair. Based on a historical survey of these marine life habitats, I have found that the most durable enclosures have been constructed of poured-in-place concrete. Specifically, the greatest success has been with 5,000 lb/in$^2$ Type II concrete, air entrained at the option of the structural engineer, with standard deformed rebar. This, combined with conservative coverage of rebar and thoughtful placement of joints and their design, will obviate much of the concern for such expensive extras such as epoxy-coated rebar, liner systems, and cathodic protection. As a case in point, recently a 20-year-old pool complex was torn down at a major marine park to make room for an expansion area. The pools were constructed as recommended above, without epoxy-coated rebar and other extras. These dated pools were still sound, and most of the rebar exposed during demolition remained uncorroded.

### *Concrete Detailing*

For the sake of the animals, concrete surface detailing is very important. Consideration must be made as to where the animals will be for different types of activities such as training, handling, feeding, husbandry, etc. It is very important to provide chamfered corners and rounded edges in areas of pools where an animal is likely to lean against an edge. To assure smoothness, defects in concrete formwork have to be stoned down and removed. Shallow areas where dolphins may slide out for presentation or medical examinations should be smooth to reduce the chance of abrasions.

### *Pool Coating*

A second area of material consideration is pool coatings. It is important to understand what a pool coating function should be. Pool coatings are not required, nor do they ensure a safe or clean pool wall, nor are they a cure for bad concrete. Quite simply, pool coatings have positive and negative aspects that must be weighed during the habitat-design phase.

From the animal's perspective, the coating should help create a smoother wall surface to reduce chance of abrasion. From management's viewpoint, the coating should make it easier to control the growth of algae. The coating system becomes a liability to the habitat when its color is too intense or when it fails to stay adhered to the concrete substrate. Brightness creates eye strain, possibly leading to eye disorder, and coating adhesive failure leads to the possible ingestion of nondigestible material.

If a coating system is to be applied, it is best to use a 100-percent solid unthinned epoxy applied directly to a properly prepared concrete surface. Epoxy is the most available coating system with the most successful history of adhesion to concrete in saltwater environments. I especially do not recommend fiberglass mats because they tend to hide adhesive failure and could enhance delamination from concrete substrates in the event the substrate cracks from hydration, movement, or shrinkage.

## Containment and Separation

Containment and separation of animals is a response to both physical and behavioral needs. Pod or colony social structure, mating cycles, and/or mixing of potentially adversarial species in relatively close confines—compared to the natural environment—make containment and separation two of the most important design considerations. Claims of success in the attempt to design facilities to address these issues must be viewed with as much skepticism as the answer to the "terrible two's" of childhood. In managing pinnipeds and cetaceans, we are dealing with intelligent life. We cannot lay their behavioral health initially on the doorstep of the facility's design. Most of the success of an animal's adaption to an artificial environment rests with the husbandry staff's procedures (fig. 1). The habitat should act as a tool for the curators to do their best in helping the animals to adapt. The scope of this paper allows for discussion of only a few of the physical features of a habitat that play a part in the adaption process.

**Figure 1**—Fancy facilities are no substitute for caring, knowledgeable employees. At the Aquarium for Wildlife Conservation in Brooklyn, NY, handlers convinced this young, still-nursing beluga whale to let them pump antibiotics right down its throat. The trick? Knowing that belugas love to have their tongue and gums stroked. (This shot and the other two in this chapter were furnished by fellow contributor Lou Garibaldi; all images are the property of the New York Zoological Society/Wildilfe Conservation Society and are reproduced by permission.)

Assuming there is more than one pool, the first consideration is the general arrangement of the pools. There should be a primary habitat that at least meets USDA standards but, hopefully, would be larger; a secondary holding and isolation pool of the same consideration; and a medical pool designed specifically to meet emergency husbandry needs. Ideally, the pools are arranged so there are two gates per pool, with each of the gates going to a different pool in order to maximize the options for the animals to move or be moved if the need arises. If it is a one-pool primary habitat that will hold multiple numbers of one or more species, then installing some sort of an island may be wise to help an animal escape a confrontation. Pool size alone may achieve the same purpose of providing separation, with the size needed for separation determined by the species, behavior, social mix, etc. It is advisable to have one or more of the pools, or a portion of the single large pool, away from the public. There may be some validity to the thought that the inhabitants need to remove themselves from the gawking guests (who may overstimulate them) or just because of the normal protectiveness a mother may display around its young.

In the case of pinnipeds, they seem to prefer a more dense social environment. Even still, retreat and escape options are an important part of the habitat. Haul-out space, for example, beyond being a requirement, needs to be broken up in order to reduce the chance that one dominant animal controls all the prime real estate and waterfront property.

Gates and barriers in the pool or above, obviously play a large role in animal constraint and separation. Without getting into the particulars for any one species, here are several basic observations that can serve as tools to evaluate a barrier's effectiveness.

To design a gate or barrier, the first step is to know well the animal that the gate or barrier is supposed to restrain. For instance, will it charge the gate? Chew on it? Jump over it? Go through it? Open it? Obviously, if the facility designers are trying to convince you they can keep a male killer whale away from a female with just a floating fish net, you will want to challenge their theory.

If there are multiple animals in the pools, there will be a lot of activity around the gateways. Consideration needs to be given to designing gates that present rounded edges; in other words, avoid projections, square-edged frame members, or anything the animals might be able to hook a tooth on, etc. The most durable fabrication material is Type 316 stainless steel pipe framing.

## Environmental Control

For the purposes of this paper, "environmental" means water quality, weather protection, and biologic intrusions.

Water quality in marine mammal facilities is well covered in other papers. I will, however, address a few items. Pool configuration should be designed to avoid dead spots on the surface that collect oils, fecal materials, and debris. Skimmers and inlets should be located on opposite sides of a pool so the water is drawn in one direction across the pool surface. If there is a prevailing breeze which will push debris on the pool surface, it should also be accounted for in the design by locating the skimmers on the downwind side of the pool. For pinniped haul-out areas, it is best to have them drain away from the pools to avoid adding their fecal load directly to the pool.

However important it is to create a degree of flow in a pool, consideration also has to be given to too much velocity in certain conditions. For medical pools, the amount of water movement must be kept at levels low enough to avoid adverse effects on a sick or weakened animal.

The need for protection from weather varies by location and species. For hot, sunny climates, shade is advisable for pool areas where the animals will most likely be on the surface for extended periods of time. Such locations would be pools of minimum depths, and minimum horizontal distances (MHD's), medical pools, and gateways. Whether the shade structure needs to shed water must also be considered: if it should, water must not run off the structure and into the pool. In the design of outdoor pinniped holding facilities, shade can be very important to enclosures that have solid walls on all four sides. Although solid walls inhibit ventilation and should be avoided when possible, they are necessary for the largest pinniped species such as walrus, stellars, and elephant seals.

In cold environments, cetaceans are subject to damage from windchill, ice, or frostbite that can be caused by inadequate swim areas or anything that may keep them on the water surface for prolonged periods of time.

In the wild, pinnipeds live and die in fairly cold climates. But parks or zoos cannot afford the negative scrutiny of any animal dying unnecessarily from a habitat condition that could have been better. As an example, one might note that, in the wild, the sea lions often sleep on rocks assume from that fact that concrete floors are adequate under any condition of captivity. But the observation of sleeping on a cold surface in the wild is not necessarily accurate. In the wild, sea lions often sleep on top of each other and on less dense surfaces, such as sand, aquatic vegetation, etc., that tend to reduce heat loss. More observant curators provide wooden platforms for both young and sick animals to sleep on, thereby giving them a measure of insulation. Haul-out areas with wooden platforms protect the animals from the direct exposure to cold concrete.

Another environmental consideration is lightning. In lightning-prone States such as Texas and Florida, most building authorities require lightning protection. But managers of marine mammal facilities need to check to see if the pool rebar is lightning protected or bonded.

Windborne dust, leaves, and debris should be considered: if necessary, indoor holding areas should be provided for extreme conditions. Please also note that if a confined space is used, be sure to provide good ventilation at the water surface, especially if chlorine is used as a disinfectant in the pool. Chlorine gas, which is heavier than air, can build up on the surface of the water and cause respiratory distress in the pool's inhabitants.

## Biologic Intrusion

Biologic intrusion includes airborne bacteria, plant materials, insects, pests, and people. When it is known that such extreme conditions as monsoons, dust storms, or acid rain exist, provisions should be considered for an indoor, environmentally controlled habitat.

As they move to create the immersion experience for guests, exhibit designers need to be aware that many plant materials can be either toxic or nondigestible to marine mammals. While in the wild, dolphins and pinnipeds may pay little attention to a palm frond or a leaf floating at a shore edge, but in an artificial environment, where they may have less daily environmental stimulation, these species might find such objects of interest and might swallow them.

Insects may be more of a problem for pinnipeds than cetaceans, and, at best, insects can be controlled but never eliminated. In outdoor facilities, structure cannot contribute much to insect control. However, some helpful ideas include shade cloth to serve as both shade and fly screen, water misters, frequent wash-downs, good air movement, bug zappers, and even the installation of predatory insect species.

Among pests, birds and vermin need to be included in a marine park's general pest-control program.

## Specific Conditions Within the Pool

### *Depth v. Minimum Horizontal Distance*

If I had to rate which is more important, minimum horizontal distance (MHD) or pool depth, I would choose MHD. Cetaceans and pinnipeds have the physiological capability for deep diving, but I do not know of any scientific study that indicates affording them that opportunity in an artificial environment is either good or bad for their health.

From my observations, it appears depth is more important to cetaceans than to pinnipeds. Besides achieving the minimum depth requirement for a certain species, depth needs can be fine-tuned. For example, where an animal is regularly fed or receives its behavioral cues, it is advisable to provide more than half the body length for the depth, in order to allow it to "stand" on its tail and not have its tail flukes drag on the bottom of the pool. Another place a whale might be in the vertical position is at split-level public viewing windows. Also, if the animal is part of a presentation, depths should be increased for reentry from jumps so it does not hit the bottom.

Again, it is important to be sensitive to the animal being displayed. Consider how much a white-sided dolphin loves to jump and dive, but a beluga whale stays pretty much in the water.

On the other hand, the depth throughout the exhibit does not have to be consistent. Diversity of depths can add interest to a pool for the animals, and from a budgeting consideration, can maximize horizontal swimming areas without adding large volumes of water.

### *Shelves*

Shallow areas can serve a wide variety of functions responding to animal needs. The most important I have seen is the shallow shelf around the perimeter of a pool. It has proved effective in keeping dolphins from surfing the pool wall and accidentally sliding outside the pool, which is life threatening to the animal and very dangerous for spectators.

Wide shelves are also excellent medical examination and sampling areas, as well as play areas for the animals. Even killer whales can have fun in 6 inches of water. Just make sure the shelf is not so wide the animal cannot get back into deep water.

### *Rockwork*

Rockwork, artificial reef, and the like must always be considered from the animal's perspective first. I feel designers are guilty of two errors in this regard: responding to emotional pressure to make the exhibit look more natural to the guest and assuming the animal will appreciate the esthetically pleasing effort in a human way.

Rockwork comes three ways for saltwater environments: gunnite, fiberglass, and a combination of fiberglass and cement grout cast in molds. Regardless of which is used, the basic criteria—durability and potential for ingestion, abrasion, or injury—must each be considered. In the design of rockwork for pinnipeds (figs. 2 and 3), much more latitude is possible than in that for cetaceans (i.e., for pinnipeds, the sharper forms of rock from molds can be used). However, gunnite is still important to the concept for strength. Remember these animals can weigh a couple hundred pounds and jump a lot on the rock, and they can also bite and chew it. Also, be concerned about rockwork that overhangs rockwork below. If a pinniped can get on the upper shelf and fall off onto a hard surface, it is obviously risking injury.

Even so, the employment of rockwork for cetacean habitats is also on the increase. Managers of

**Figure 2**—The Sea Cliffs exhibit at the Aquarium for Wildlife Conservation is home to Tiipaq, a Pacific walrus shown here at 11 months of age. This habitat was designed to resemble the granitic rock shoreline at Round Island in the Bering Sea, where walruses are often found basking in the sun.

**Figure 3**—Tiipaq's home, the walrus enclosure at Sea Cliffs, is in the foreground. Extending sequentially into the distance are the harbor seal, northern fur seal, sea otter, and penguin exhibits. The artificial rock habitats mimic the geology of the coastline where these animals live in the wild.

cetacean habitats should be more sensitive about abrasion because cetaceans' skin is not as tough as pinnipeds'. Rockwork should be kept off the perimeter walls of the pool, where dolphins tend to swim when they are doing fast laps.

Curators need to remember that adding rockwork can increase pool maintenance. Rockwork tends to harbor more algae growth simply because it may be harder to see than on flat surfaces, and rockwork can trap debris that may not make it to skimmers and drains. Also, rockwork needs to be considered in conjunction with the water-sterilization methods used. Some extra thought needs to be given to the notion of rockwork and its cleansability (fig. 4) if the curator elects to try doing a habitat without a residual chlorine disinfectant in the habitat itself.

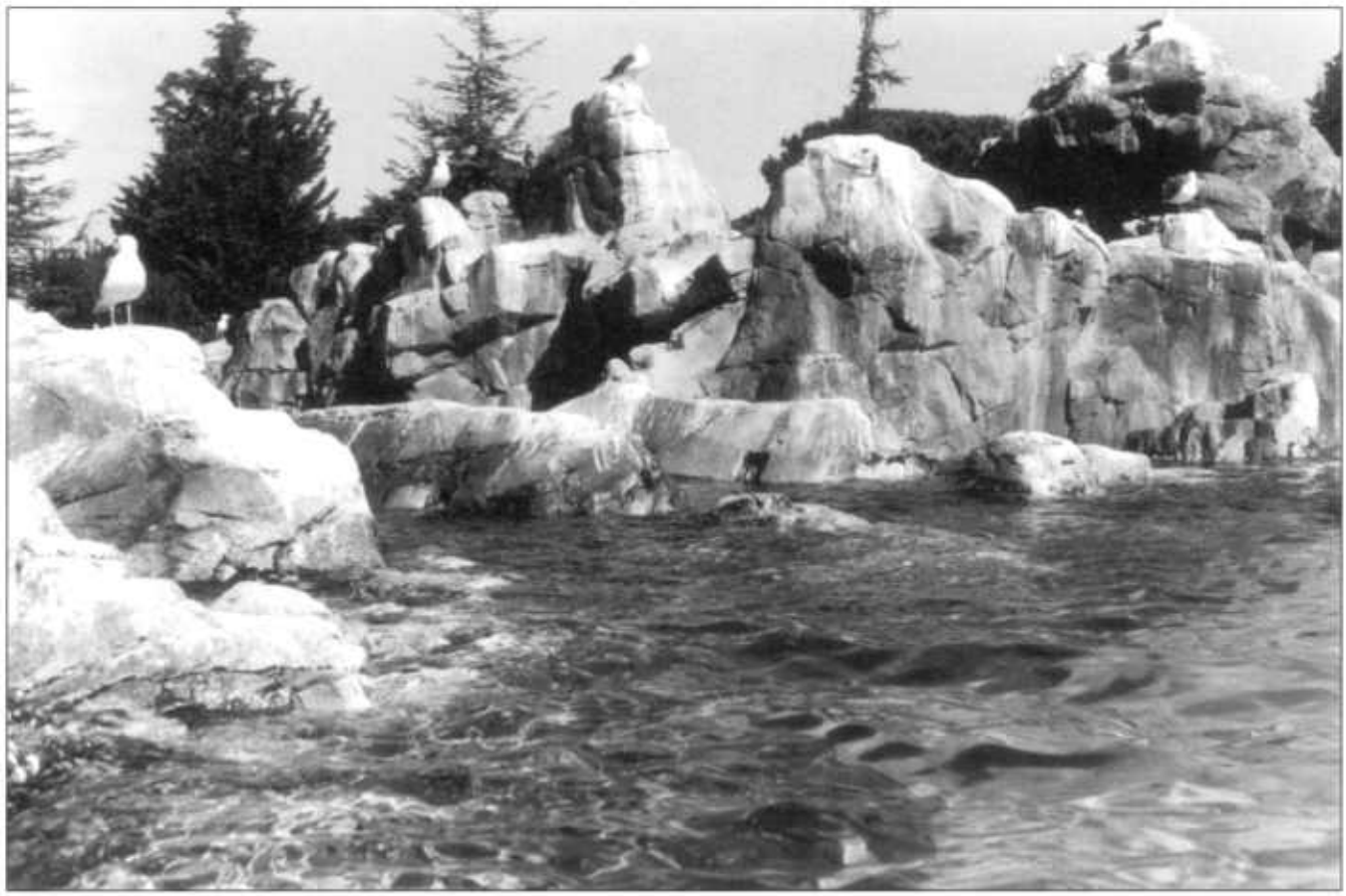

**Figure 4**—Seagulls loaf on the rock habitat surrounding a pool at Sea World in San Diego. (APHIS photo by Ann Czapiewski.)

## *Acoustics*

More attention is now being given to the acoustic environment of dolphins. Studies have been done on this subject by Hubbs Research Institute of Sea World, San Diego, CA, and possibly other investigators. The findings of the Hubbs team have yet to be interpreted, but I think the main thing is to guard against putting the dolphin in our shoes. One phrase I hear from time to time is, "I don't think dolphins like that," or "Vertical pool walls are confusing to their echolocation." Although we should respect dolphins as an intelligent life form, we have to be careful not to give them human personalities, likes, dislikes, etc.

Are vertical walls part of their natural environment? Rarely, but that does not mean these are a health factor. To my knowledge, no one has proved that vertical walls make dolphins neurotic or cause their death. Ideally, however, vertical walls should be minimized whenever possible, when it does not compromise other more demanding habitat criteria, until this notion is proven wrong.

Noise pollution is difficult to define; however, I can imagine that if I were a dolphin, the constant pounding of a pump or some other noisy equipment might be irritating. Again, it is important to first respond to what the animals react to, not to what critics (who may have no data to support their claims) may say. Remember that there are many ambient sounds in the ocean—

from crabs clicking to fish croaking to waves crashing—that add acoustic variety to the animal's life. I do not feel the environment should be acoustically sterile, as intelligent life normally seek environmental stimulation as part of its total health.

What about rockwork and acoustics? From this perspective, rockwork would add some echolocation variety to the environment, but for how long? Acoustic signatures of an environment may become as boring to a dolphin as recurrent sounds in the ambient environment could become to humans.

There are strong indications that dolphins are always looking for something to play with; obviously, sound is a big part of this. It is always worth considering the acoustic aspects of the elements that are part of that animal's environment—the shape of the pool, decorative treatments, filter systems, underwater tone system, locking mechanisms on gates, or adjacent building functions and guest activities. Any of these can be a factor in the effect the total habitat has on the health of its animal inhabitant.

### *Waves*

Waves are beginning to be introduced into aquatic environments. Personally, being a surfer and having seen both sea lions and dolphins playing in the surf, I think waves are a great idea that adds more diversity to an environment. However, the negatives of waves remain to be determined until we see how animals react in the artificial environment and after we have tried several different designs.

## Summary

There are far fewer absolutes than variables in the design of a successful marine habitat. Beyond the minimum criteria, there are many combinations of these variables to consider, open-mindedly and objectively, that may be impossible to quantify. I remember when I was working with a client and his curator in the design of a dolphin habitat. After our presentation, the client concluded that into "X" volume of water he could put "Y" number of dolphins in his habitat. We said, "No, it's not that simple." Hopefully, this paper illuminates some of the reasons why marine mammal habitat design is not simple. I also hope this paper helps to open thinking about habitat design and helps all who are involved in this process think analytically, honestly, and objectively in this evolving process.

# Reviewing Life Support Systems for Marine Mammals— What To Look For

By Patrick A. Case, P.E.

**Abstract**—This paper provides APHIS marine mammal specialist veterinarians a general background of life support systems (LSS's) for marine mammals and their design and identifies a number of issues concerning the operation and maintenance of LSS's that should be of concern to inspectors in determining the adequacy of a facility. Overall water-quality objectives are discussed, as well as factors that influence an LSS design. The basic processes of filtration and disinfection are discussed, along with design and operating trends in both areas. With the above as a background, this paper concludes by listing 11 key areas of an LSS operation and maintenance program that will indicate whether the facilities are being operated consistently and reliably.

The purpose of this paper is to provide APHIS marine mammal specialist veterinarians a basic background of what influences the design of life support systems for marine mammals, and what to look for in a properly designed, operated, and maintained LSS. The following viewpoints are presented from the perspective of a designer specializing in LSS design and operation.

First, it must be recognized that there is hardly a consensus of what constitutes a properly designed LSS. While there are some absolutes, the configuration of the system, type of filters, type of disinfection system, whether the system is automated or manually controlled, and the materials of construction may vary considerably. Given two very different LSS's, both of which meet water-quality objectives, one system cannot be deemed right and the other wrong, or one system superior to another. To explain differences in systems, it is necessary to review water-quality objectives, how systems get designed the way they are, and how the engineer accomplishes the water-quality objectives within the limitations of the project. With this background, the inspector can look at a facility in its proper perspective and focus on critical issues.

## Water-Quality Objectives

The overall objective of the LSS is to maintain animal-enclosure water quality that is healthy for the animals. Inasmuch as most facilities holding marine mammals have public display as one of their primary purposes, the LSS must also provide an aquatic environment that is esthetically pleasing to the viewing public. Although the requirement that the water be esthetically pleasing—free of "objective" turbidity and color—is not part of the USDA standards, it is nevertheless an extremely important factor. Nothing will cause the public to question the healthful aspects of the water faster than a perception of "dirty" water (fig. 1). In fact, how the water looks is one of the first things an inspector should observe. Although clean-looking water does not mean the water is chemically or biologically healthy, turbid or colored water is certainly an indication there is a problem with the water quality, and the LSS in general.

With regard to USDA water-quality standards, they may be summarized as follows:

- The water shall not be detrimental to the health of the animals;
- Coliform bacteria count shall not exceed 1,000 MPN per 100 mL;
- Added chemicals shall not cause harm or discomfort to the animals;
- Coliform and chemical additives shall be tested for and recorded;
- Proper salinity shall be maintained; and
- Adequate water quality shall be maintained by adequate filtration, chemical treatment, etc.

Notwithstanding the "catch-all" phrases of the above requirements, a specific requirement that temperature control be provided for should be added.

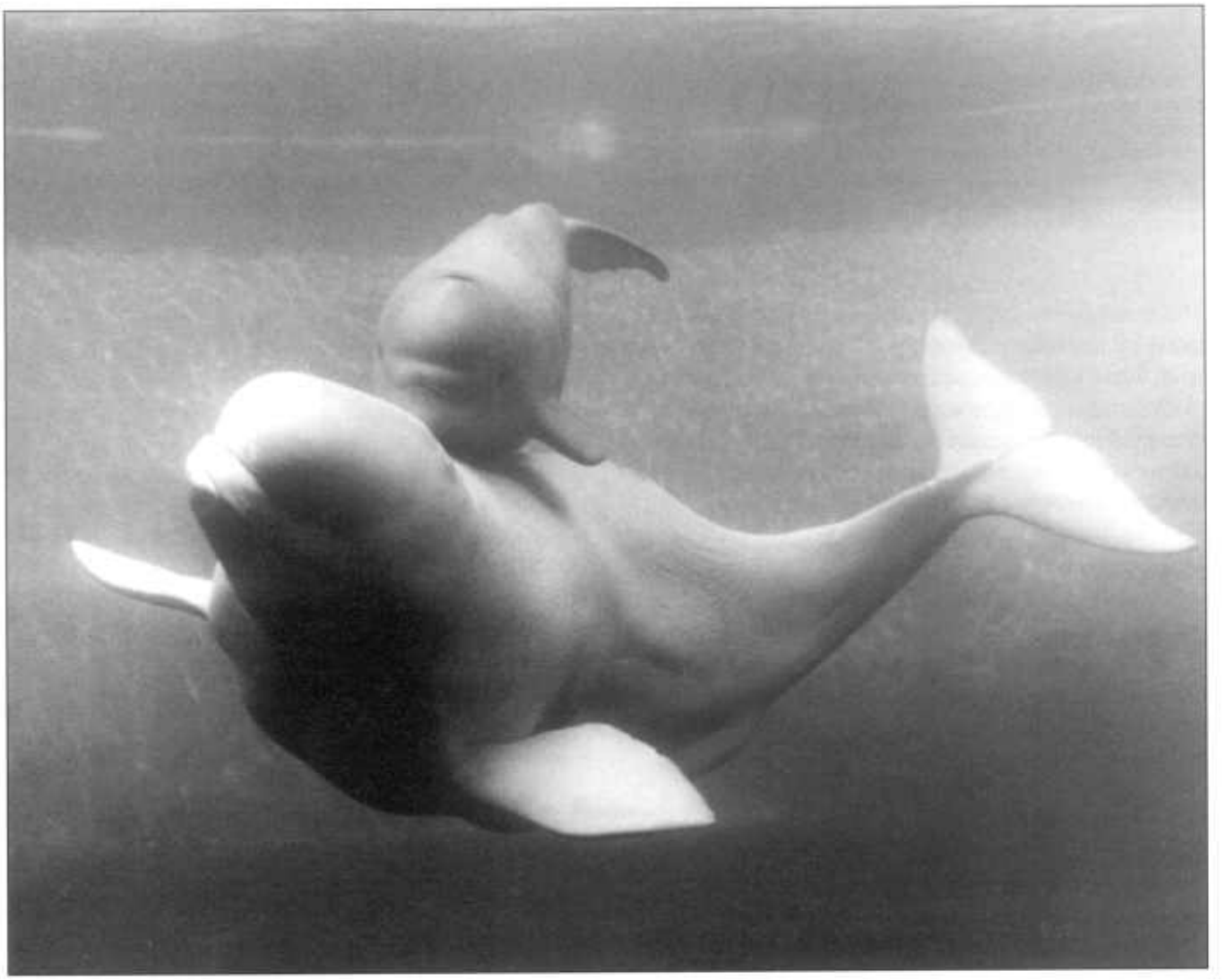

**Figure 1**—This photo of a beluga whale and her 2-day-old calf was taken under low light conditions. Here the water is murky because of nursing activity, not defective filtration. (This photo, provided by chapter author Louis Garibaldi, is the property of the New York Zoological Society/Wildlife Conservation Society and is reproduced by permission.)

## The Realities of LSS Design

Why do systems get designed the way they are? There are a number of factors that influence how an LSS is designed, what processes it includes, the physical configuration, whether it is automated or not, etc. These factors include (1) architectural constraints, (2) budget constraints, (3) direction from the owner reflecting its discretion and philosophy, (4) state-of-the-art at the time of the design, and (5) knowledge of the designer.

Architectural constraints will determine whether the LSS is in close proximity to the animal enclosure or a considerable distance away; whether the LSS is located outdoors, in the basement, or someplace else; where the ozone towers are and how accessible they are; the general configuration of the LSS area, overhead clearances, accesses, etc. Budgetary constraints may determine if a facility has such components as an ammonia removal system or automation, and what degree of redundancy is built into the system.

As frequently occurs with an existing institution, the owner's personnel may have very strong ideas of how the LSS should be configured and sized and what processes it should include, as well as what the materials specifications should be. Rightly or wrongly, the designer is often pressured into designing the system a certain way or making compromises simply to satisfy the owner. With regard to the state-of-the-art factor, this industry has been growing by leaps and bounds over the past 10 years. Often a design is "last year's model" before construction is complete.

The last factor, knowledge of the designer, is fairly self-explanatory. Very few engineers would pass up designing the LSS for a marine mammal facility just because they have no prior experience. An all-too-frequent occurrence is for the novice to approach designing the filtration system of an animal habitat as if it were a swimming pool, without regard for the type and number of animals in the exhibit, food and fecal load, and other factors that must be taken into consideration. Ozonation and other support facilities are added to emulate what may have been seen at other facilities toured during the design process. A common result is a system that does not work.

## LSS Basics

The LSS must be somewhat conservatively designed, incorporate redundancy, be comprised of reliable processes and equipment, and be easy to operate and maintain. Owners and prudent designers alike are very cautious in the consideration of new processes and generally shun complexities. However, with their desire to create distinctive exhibitry and provide better animal health care, owners of facilities are continually asking more from the LSS. As a result, systems may now incorporate ammonia removal, exhibits and LSS's are becoming much larger to enhance the presentation and successful breeding and rearing of animals, and ozone is being incorporated in virtually all systems.

Nevertheless, the main interest of facilities keeping marine mammals is the husbandry of the animals, not the operation of unnecessarily complex and gadget-plagued LSS's. With that in mind, the LSS should be fairly simple and straightforward.

### *Filtration*

Filtration for marine mammal exhibits in the United States is provided by pressure sand filters. The advantages of pressure sand filters are that they produce water of very good quality, are easy to operate, require minimum labor, are readily automated, minimize space requirements, and do not require the purchase and disposal of an expendable filter medium (e.g., diatomaceous earth). Pressure sand filters come in various configurations, including vertical and horizontal arrangements, and may be anywhere from 4 to 12 feet in diameter. Nominal filtration flow rates are approximately 12 gal/min/ft$^2$ of filter surface area and approximately 15 to 18 gal/min/ft$^2$ in the backwash mode.

The objective of filtration is to remove feces, uneaten food, and other solids from the water in a reasonable period of time to minimize the degree of ammonification and improve water clarity and the effectiveness of the disinfection process.

The amount of filtration required is commensurate with the type and number of animals in a habitat. The amount of filtration is commonly referred to in terms of "turnover," which is the theoretical period of time required to filter all water in the animal enclosure. The turnover in an exhibit may range from 30 minutes to more than 3 hours. As some broad examples, for an enclosure less than 100,000 gal, the turnover may be 30 to 45 minutes; 100,000 to 500,000 gal, 60 to 90 minutes; 500,000 to 1 million gal, 1 to 2 hours; and 2 to 5 million gal, 3 to 3.5 hours. Again, however, these values may vary considerably, depending on a number of factors.

While filters fabricated of steel, whether vertical or horizontal, are still common, fiberglass filters are becoming more popular because of their resistance to corrosion. Historically, fiberglass filters have been limited to an approximate diameter of 4 feet; however, filters with a diameter up to 8 feet, both vertical and horizontal, are now being utilized. Neither steel nor fiberglass filters are superior to one another; each has advantages and disadvantages.

### *Disinfection*

Ozone and chlorine are used almost exclusively for the disinfection of marine mammal enclosures. Ozone, which has been shown to be very effective for killing bacteria and deactivating viruses, is used as the primary disinfectant. Ozone also enhances the clarity of the water and is effective for oxidizing dissolved organic matter. Chlorine is used to maintain a residual disinfectant in the pool.

Managers at a number of facilities have found they are able to maintain coliform counts within acceptable ranges with the use of ozone alone (i.e., without the need for chlorine). Indeed, for certain mammals that do not have a tolerance for chlorine (e.g., sea otters) ozone must be relied upon exclusively for disinfection. The levels of chlorine maintained in enclosures can vary significantly. Closed systems may be able to maintain only very low concentrations of free chlorine (i.e., virtually all chlorine is in combined forms), while open systems routinely maintain free chlorine at 0.6 to 1.2 mg/L.

As facilities become more comfortable with relying on ozone alone for disinfection, inspectors should more often see the use of only minimal chlorine as necessary to reduce the growth of algae, or no chlorine at all. Another trend in the use of chlorine and ozone is greater preference for liquid chlorine (sodium hypochlorite) over gas because the liquid is generally considered safer.

## Evaluation of Life Support Systems

Based on the above general discussion of LSS's and their design, the APHIS inspector is in a better position to evaluate the facility beyond accepting USDA water-quality reports at face value. He or she can focus on observing trends and shortcomings in the operation and maintenance of the facility that may forecast impending problems. The remainder of this section suggests areas and issues recommended for evaluation in determining the suitability of a facility.

In evaluating the LSS for any marine mammal facility, the following factors should be considered:

■ **What does the water look like: Is it turbid (greater than 0.2 nephelometric turbidity unit [NTU]) or green?** These may be signs the system is overloaded with animals or is not being operated correctly.

■ **Are there stated objectives for water-quality parameters?** Lacking such information, it is impossible for the operator to know what water-quality parameters he or she is expected to maintain.

■ **Does the amount of filtration look reasonable for the size of the pool and the number of animals?** It is not difficult to estimate the gallonage of a pool. One can either ask the operator for the dimensions or pace off the pool for a rough estimate (remember, 1 $ft^3$ = 7.48 gal). The turnover rate of the system is determined by dividing the volume of the pool by the total filtration flow rate in gal/min.

■ **Is there an accumulation of debris on the bottom or floating materials on the surface?** The presence of debris is a clue that the facility is being operated and maintained poorly. Also, the accumulation of debris on the bottom contributes to the general deterioration of water quality, the consumption of chlorine, and inefficiency of disinfection.

■ **Are there trained operators responsible for the system, or is LSS management delegated to the maintenance department?** Do the operators really know how the system works? Ask them to explain! Operation of the LSS may be left up to the maintenance department or keepers, neither of whom often has the knowledge, dedication, or time to give the LSS the attention it needs.

■ **Is there an operating manual that addresses emergency procedures, safety and housekeeping, and water-quality testing, as well as normal operation? Is the operating manual written in a clear and concise manner and in adequate detail? Are copies of the operating manual readily available to the operators?** Marine mammal facilities often do not have an operating manual for their system, and instructions are communicated only verbally on a day-to-day basis, as well as to the next generation of operators. As a result, the proper operating procedure degrades over time; soon the operators are doing something only because that's what they were told, without knowing why they are doing it.

■ **Look at the records.** Are there records? Is the recordkeeping hit-and-miss? Note how often the water of the exhibit is being dumped (i.e., thrown away). Wholesale dumping of water from a pool indicates deterioration of water quality due to the LSS being taxed or the operator's lack of knowledge of how to rectify water-quality problems. Thus the problem is "thrown away," and the operator starts over.

■ **Is all the equipment operational?** Or is the equipment in disrepair or corroded, and with no obvious repair or preventive maintenance program? In brief, is the system limping along like an accident waiting to happen? Deferred maintenance, which may even lead to shutting down a piece of equipment for long periods of time, generally indicates that inadequate resources are committed to the facility for maintenance and/or manpower. Sooner or later, such a situation generally leads to the water quality's suffering as well.

■ **Are there written operating and maintenance instructions for the major pieces of equipment, and are these instructions available to appropriate personnel?** Is there a planned schedule for maintaining equipment?

■ **Does the LSS incorporate adequate heating and cooling (this is not a specific USDA requirement), backup emergency power, redundancy, and controls for chemical additions?** These features are necessary for consistent water quality throughout the year, as well as from day to day.

■ **Is there an operating program that incorporates an organization chart and sense of accountability between animal husbandry, water-quality testing, LSS operations, and management?** Without such a system, there are no checks and balances between the personnel responsible for testing the water-quality parameters and those responsible for making proper adjustments to the LSS to attain the established water-quality objective.

While the above areas of concern are certainly not comprehensive, answering these questions will go a long way in determining if the LSS is well operated and maintained or is running on a shoestring, employing crisis management. Needless to say, positive responses to the above questions and concerns will describe a marine mammal enclosure that provides a healthy environment for the animals when the inspector is not around, as well as when he or she is. Negative responses indicate the opposite.

## Summary

There is hardly a consensus of what constitutes a properly designed life support system for marine mammals. But the overall objective of the LSS is clear: to maintain animal-enclosure water quality that is healthy for the animals. Factors that influence the design of LSS's include architectural and budgetary constraints, direction from the owner, state-of-the-art at the time of the design, and the designer's knowledge. LSS's must be somewhat conservatively designed, incorporate redundancy, be comprised of reliable processes that incorporate reliable equipment, and be easy to operate and maintain.

Filtration for marine mammal enclosures in the United States is provided by pressure sand filters. The amount of filtration required is commensurate with the type and number of animals being kept. The "turnover" of water in an enclosure may range from 30 minutes to more than 3 hours.

Ozone and chlorine are used almost exclusively for the disinfection of marine mammal enclosures—ozone as a primary disinfectant and chlorine to maintain a disinfectant in the pools and minimize the growth of algae. As managers of facilities become more comfortable about relying on ozone alone for disinfection, the use of chlorine will be reduced or eliminated.

In evaluating the LSS for a marine mammal facility, the following factors should be considered:

■ What does the water look like: Is it turbid or green?

■ Are there standard objectives for water quality?

■ Does the amount filtration look reasonable for the size of the pool and the number of animals?

- Is there an accumulation of debris on the bottom or floating materials on the surface?
- Are trained operators responsible for this system, or is managing it delegated to the maintenance department?
- Does this system have a readily available operating manual that addresses emergency procedures, safety and housekeeping, and water-quality testing, as well as normal operations?
- Are there written operating and maintenance instructions for the major pieces of equipment, and are these instructions available to appropriate personnel? Is there a planned schedule for maintaining equipment?
- Is all the equipment operational?
- Does the LSS incorporate adequate heating and cooling, backup emergency power, redundancy, and controls for chemical additions?
- Is there an operating program that delineates accountability between animal husbandry, water-quality testing, LSS operations, and management?

# Marine Mammal Information Resources at the Animal Welfare Information Center

By Michael D. Kreger

The Animal Welfare Information Center (AWIC) was established in 1986 as mandated by Congress in the 1985 amendments to the Animal Welfare Act (AWA). AWIC is located in Beltsville, MD, at the U.S. Department of Agriculture's (USDA) National Agricultural Library (NAL). Among the Center's objectives is to provide to individuals or institutions information on any animal covered under the AWA. This charge includes marine mammals, which are covered in the 1970 amendments to the Act with regulations concerning their care in captivity and during transport.

AWIC has a variety of resources available to anyone requesting information on marine mammals. The patron may ask for AWIC-produced publications, specific contacts or literature references, or a literature search in response to a particular question. Articles indexed from the NAL collection are cited in the NAL data base AGRICOLA. NAL has a collection of marine mammal reports, conference proceedings, journals, books, and audiovisuals, all of which are available to U.S. libraries and individuals through interlibrary loan. Interlibrary loan information is available from NAL's site on the World Wide Web at

http://www.nal.usda.gov/ddsb

AWIC annually responds to a large number of information requests (more than 2,000 requests in 1996). Often, this work requires the technical information specialist to search for relevant articles through CD–ROM (compact disk), online data bases, and websites. Articles relating to environmental enrichment of dolphins or pinniped nutrition, for example, can be searched by species or general subject area. The patron will receive a copy of the bibliographic citations. If he or she has access to data bases, the AWIC staff can provide an appropriate search strategy. Requests for information are kept confidential. While AWIC does not charge for searches of CD's, the World Wide Web, or brief online searches of multiple data bases, the Center must recover costs for running extensive online searches of multiple data bases. Searches that cost AWIC more than $25 are billed to non-USDA patrons.

Patrons can also attend the AWIC workshop "Meeting the Information Requirements of the Animal Welfare Act," which is held several times a year at NAL. The workshop is geared toward veterinarians, researchers, information providers, and members of Institutional Animal Care and Use Committees (IACUC). It provides an introduction to the specific information requirements of the Act for both IACUC, researchers, and inspectors working for USDA's Animal and Plant Health Inspection Service's (APHIS) Animal Care program. The workshop also offers a discussion and regulatory update given by an APHIS Animal Care staff member, an introduction to data bases, hands-on training in data-base searching, and use of animal-related listserves and websites on the Internet. Attendance is limited to 20 participants per workshop, so early registration is encouraged.

An inhouse file system contains paper articles on marine mammals and other animal care topics taken from both the scientific and popular press. AWIC also archives relevant State and Federal laws and promotional materials from nonprofit animal-interest groups. Visitors to AWIC are welcome to use the files by appointment.

The free quarterly Animal Welfare Information Center Bulletin provides articles that focus on animal care and use, alternative models for research and education, environmental enrichment, funding sources, and regulatory issues and updates. Although most articles may be only indirectly relevant to marine-mammal concerns, past articles have included marine-mammal training, negotiated rulemaking in developing regulations, and the history of cetaceans in captivity. Any regulatory change or proposal affecting marine-mammal coverage in the Animal Welfare Act is printed in the bulletin. Professionals from the marine- mammal care community are invited to contribute articles.

AWIC produces and distributes bibliographies and information resource guides that relate to stress, animal care and use committees, animal models for research, facility design, and legislation. Readers may contact AWIC at the address given below for a list of the more than 60 publications available.

A recent publication is "Handling Fish Fed to Fish-Eating Animals: A Manual of Standard Operating Procedures," by Dr. Susan Crissey, director of nutrition services at the Brookfield Zoo. The popular manual is available in hard copy from AWIC and can also be found on the AWIC website. Funding for the publication came from APHIS–Animal Care and the Brookfield Zoo.

AWIC maintains a website that contains interactive documents relating to the AWA, animal care and use guidelines and policies, past and current issues of the Animal Welfare Information Center Bulletin, AWIC bibliographies and resource guides, workshop information, and links to other related websites. The workshop registration form can be completed and sent electronically to AWIC, and patron requests can be taken from the site. The website is constantly being updated with new information and graphic enhancements. The site address is http://www.nal.usda.gov/awic

The services of AWIC are available to everyone. In fiscal year 1997, AWIC answered more than 2,500 requests for information, distributed more than 38,600 publications, and provided information to more than 3,800 people through outreach and training. There is no charge for most literature searches, and all publications, workshops, and newsletter subscriptions are free of charge.

AWIC is open during normal NAL hours of operation from Monday through Friday, 8 a.m. to 4:30 p.m.

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# Appendix 1
# Marine Mammal Water Quality Standards, Title 9 Code of Federal Regulations, Subchapter A: Animal Welfare, Section 3.106

## § 3.106 Water quality.

**(a) General.** The primary enclosure shall not contain water which would be detrimental to the health of the marine mammal contained therein.

**(b) Bacterial standards.**

(1) The coliform bacteria count of the primary enclosure pool shall not exceed 1,000 MPN (most probable number) per 100 ml. of water. Should a coliform bacterial count exceed 1,000 MPN, two subsequent samples may be taken at 48-hour intervals and averaged with the first sample. If such average count does not fall below 1,000 MPN, then the water in the pool shall be deemed unsatisfactory, and the condition must be corrected immediately.

(2) When the water is chemically treated, the chemicals shall be added so as not to cause harm or discomfort to the marine mammals.

(3) Water samples shall be taken and tested at least weekly for coliform count and at least daily for pH and any chemical additives (e.g. chlorine and copper) that are added to the water to maintain water quality standards. Facilities using natural seawater shall be exempt from pH and chemical testing unless chemicals are added to maintain water quality. However, they are required to test for coliforms. Records must be kept documenting the time when all such samples were taken and the results of the sampling. Records of all such test results shall be maintained by management for a 1-year period and must be made available for inspection purposes on request.

**(c) Salinity.** Primary enclosure pools of water shall be salinized for marine cetaceans as well as for those other marine mammals which require salinized water for their good health and well-being. The salinity of the water in such pools shall be maintained within a range of 15-36 parts per thousand.

**(d) Filtration and water flow.** Water quality must be maintained by filtration, chemical treatment, or other means so as to comply with the water quality standards specified in this section.

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