

# The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives

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## Chapter 6

# Water Quality and Life Support Systems for Large Elasmobranch Exhibits

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*Abstract:* High water quality and effective water treatment are an essential part of any successful husbandry program for elasmobranchs. Water sources, salt constituents, and contaminants can all contribute to problems encountered during the mixing of artificial seawaters, preparation of natural seawater, system start-up for elasmobranch LSSs, and daily LSS operation. Critical water quality concerns such as dissolved oxygen concentrations and gas supersaturation must be understood so that potential disasters are considered and prevented during LSS design. LSS objectives such as particulate removal, dissolved organic removal, biological filtration, chemical filtration, and sterilization are essential for optimum water quality and a variety of system designs accommodate these demands. Rapid sand filters, foam fractionators, algal turf scrubbers, biological filters, ozone contact systems, and degassing systems possess overlapping attributes, and various combinations of these LSS elements will achieve effective water treatment. While system designers frequently disagree about how these elements should be arranged, and which elements provide the best result, all concur that effective systems should provide acceptable water clarity, biological filtration, removal of dissolved organics, minimization of bacterial pathogens, and effective gas balance.

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High water quality and effective water treatment techniques are an essential part of any successful husbandry program for elasmobranchs. If water quality is high many possible husbandry challenges are eased. It is therefore incumbent upon elasmobranch husbandry personnel to strive for optimal water conditions.

This chapter has been divided into three sections. The first section reviews general water quality issues as they pertain to elasmobranchs. The second section gives an overview of generic LSS (life support system) components. The third section describes different LSS philosophies used for elasmobranch exhibits resulting in diverse arrangements of the components described in section two.

### WATER QUALITY

Water quality parameters for elasmobranch systems do not differ significantly from those considered desirable for bony fishes. Maintaining water quality parameters for elasmobranchs is generally more challenging because large volumes of water must be processed. With notable exceptions, cartilaginous fishes are sensitive to therapeutic copper, organophosphates, and low salinity. A thorough review of general water quality issues can be found in Spotte (1992). Suggested water quality parameter limits for elasmobranch exhibits have been summarized in Table 6.1.

**Table 6.1.** Recommended basic water parameter limits for elasmobranch exhibits. Where possible water parameters, in particular temperature, should be restricted to natural ranges encountered by wild conspecifics. Major elements (i.e., Na, Cl, Mg, K, SO<sub>4</sub>, and Ca) should be maintained at levels  $\pm 15\%$  of natural values. The ORP range given is suitable if other parameters that affect ORP (e.g., total residual oxidants, ozone concentrations, etc.) are within safe limits. The value quoted for nitrate should be considered an operational goal rather than an absolute.

Parameter	Range	Units
Salinity	25.0-35.0	g l <sup>-1</sup> (ppt)
pH	8.0-8.4	
DO (dissolved oxygen)	85-98	% saturation
Turbidity	<0.15	NTU
ORP	250-380	mV
Ammonia, unionized (at 10°C)	<0.1	mg l <sup>-1</sup>
Ammonia, unionized (at 17°C)	<0.2	mg l <sup>-1</sup>
Ammonia, unionized (at 28°C)	<0.3	mg l <sup>-1</sup>
Nitrites	<0.1	mg l <sup>-1</sup>
Nitrate (as nitrate nitrogen)	<70.0	mg l <sup>-1</sup>
Total coliform	<1000	ufc 100 ml <sup>-1</sup>
Copper	<0.01	mg l <sup>-1</sup>
Zinc	<0.01	mg l <sup>-1</sup>
Nickel	<0.01	mg l <sup>-1</sup>
Iron	<0.03	mg l <sup>-1</sup>

## Temperature

The water quality parameters of an animal's natural habitat should be used to determine temperature limits for a display. Typically, non-biologist exhibit designers create specimen "wish lists" based on physical appearances rather than water quality and husbandry requirements. It is unwise to mix animals having a purely temperate distribution with those that require high tropical temperatures. For example, keeping zebra sharks (*Stegostoma fasciatum*) and leopard sharks (*Triakis semifasciata*) in the same exhibit is a risky long-term arrangement (geographic confusion aside) because overlap in their natural temperature preferences is minimal at best. The health of one or both species will be compromised.

## Nitrogenous wastes

Ammonia (NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup>) and nitrite (NO<sub>2</sub><sup>-</sup>) concentrations considered safe for fishes are

quite low and are often derived from studies based on salmonids, fishes particularly sensitive to these compounds. Most marine fishes, including sharks, tolerate somewhat higher concentrations of these nitrogen compounds. Regardless, there is no logical reason to expose elasmobranchs to water parameters more extreme than those considered safe for salmonids and other teleosts.

Project managers must allow sufficient time for biofilter stabilization and a subsequent gradual stocking period when developing time lines for opening new exhibits. Use of biofilter seeding techniques and a 4-5 week maturation period will allow workers to avoid the accumulation of dangerous levels of ammonia and nitrite. Since nitrite levels will often increase near the end of biofilter maturation, this waste product must be monitored closely.

We recommend nitrite levels  $\leq 0.10$  mg l<sup>-1</sup> (=ppm). Properly designed and operated LSSs will rarely allow nitrite to exceed this value. Based on

observations by one of the authors (Mohan) short-term (i.e., 3-5 days) exposure to nitrite levels as high as 0.50 mg l<sup>-1</sup> seems to be relatively safe for a number of species (e.g., California bat rays, *Myliobatis californica*; sand tiger sharks, *Carcharias taurus*; sandbar sharks, *Carcharhinus plumbeus*; lemon sharks, *Negaprion brevirostris*; blacktip reef sharks, *Carcharhinus melanopterus*; and whitetip reef sharks, *Triaenodon obesus*). Nitrite reacts with ozone almost immediately and is unlikely to be of great concern in ozonated systems. However, attempts to compensate for poor nitrification in new systems by increasing ozone dosing may lead to disaster once nitrite is depleted and excess ozone enters the exhibit (Johnson, 2001).

### Alkalinity and pH

Alkalinity and pH tend to be low in systems that have high animal loads or poor facilities for the removal of organics. Sodium bicarbonate (NaHCO<sub>3</sub>) and sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), or soda ash, can be used to make adjustments in systems where pH and alkalinity are already relatively stable. Some facilities experiencing chronically low pH use dosing pumps to inject sodium hydroxide (NaOH), or caustic soda, on a continuous basis. These chemicals represent a temporary solution, requiring constant monitoring and dosing, as they modify pH but do not act as true buffers. Systems challenged by decreasing pH may benefit from the addition of foam fractionation and better gas balance facilities. Foam fractionation and improved gas balance enabled SeaWorld Ohio, USA to maintain a pH of 8.0 in a 1,500 m<sup>3</sup> system. The system contained 25 large sharks, two adult sawfish, two sea turtles, and several hundred teleosts. Additions of sodium carbonate were required for this system, although they were infrequent (11.5-23 kg month<sup>-1</sup>). In general, a pH range of 8.0-8.4 is recommended for elasmobranch exhibits.

### Artificial seawater and salinity

It can be economical to make artificial seawater from scratch, but only if large quantities are needed. The reagent and analytical grade chemicals used to provide trace elements require an initial outlay of funds, but some of these small quantities will last for many years. Bulk quantities of major salts such as food grade sodium chloride, magnesium sulfate, magnesium chloride, calcium chloride, and potassium chloride are readily available.

Over the years the number of trace element components used in artificial seawater formulas has dwindled. Nickel, vanadium, titanium, rubidium, iron, boron, and other elements formerly added to general-use artificial seawater are now often omitted. Many of these elements are already present as contaminants in the major ingredients of artificial seawater. Infinitesimal quantities are probably present in tap water, or may enter systems via food.

Many facilities tailor their institutional salt formulas to meet special requirements. For example, phosphate (HPO<sub>4</sub><sup>2-</sup>) need not be added to established aquarium systems because it typically accumulates to unnaturally high levels through input via food. However, low levels are thought to be required by nitrifying bacteria during biofilter maturation and should always be included when an exhibit is initially filled.

Many aquariums have stopped adding bromide to their artificial seawater mixes because it reacts during aggressive ozonation (i.e., when ORP is >400 mV) to form long-term residual oxidants that are harmful to fishes. Although this is one of the most abundant minor elements, it does not appear to be required by marine fishes at the levels present in natural seawater.

The expected composition of seawater in a closed system can change as a result of interactions with LSS components or exhibit decoration. When a standard artificial seawater formula is mixed using hard tap water, the resulting calcium and magnesium levels may be higher than desired or found in natural seawaters. Institutional salt formulas can be easily adjusted to include less calcium and magnesium. Periodic testing of the artificial seawater in established systems may indicate an accumulation or depletion of some elements through salt contamination, product variation, mixing errors, biological activity, or the composition of local tap water. Special batches of artificial seawater tailored to correct these imbalances can be formulated and used during subsequent water changes.

The leaching of un-reacted construction materials can affect the composition of artificial seawater. Concrete rockwork is known to leach calcium into the water, while simultaneously causing the precipitation of both calcium and magnesium (Grguric et al., 1999). This precipitation is typically easy to correct, through minor adjustments to artificial seawater, if major seawater elements are checked periodically. Severe imbalances can

occur if concrete mixes are used that are incompatible with seawater. Despite assurances from the manufacturer that a product was suitable for marine applications, SeaWorld (Busch Entertainment Corporation, St. Louis, USA) discovered that one commercial concrete mix rapidly crumbled when exposed to seawater. One of the authors (Mohan) observed that calcium concentrations in system water increased to several times their normal value (i.e., >1,000 mg l<sup>-1</sup>) during experimental trials with the concrete.

Contaminated raw materials can sometimes cause problems in artificial seawater mixes. Unexpectedly high levels of manganese, nickel, ammonia, or cyanide compounds have been detected in low quality components supplied to aquariums. This problem can occur if: low-grade materials are purchased in error because of lack of research, orders are substituted by purchasing agents attempting to save money, or shippers accidentally send the wrong material. The use of specific raw materials, whose safety record has been proven by other aquariums, is a wise precaution.

While salinity tolerances for elasmobranchs are not well documented, examples of broad euryhaline activity in wild sharks have been observed. The bull shark (*Carcharhinus leucas*) is found at salinities of 0-53 g l<sup>-1</sup> (=ppt), while blacktip reef sharks have been found in brackish estuaries and freshwater lakes (Compagno, 1984). Both sandbar sharks and blacktip sharks (*Carcharhinus limbatus*) are common at the entrance of rivers, where they experience lower salinities, but neither species is known to ascend rivers into freshwater (Compagno, 1984). Merson and Pratt (2001) routinely collected young sandbar sharks in Delaware Bay at salinities as low as 22.8 g l<sup>-1</sup>. Similarly, cownose rays (*Rhinoptera bonasus*) have been found in lower salinity waters of the Chesapeake Bay. The species mentioned above can be maintained long-term at salinities of >25-26 g l<sup>-1</sup> (Thoney, pers. com.).

While freshwater dips have been routinely used for parasite removal on a variety of elasmobranchs (Henningsen, pers. com.), the New England Aquarium, Boston, USA, reported that yellow stingrays (*Urobatis jamaicensis*) and bamboo sharks (*Chiloscyllium* spp.) were extremely intolerant of freshwater; although it has been observed that the latter can be kept safely at 15 g l<sup>-1</sup> for extended periods (Bailey, pers. com.).

The Aquarium of the Pacific, Long Beach, USA, successfully acclimated sandbar sharks, blacktip

reef sharks, whitetip reef sharks, and zebra sharks to salinities as low as 14 g l<sup>-1</sup> (Clarkson, pers. com.). Most sharks exhibited a reduced interest in food during a slow acclimation over a one-week period. After one month, salinities were increased rapidly to normal levels. Two mortalities were observed; a juvenile sandbar shark and an adult whitetip reef shark. These unexpected deaths could have resulted from the rapid change in salinity, or the combined effects of the rapid change and long-term maintenance at a lower salinity.

Low salinities are not tolerated well by sand tiger sharks for extended periods of time. During aquarium startup, Moody Gardens, Galveston, USA, lowered shark exhibit salinity to 15 g l<sup>-1</sup> for 1.5 months and further reduced it to 11 g l<sup>-1</sup> for one week. Behavioral side-effects, including inappetence, lethargy, changes in ventilation, and a tendency to remain near the pool bottom indicated that long-term brackish conditions were unsuitable for sand tiger sharks. These signs disappeared when salt concentration was raised to >22 g l<sup>-1</sup> (Zoller, pers. com.). Introduction of freshly transported specimens to low salinity conditions appeared to be contraindicated. Sand tiger sharks are often collected at 25 g l<sup>-1</sup> in the Delaware Bay and routinely displayed in the same salinity at the National Aquarium in Baltimore (USA) and other facilities (Henningsen, pers. com.). Every attempt should be made to maintain elasmobranchs within the salinity ranges at which they naturally occur.

### Gas balance

The proportions of dissolved gases in exhibit water should approach normal atmospheric values. Respiration and photosynthesis within a LSS and display may impact oxygen (O<sub>2</sub>) and carbon dioxide (CO<sub>2</sub>) concentrations as well as their ratio to one another.

### Dissolved oxygen

Daily testing of dissolved oxygen (DO) levels is recommended for all systems. Under-oxygenation can occur, for a variety of reasons, in systems that otherwise appear to function normally. Where ozone contact chambers or degas towers are the primary sources of aeration, plugged or defective diffuser systems or venturis (=eductors) can lower oxygen concentrations. Reduced flow through these towers, as a result of a faulty or a stopped pump, can lower oxygen concentrations. Where

foam fractionators are an important source of aeration, pump or venturi failure can cause similar problems. This risk is reduced if multiple foam fractionators are used. Employing a biotower (i.e., a wet-dry trickle biofilter) as the primary source of aeration can reduce the risk of under-oxygenation. When water flowing through the biotower is mixed with ambient air, a constant source of oxygen and nitrogen is supplied at atmospheric pressures, excess carbon dioxide from fish and bacterial respiration exits the system, and gas levels in the water can return to natural atmospheric partial pressures.

Some species tolerate low oxygen better than others. For example, the sand tiger shark is more tolerant of low oxygen concentrations than the sandbar shark. At many facilities (e.g., SeaWorld Ohio) oxygen saturation levels are maintained at 85-95%. Some facilities choose to maintain oxygen saturation levels >100% (i.e., oxygen supersaturation only; nitrogen saturation must be maintained at, or below, atmospheric concentrations). These levels can only be achieved through heavy ozonation or oxygen enrichment.

### *Supersaturation*

Pressurized seawater can hold more dissolved gases than seawater at one atmosphere. When pressure is removed (e.g., as water moves from a pump into an exhibit), these gases become supersaturated and will gradually come out of solution. As supersaturated gases come out of solution in the blood-stream of fishes it causes emboli. The resulting condition, called gas bubble disease or the bends, causes tissue damage, organ failure, or death. Theoretically, oxygen and carbon dioxide can be supersaturated without harming fishes. However, emboli will develop when the sum of the partial pressures of all dissolved gases exceeds atmospheric pressure (www1). Nitrogen dissolves or leaves solution slowly by comparison to other gases. Although the total pressure of all dissolved gases determines whether supersaturation will occur, nitrogen is the most dangerous individual gas causing "bends" when it comes out of solution inside an animal. High concentrations of nitrogen (>110% saturation) should be avoided at all costs.

Various measurement devices can be used to evaluate total dissolved gas levels. Gasometers and satumeters measure total gas pressure (TGP; the sum of the partial pressures of

atmospheric gases measured in mm Hg) or dissolved gas tension ( $\Delta P$ ; the difference between TGP and atmospheric pressure), representing the force available to inflate bubbles or emboli and determine if gas bubble disease will occur at a given depth (Bouck, 1980). Manometer-based devices measure  $\Delta P$  directly, while those employing an electronic pressure transducer, calibrated to absolute pressure, measure TGP. Atmospheric pressure (pAtm) must be known to extract  $\Delta P$  values from TGP measurements as follows (www1):

$$\text{TGP} = \Delta P + \text{pAtm}$$

Most commercially produced gasometers are somewhat expensive. However, Bouck (1982) has described the construction of an inexpensive gasometer that will operate continuously and can be built from materials familiar to aquarists.

Data in the literature are often reported as percent total gas pressure (TGP%), or percent saturation. TGP% is calculated as follows:

$$\text{TGP\%} = (\Delta P + \text{pAtm}) \times (100 / \text{pAtm})$$

Saturation levels above 110% will usually cause gas bubble disease, while levels under 102% are typically safe for fish systems (Wedemeyer et al., 1976). Oxygen saturation values can be a predictor of nitrogen levels in systems where excess oxygen is not introduced (naturally or artificially), or where air entrainment is not occurring in oxygen-deficient parts of an LSS. The use of meters that measure total gas saturation is the only way to accurately determine whether nitrogen supersaturation is occurring (following a comparison with DO measurements). Independently acceptable TGP and oxygen saturation levels may hide nitrogen supersaturation capable of damaging fishes. Atlantic menhaden (*Brevoortia tyrannus*) are known to exhibit erratic swimming behavior at 95% TGP accompanied by 105% nitrogen saturation (Weitkamp and Katz, 1980).

Oxygen-specific supersaturation is relatively benign, but in outdoor ponds it can lead to hypercapnia and cause gill damage to fishes through the retention of excess carbon dioxide. This phenomenon is rare (as CO<sub>2</sub> diffuses rapidly) and is usually only observed in aquaculture ponds where algal photosynthesis dramatically increases dissolved oxygen levels (www2). Designers of outdoor elasmobranch systems should consider this problem when sizing gas-balance devices.

The symptoms of supersaturation may be subtle, or in serious cases, disturbingly obvious. Sand tiger sharks will develop rapidly spreading white patches on their skin as bubbles accumulate in their subcutaneous capillary beds. If caught immediately, this is a reversible condition. An effervescence at the surface of the water and unexplained bubble accumulation on algae-free walls or substrate are good indications that supersaturation is occurring.

Good system design and regular testing of dissolved gas levels is the best way to prevent nitrogen supersaturation events. The use of a biotower, with ambient air mixing, allows for the re-equilibration of any gas that may exceed its ambient partial pressure.

Water depth may offer some protection against supersaturation in closed systems. Weitkamp and Katz (1980) note that each meter of depth compensates for about 10% of near-surface saturation levels. Where 120% TGP is observed near the surface, the actual environment at two meters is closer to 100% saturation. This situation suggests that exhibit animals preferring upper regions of displays may become the first victims of supersaturation events, and in some cases seek refuge in lower parts of the exhibit.

Supersaturation can be caused by a number of equipment or design failures. A leak on the suction side of a pump is probably the most common scenario. Such leaks appear dry during system operation, but suck air into the system. These bubbles are then forced into solution by the high pressures within the pump. When the system is shut off, water often (but not always) leaks from the compromised piping.

Another common cause of supersaturation is entrained air bubbles that are forced into solution at a high-pressure area within LSS piping. Pumps, plumbing restrictions, and venturis are common problem areas. Improperly designed systems, where air bubbles can enter a water intake, can cause supersaturation events. Gravity sand filters are prone to this problem when float valves are employed to control water levels, as air may be sucked through the sand bed if the valve sticks open. Throttling valves placed between degas chambers and exhibits is another location where pressure drops occur and may cause supersaturation as a result of air entrainment through valve restrictions (Linn, pers. com.).

A less common source of supersaturation in closed systems is the introduction of bubbles at

depth. Waterfalls that cascade into deep pools, and diffusers or venturis located at the bottom of deep tanks or foam fractionators, can all result in supersaturation.  $\Delta P$  increases of 73 mm Hg are added for every meter of depth in plunge pools beneath dams or waterfalls (Colt, 1986). In general, air-stones or venturis can have similar effects on gas pressure (www1) depending on the depth at which they are located in a pool, foam fractionator, or ozone contact chamber. LaBonne (pers. com.) suggests that adding air at a depth of 1.8-2.4 m, the maximum depth of many commercial foam fractionators, is probably safe. However, fine diffusers may produce supersaturation when placed deeper than a meter, while coarse and medium bubble diffusers could cause gas saturation problems at depths exceeding two meters (www2). Since it may not be practical to inject air at depths less than a meter in many foam fractionators, provision for post-treatment gas balance should be made. Placing a degas chamber or biotower at the end of the process stream is an excellent way to protect systems against supersaturation.

Facilities using well water should be especially mindful of dissolved gas issues. There have been many reports of wells producing water with dissolved nitrogen levels of up to 180% saturation (www1).

The addition of gas-saturated cold water to a warmer system can cause supersaturation (Powell, pers. com.), as can rapidly heating or adding salts to saturated water (www2). Rapid heating of saturated water, starting at 0°C, can produce a  $\Delta P$  increase of 20 mm Hg °C<sup>-1</sup> (Colt, 1984), while water initially held at 15°C experiences a  $\Delta P$  increase of 15 mm Hg °C<sup>-1</sup> with rising temperature (www1).

A number of freak conditions that can result in supersaturation are often overlooked. Johnson (pers. com.) warns that systems on the verge of supersaturation can be pushed over the edge by the passage of a severe low-pressure storm front. It has been suggested (www2) that this phenomenon played a role in the death of millions of marine fishes during a hurricane in 1992. The passage of a storm front typically causes changes in barometric pressure of +5 to -17 mm Hg (Craig and Weiss, 1971). A 17 mm Hg decrease in barometric pressure produces a 17 mm Hg increase in  $\Delta P$ .

Variations in atmospheric pressure may affect dissolved oxygen measurements. A table of  $\Delta P$

vs. altitude (and associated atmospheric pressures) is available on the world wide web (www1). Rapid changes in altitude, pressure drops during take-off (Jewell, pers. com.), and re-pressurization after landing at a high altitude (Lerner, pers. com.) may all cause supersaturation in bodies of water carried aboard aircraft.

### Toxicants

Harmful materials can enter an exhibit in a variety of unexpected ways. As noted above, a variety of harmful compounds have occasionally been found in low-grade chemicals used to make artificial seawater. Human error is implicated in virtually all other situations that result in the introduction of toxic materials to aquarium systems. The improper application of protective coatings, poor fabrication of exhibit decoration, LSS component materials, and general building maintenance activities are usually implicated as the source of toxicants.

Properly designed and maintained systems are less likely to experience contamination by toxicants. Should a proactive approach to the management of toxic chemicals fail, the United States Environmental Protection Agency's Ecotox searchable internet database (www3) will help identify harmful levels of various substances. The National Institute of Health's National Library of Medicine hosts a similar site (www4).

#### *Volatile organic compounds (VOCs)*

Volatile organic compounds have been implicated in the mortality of sharks in new exhibits (Rasmussen and Garner, 1999). Appropriate planning for any painting or coating activities in and around exhibits will minimize organic chemical contamination. Floor sealants, paints, and adhesives should always be evaluated before use. Every attempt should be made to use products that have been successfully applied elsewhere or tested on sample fishes. Ironically, materials suitable for drinking water may be unsuitable for aquarium use (Atz, 1970). For example, a polysulfide-based material, formulated as a liner for potable water tanks, was tested by one of the authors (Mohan) and found to be lethal to goldfish (*Carassius auratus*).

Epoxy compounds used to manufacture artificial coral and other tank décor can be toxic if the mix ratio is incorrect. Even fresh, correctly made

corals can be expected to release volatiles. It may be wise to place recently manufactured corals in direct sunlight for days or weeks before use. Pre-filling new exhibits with freshwater, which is discarded before the final fill with seawater, is another worthwhile preventative measure. Filling, flushing, circulating, and dumping are advisable for any open or semi-open systems. Where natural seawater is abundant it makes sense to use this cheap natural resource for the initial fill. The precautionary use of activated carbon during initial operation of the LSS is recommended.

#### *Concrete alkalis*

New concrete, especially uncoated surfaces such as rockwork, will typically leach alkalis. There are many opinions as to how to neutralize these leachates (Choromanski, pers. com.). Some workers simply pre-fill the exhibit with domestic freshwater for 5-10 days, drain it, refill with seawater, and then add acid to bring pH into normally expected ranges (i.e., 8.0-8.4). Others reduce pH to 3.0-5.0 during the initial freshwater bath for 1-45 days. Muriatic acid is the most commonly used pH reducer. Concrete type and volume, as well as system type (i.e., open, closed, etc.) may determine which of these methods should be used.

Inventors of the reef ball (Reef Ball Development Group, USA), an artificial reef module that is widely used in natural waters, suggest a more proactive method of neutralizing alkalis. Micro-silicates added to a Portland II concrete mix reacts with excess calcium hydroxide and results in a finished product having a pH of 8.3 (Barber, pers. com.; www5). While the addition of micro-silicates increases concrete cost, it minimizes the need for post-construction pH adjustments. Its use should be considered for systems that incorporate large volumes of concrete rockwork.

#### *Metals and metalloids*

Harmful metal ions can be introduced into closed aquarium systems if equipment containing inappropriate materials is specified during LSS design. Antifouling paints intended for watercraft contain copper, other metals, or other poisons and should never be used in aquarium systems.

Metals such as lead, copper, zinc, and nickel are generally less toxic in marine systems than in freshwater, largely because of the protective effect

of high pH, hardness, and alkalinity (Sorensen, 1991). Although significant amounts of dissolved metals precipitate at the high pHs typical of marine systems, the corrosive effects of saltwater can make metal accumulation from LSS equipment or other hardware a serious problem. Any large metallic LSS components placed in direct contact with seawater should be made of titanium. Metal contamination of aquarium seawater is not a serious concern in systems where water is continuously replaced with raw seawater. Stainless steel pumps with magnesium cathodic protection have been used successfully in such situations (Powell, pers. com.).

Elevated levels of nickel have occasionally been attributed to the use of nickel-copper alloys (e.g., Monel<sup>®</sup>, Inco Corporation, USA) that may be used in valve components and pump shafts. While these alloys are recommended for marine applications they have been reported to cause elevated nickel concentrations in closed systems (Davis, pers. com.). Certain grades of stainless steel can be a source of nickel and other heavy metals (e.g., chromium, etc.). Davis (pers. com.) observed that threaded metal rods used to secure the lid of cartridge filters were a source of nickel contamination for small aquariums at SeaWorld Orlando. Nickel contamination was successfully removed using activated carbon.

High zinc levels in closed systems can usually be traced to the inappropriate use of sacrificial zinc anodes in rapid sand filters. Aluminum or magnesium-based anodes should be used in closed-system seawater LSSs. The immersion of any galvanized materials will lead to zinc contamination. Hughes (1968) observed that 100% of striped bass (*Morone saxatilis*) fingerlings survived 96 hours at 0.05 mg l<sup>-1</sup> of zinc, but half died within 48 hours at 0.10 mg l<sup>-1</sup> of zinc. Brungs (1969) reported that 0.18 mg l<sup>-1</sup> of zinc inhibited reproduction in fathead minnows (*Pimphales promelas*) but did not affect growth or maturation. Gill damage occurred at 0.80 mg l<sup>-1</sup> of zinc in rainbow trout, *Oncorhynchus mykiss* (Brown et al., 1968). There is evidence to suggest that gender may affect elasmobranch susceptibility to zinc poisoning. Crespo et al. (1979) reported that male smallspotted catsharks (*Scyliorhinus canicula*) accumulate up to three times more zinc in their gill arches than females. Zinc intoxication compromises equilibrium and damages gills, kidneys, liver, and muscle tissue (Sorensen, 1991). Magnesium competes with zinc and may be somewhat protective in hard water (Zitko and Carson, 1976), especially in marine systems.

Copper contamination typically results from errors in LSS design or repair. The use of copper or brass fittings in LSS piping can produce elevated copper levels in closed systems (Atz, 1970). The accidental use of pumps with bronze components can cause copper contamination. Coating copper LSS components will not dependably protect specimens from intoxication. Stonecypher et al. (1992) observed that vinyl-coated copper chiller coils leached dangerous levels of copper (i.e., exceeding therapeutic levels) in little more than a week. Copper contamination can come from unexpected sources. One of the authors (Mohan) has observed a cracked glass submersible heater continue to operate while flooded and found that it shed copper ions into the surrounding water. Some facilities (e.g., the Pittsburgh Zoo and Aquarium, Pittsburgh, USA) are supplied with domestic freshwater from reservoirs containing seasonally toxic levels of copper (Billin, pers. com.). New facilities should thoroughly test their water supply before it is used to make water exchanges in animal exhibits. Unlike exposure to zinc, the difference between safe and fatal copper concentrations is quite small (Brungs, 1969).

Iron is unlikely to be a problem in marine systems unless highly contaminated well water is piped directly into an occupied exhibit. Iron precipitates in alkaline seawater (Anon, 1972) and the iron hydroxide flocs are generally removed by mechanical filtration. At SeaWorld Ohio iron removal was accomplished using a water softener that improved iron-laden well water from 3.0-5.0 mg l<sup>-1</sup> to  $\leq 0.30$  mg l<sup>-1</sup>. This water was considered acceptable for makeup and was routinely used for water exchanges in freshwater systems. The tendency for iron to precipitate in seawater means that small amounts of exposed rebar should not be viewed as an important source of iron contamination.

Arsenic contamination is a potential concern when chronic leaks occur in roofs constructed from chemically treated lumber containing chromated copper arsenate (CCA). Ash from burnt CCA lumber is highly toxic: one tablespoon contains a lethal human dose. The construction of outdoor shade structures using CCA-treated wood is contraindicated unless these shelters are designed to minimize the introduction of condensate and rainwater into system water. Fly ash collected by electrostatic precipitators on coal-burning power plants contains high levels of arsenic. The no-effect level for fish exposure to disodium arsenate is <1.0 mg l<sup>-1</sup> (Sorensen, 1991). Arsenic is known to replace phosphate in normal

metabolic reactions, interfering with respiration and other processes. Death usually occurs as a result of excess mucus production, especially on the gills, and subsequent suffocation (i.e., coagulation film anoxia).

Interactions between different metals are a concern, especially those involving copper, zinc, and perhaps nickel, all of which may be found at low levels in most marine aquarium systems. In laboratory studies using highly toxic concentrations of metals, a synergistic effect between zinc and copper has been reported (LaRoche, 1972). At the doses of metals likely to be encountered in aquariums, the effects of copper and zinc are probably additive, rather than synergistic (Lloyd, 1961). The presence of excess zinc in a quarantine system will therefore increase the toxicity of copper added as a therapeutic. For this reason, we recommend that metal levels in aquariums be checked before the initiation of any copper therapy. The removal of excess heavy metal ions prior to copper addition can be accomplished using activated carbon (Davis, pers. com.). It may be wise to discontinue the use of vitamins and trace element solutions containing heavy metals during such treatments.

### *Chlorine*

Shocking system water with chlorine, to sterilize the water or reduce nitrogen levels (i.e., through breakpoint chlorination), should be avoided where possible. Incomplete neutralization of chlorinated water introduces toxic chlorine into elasmobranch systems. Less well known is the danger of using excessive amounts of sodium thiosulfate to neutralize chlorinated or chloramine-treated water. Reactions resulting in dangerously low pH and dissolved oxygen levels have been observed in systems where residual thiosulfate is present (Linn, pers. com.). Chen (1974) notes that slightly acidified dilute solutions of thiosulfates decompose to sulfite, free sulfur, and polythionate. Sulfur formation is signaled by the precipitation of a milky-white colloid.

If chlorinated or chloramine-treated city water is used for large water exchanges, it should be passed through a carbon filter prior to use. If chlorine alone is present, aeration can be employed for its removal (Wheaton, 1977). The judicious use of chlorinated tap water during routine pressure-washing of rockwork is usually not harmful. For example, two pressure washers (DSL 4200E, Clarke-Delco, USA) were routinely

used to clean the Shark Experience at SeaWorld Ohio adding 3.6 m<sup>3</sup> (i.e., <0.25% exhibit volume) of chlorinated (i.e., without chloramines) domestic water to the exhibit. These cleaning sessions occurred on a weekly basis and lasted for two hours duration.

### **ORP (Oxidative Redox Potential)**

As a chemical process, reduction-oxidation (redox) reactions are those in which electrons are transferred back and forth between chemical species. An oxidized species is one that donates an electron, a reduced species is one that accepts it. When a pathogen is oxidized a chemical component in its cell wall gives up an electron. When sufficient electrons have been lost, the cell's functions deteriorate or the cell wall disintegrates and eventually the organism is killed. Water that has been treated with an oxidant such as chlorine or ozone has a greater opportunity to allow these kinds of reactions to occur. ORP (oxidative redox potential) is a measure of the potential for oxidation-reduction reactions to take place and is measured in millivolts (mV).

Cohrs (2002) provides a good introduction and reference to ORP. Informally, ORP is used in the industry to describe the relative cleanliness of seawater. Raw or new artificial seawater has an ORP of ~250 mV. When aquatic animals and food are added to this water, ORP will drop further (i.e., the opportunity for redox reactions to occur will decrease). Therefore, pathogens and other unwanted chemical species are less likely to be oxidized. Conversely, when an oxidant such as ozone or chlorine is added to the water, ORP rises and the likelihood of pathogens and other unwanted chemical species being oxidized increases.

Different contaminants require different levels of oxidation for destruction. Many bacteria will be killed at an ORP as low as 400 mV, while other micro-organisms require an ORP as high as 800-900 mV and exposure for several minutes before they are destroyed. Unfortunately, an ORP of 800 mV is extremely unhealthy for the aquatic species on display. Even an ORP of 450 mV is harmful if exposure lasts for more than a few hours. Therefore, if oxidants (e.g., chlorine, ozone, etc.) are applied, they must be applied with a strategy to return oxidized water to the exhibit at a low ORP and toxic residual oxidants must be absent. The safe upper limit for ORP within an exhibit is generally regarded to be ~380 mV or less. On

the other hand, an ORP of  $\leq 250$  mV typically indicates an aquatic environment containing excess dissolved organics or other unwanted constituents.

## LIFE SUPPORT SYSTEM COMPONENTS

### Water supply

Water supplies for recirculating aquarium LSSs can be classified into three basic types: (1) flow-through systems; (2) closed systems; and (3) semi-closed (or semi-open) systems.

#### *Flow-through systems*

Flow-through systems receive a constant flow of new seawater that continually displaces water recirculating in the tank. The volume of influent replacement water (measured per day), compared to the total system volume, defines the percentage of flow-through. For example, 150 m<sup>3</sup> of new water added to a 1,000 m<sup>3</sup> exhibit each day is equivalent to a 15% flow-through or blow-down. These systems are typically outfitted with minimal water treatment, often limited to mechanical filtration, relying instead on the steady flow of replacement water to maintain water quality. Although, theoretically, the water source could be artificial, large elasmobranch systems usually draw new water from a natural source.

#### *Closed systems*

At the other end of the theoretical spectrum are closed systems, which by definition never undergo any water change or receive any replacement water. In reality, these systems do not exist. The replacement of losses resulting from backwash recovery, or evaporation, prevents any system from ever being truly closed.

#### *Semi-closed systems*

Semi-closed systems are the most common. Although these systems are not provided with a steady flow of replacement water, they do receive periodic water changes (natural or artificial) in batches. The volume and frequency of water changes vary greatly from system to system, and depends largely on system design and stocking levels. Aquariums with semi-closed systems designed to receive large contributions of natural

seawater are sometimes supplied with under-engineered LSSs. These systems may prove to be inadequate during emergency periods when they must operate closed (LaBonne, pers. com.).

The maximum time a semi-closed system may be required to operate closed should be clearly quantified during LSS design. To achieve this goal, system design should be based on defined biological loads. Where natural seawater is used, the expected need for nitrification and gas balance should be weighed against historical data for quality of source water. If interruptions in source water are common, the exhibit may need to be designed as a fully functional closed system.

### Mechanical filtration

If no provision for particulate removal is made, water clarity will be severely compromised, particularly for systems with long sight-lines.

Bubble-bead filters trap particulates but are designed for high-load systems (e.g., high density aquaculture) and are not as effective for particulate removal in low-load systems (i.e., a typical elasmobranch exhibit). Diatomaceous earth (DE) filters were extremely common in large systems built for marine mammals in the 1960's, but are infrequently used today because of operational difficulty (i.e., rapid soiling creating low flow conditions) and hazardous material concerns (i.e., from ultra-fine DE powder). Gravity sand filters have been widely used in Asian facilities. While effective at removal of particulates, gravity sand filters tend to be labor intensive, are prone to organic fouling, and require as much space as the exhibits they serve (Gendron, pers. com.).

Rapid sand filters are the most appropriate and most common form of mechanical filtration used for large elasmobranch systems. Rapid sand filters are designed to trap particulate material, improving water clarity, but are not capable of removing dissolved organic molecules. Foam fractionators will remove both dissolved organics and particulates. However, foam fractionators are not as efficient as rapid sand filters at removing all types and sizes of particulate contaminants. Thus, acting alone, foam fractionators may not produce sufficient clarity for large elasmobranch exhibits.

In general, a disadvantage of mechanical filters is that water is continually filtered through

contaminants trapped in the media (i.e., sand, diatomaceous earth, beads, etc.). Trapped accumulated particulates will release organic carbon back into solution and thus system water. Films of heterotrophic bacteria consume the hydrolyzed carbon, release carbon dioxide, and thereby lower system pH (Hovanec, 1995). Thus, although rapid sand filters are the most appropriate means for particulate removal for elasmobranch systems, some provision for facilitating gas exchange and removing dissolved organics must be considered.

There are two ways to alleviate the negative effects of trapped contaminants in mechanical filters. First, perform filter backwashes (or exchange media) on a timed interval rather than relying on the cue of pressure differentials. Employing this type of schedule will reduce the period of time that water is flowing through increasing amounts of trapped contaminants. Second, incorporate foam fractionators and/or biotowers (equipped to allow the mixing of ambient air with system water) into the LSS. If no provision is made for ambient air mixing or dissolved organics removal, the signs of accumulated  $\text{CO}_2$  and dissolved organics will begin to occur—i.e., pH and ORP will have a tendency to drop. Adequate ventilation is an important component of any gas-balance device (Powell, pers. com.). These principles have been successfully applied at SeaWorld Ohio for the Shark Encounter exhibit, where each of the four sand filters (surface area  $10.2 \text{ m}^2$ ) were operated on a staggered 140-hour backwash cycle. This schedule is a compromise between daily backwashing to remove organic solids, and a longer interval to optimize water clarity. Sand filtration for the exhibit has been used in conjunction with foam fractionators, resulting in acceptable water clarity, a stable pH of 8.0, and minimal nitrate ( $\text{NO}_3^-$ ) accumulation. If no provision is made for the removal of dissolved organics and ambient air mixing, signs of both accumulated organo-carbon compounds and carbon dioxide will be evident (i.e., decreasing pH and ORP).

Highly loaded rapid sand filters (e.g., rapid sand filters for stingray pools where the public is encouraged to feed the animals) should be emptied and re-bedded periodically. Lightly loaded filters may not become fouled, but need to be re-bedded at some point in their operational life as media gradually wears away during backwashes (Linn, pers. com.).

### Biological filtration (nitrifying filters)

Nitrifying bacteria form biological films on filter media and oxidize the accumulated toxic nitrogen compounds, ammonia ( $\text{NH}_3/\text{NH}_4^+$ ) and nitrite ( $\text{NO}_2^-$ ), into nitrate. This process is known as nitrification. Feeding rates (i.e., amount of food added per day) will determine initial ammonia production, and minimum biological media requirements can be calculated if ammonia conversion rates for specific media are known.

Hovanec (1995) contains a good review of the various types of biological filtration currently in use. Rapid sand filters provide nitrification in most traditional systems. Other LSSs may have under-gravel filtration, or dedicated wet-dry trickle biological filters or biotowers.

Reverse flow under-gravel filtration is practical if clean, filtered water can be delivered to the substrate bed. Many under-gravel filtration systems, constructed with sand beds poured directly over a sparger (i.e., a network of water distribution pipes), have become rapidly fouled because of inefficient and uneven water distribution and shifting substrate. When buried sparger systems are used, non-calcareous pea gravel should be employed to cover the pipe network before sand beds are poured. During the construction of their elasmobranch exhibit, SeaWorld Ohio installed a perforated FRP (fiber-reinforced polyester) plate above the LSS sparger before a uniform layer of substrate was poured. The sand bed within this dependable design did not require cleaning during its 11 years of operation.

Nitrification produces carbon dioxide (as does elasmobranch respiration) so any LSS must include provisions for gas exchange or system pH will be depressed. Biological filters are susceptible to fouling with organic matter, channeling (i.e., short-circuiting) through densely packed media, and oxygen depletion because of microbial respiration. The use of biotowers is a good way to mitigate these negative effects. Biotowers designed with specific hydraulic flow rates allow water to pass in thin films over media surfaces. At ambient pressures this process facilitates a highly efficient exchange of carbon dioxide and oxygen, returning dissolved gases to natural levels, and helps relieve the negative effects of biodegradation in sand filters. Where possible, biotowers are best placed after some type of mechanical filtration component (e.g., foam fractionators or rapid sand filters) to

minimize fouling. Biotowers are a critical addition to systems where sand filtration has been reduced to a side stream process as they provide both nitrification and gas exchange.

#### *The effects of therapeutics on nitrification*

Hawke (1991) reviews the effects of therapeutic agents on biological filtration systems. Although copper sulfate is rarely used in systems with elasmobranchs, it is worth noting that copper sulfate can affect LSSs in unpredictable ways. Copper treatment can provoke a spike in ammonia concentrations, even in systems that have been treated without incident in the past. One of the authors (Mohan) has observed this phenomenon in two systems at SeaWorld Ohio, and Roger Klocek (pers. com.) reported a similar incident at the John G. Shedd Aquarium, Chicago, USA in the 1980's.

Antibiotic bath therapy is rarely used for large elasmobranch systems or quarantine pools, but it may be practical for smaller systems. Gentamycin, nifurpirinol, sulfamerazine, tetracycline, and trimethoprim appear to have little or no effect on biological filtration (Hawke, 1991). However, other antibiotics (e.g., chloramphenicol and erythromycin) may seriously inhibit nitrification.

Praziquantel (Sigma, USA), an antihelminthic treatment, has recently been used to treat large systems at the Epcot Center's Living Seas Pavilion (Orlando, USA), SeaWorld Ohio, and Omaha's Henry Doorly Zoo (Nebraska, USA). Concentrations of  $\leq 2$  mg l<sup>-1</sup> praziquantel were applied for more than one week and no adverse effects on biological filtration were observed.

#### **Denitrification**

Over time, nitrification leads to the accumulation of nitrates. Although the toxicity of nitrates is low, they should not be permitted to reach high concentrations in an elasmobranch system. The biological process of removing nitrates is called denitrification and relies on either heterotrophic or autotrophic bacterial populations.

Heterotrophic denitrification systems require a highly concentrated source of organic carbon as an energy supply for the anoxic, reducing bacteria. The concentration of organic carbon in elasmobranch LSSs is not adequate to support the heterotrophic bacteria required to reduce

nitrate concentrations. An additional source of organo-carbon (e.g., methanol) must be injected prior to the denitrification reactors. Although heterotrophic systems are more efficient than autotrophic systems, they are inherently difficult to operate because of the heavy biomass generated. Biological films clog and foul pipes, valves, flow meters, and other instrumentation within the denitrification system. Operators must frequently dismantle the system in order to prevent bio-fouling which may result in plumbing obstructions and flooding. For these reasons, heterotrophic systems are labor-intensive. In addition, many state fire codes require special handling and safety procedures for the use of methanol. Aiken (1995a) reports on a successful heterotrophic denitrification system built at the National Aquarium in Baltimore.

Autotrophic denitrification systems typically use sulfur as an energy source for the autotrophic denitrifying bacteria (i.e., *Thiobacillus* spp.). Autotrophic systems are less efficient than heterotrophic systems, but the process is simpler, less expensive, and less labor-intensive. Nitrate reduction occurs while sulfur is oxidized to sulfate. The resulting sulfate does not seriously increase sulfate concentrations within the LSS, however the resulting acid production does reduce system alkalinity. Low pH and high DO levels decrease filter efficiency by promoting nitrite accumulation (Zhang and Lampe, 1999). Thus, buffering media such as oyster shells or limestone must be added to an autotrophic denitrification system in order to maintain pH. Buffering material can either be incorporated into the sulfur reactor or added as a second component following the reactor. Furumai et al. (1996) suggest maintaining a pH above 7.4 within the denitrification vessel. Zhang and Lampe's (1999) study suggests that optimal results can be obtained using sulfur:limestone ratios of between 1:1 to 3:1 and a hydraulic retention time (i.e., the time required for a volume of water, equivalent to the size of the reactor, to pass through the reactor) of 3-10 hours. Zhang and Lampe (1999) found that autotrophic denitrification will proceed in both aerobic and anaerobic conditions, although oxygen appeared inhibitory to the process.

The first sulfur-based denitrification system employed in a public aquarium was started in 1993 at the MAAO (Musée National des Arts d'Afrique et d'Océanie) Aquarium in Paris, France. This system was installed on a 60.6 m<sup>3</sup> exhibit with a resultant reduction of nitrate from >300 mg l<sup>-1</sup> to <10 mg l<sup>-1</sup>. No negative

water quality sideeffects were observed (Hignette et al., 1997). Successful sulfur denitrification systems have been employed at the London Aquarium (UK), Tennessee Aquarium (Chattanooga, USA), SeaWorld Orlando, Discovery Cove (Orlando, USA), and the National Aquarium in Baltimore.

High biomass and/or organo-carbon concentrations typically characterize the effluent of denitrification systems. By passing denitrification effluent through foam fractionators the vast majority of organic loads will be captured and removed, preventing its release into system water. Existing foam fractionators may be employed for this purpose, or an additional foam fractionator dedicated to the denitrification plant can be used.

#### *Algal turf scrubbers*

Another means to remove nitrogen contaminants is the use of plants to naturally consume excess nutrients. Algal turf scrubbers rely on rapid growth of cultivated algae, the associated uptake of nutrients, and subsequent removal of the alga biomass to reduce nutrient levels. Reef HQ in Townsville, Australia uses both foam fractionation and frequent small water changes in conjunction with their algal turf scrubbers, and much of the harvested algae is disposed (Czaja, pers. com.). In its pure research form, this type of biological filter has been used without the export of harvested algae and protein (Adey and Loveland, 1991). As a result, experimental systems at the Smithsonian Institute (Washington, USA), the Pittsburgh Zoo (USA), and the St. Louis Zoo (USA) developed an increasingly yellow coloration over time. While algal turf scrubbers are effective at removing nutrients from system water, maintenance of the scrubbers is labor-intensive and large numbers of units would be required for a typical elasmobranch system.

#### **Foam fractionation (protein skimming)**

Foam fractionators (also known as protein skimmers) remove organic contaminants from solution while at the same time providing some disinfection via the removal of bacteria that are trapped in the skimmed foam matrix (Conway and Ross, 1980). Foam fractionators work by taking advantage of the natural attraction of surface active organic compounds to air bubbles. Air bubbles are injected into the bottom of a reactor

and as dissolved organic compounds continue to collect on the surface of the bubbles, the bubbles become stickier, stronger, and coalesce into a wet foam. The foam matrix is thus formed by the adsorption of surfactants onto the surface of the bubbles and by micro-flocculation. Foam fractionators are typically equipped with a cone and chimney designed to allow rising foam to condense and exit the system as it overflows out of the stack. The addition of relatively small doses of ozone will enhance coagulation and increase the removal of dissolved organics and bacteria.

Foam fractionation provides significant benefits to water quality, including: increased pH and a reduced dependency on buffers, increased DO concentrations, increased redox potential, improved water clarity, reduced turnover rates, reduced dependency on sand filters, and reduced frequency of sand filter backwashes (Aiken, 2000).

It is important to maintain a high air:water ratio in order to obtain maximum performance from a foam fractionator. A 1:10 air:water ratio is considered ideal when bubble size is  $\leq 0.5$  mm (Rozenblum and LaBonne, 1995). Restricting airflow to the venturi through the use of a valve will decrease the volume of air entering the venturi and decrease bubble size. The result is an overall increase in the total surface area of bubbles within the foam fractionator and an increase in operational efficiency. Bubble size, contact time (i.e., bubble retention within the water column), ozone dosage rates, and foam removal are other factors that will affect the design, size, and efficiency of a foam fractionator (Rozenblum and LaBonne, 1995).

Foam production rates appear to depend on the relative concentration of organics and antifoaming agents. The foam fractionators at Discovery Cove vary in efficiency according to a diurnal cycle that may be related to the input of body oils and suntan products from human bathers. These products appear to collapse foam columns when the exhibit is in peak use. Maximum foam production is seen during non-public hours presumably after antifoaming agents have been broken down.

One disadvantage of foam fractionators is that they require relatively frequent adjustment to ensure proper operation, unlike pressurized components such as rapid sand filters that can be left to operate without adjustment. Optimum operation can be obtained by ensuring that water

flow to the unit varies as little as possible. Stabilizing water flow to a foam fractionator can be accomplished by providing dedicated pumps that draw water directly from the exhibit, supplying water from a header tank with fixed water levels, using modulated pumps linked to level sensors within the fractionator, etc.

Supersaturation may become a problem if bubbles are allowed to exit the foam fractionator and enter a pressurized process. LaBonne (pers. com.) suggests that situations where bubbles are accidentally re-circulated through a pump and venturi should be avoided. If a fractionator discharges directly into an exhibit, pressure differentials across the venturi of  $\leq 103$ - $117$  kPa are recommended. Usually this means that the venturi intake pressure should be  $\leq 138$  kPa (LaBonne, pers. com.).

### **Flocculating agents and other additives**

The use of the strong flocculent aluminum sulfate (alum) has generally been avoided in fish systems. However, injection of alum prior to a mechanical filter may make limited use of this material possible as the floc is captured and then removed by backwashing. Organic, chitin-based flocculants, such as Sea-Klear (Vanson HaloSource, USA), are safer alternatives to alum but may be somewhat less effective. Recently, a number of facilities have used lanthanum chloride (ZeroPhos™, Vanson HaloSource, USA) to reduce phosphate levels, providing some relief from excessive algal growth, especially in outdoor exhibits. Ferric chloride has been used successfully for large elasmobranch systems, injected in-line before rapid sand filtration (Smith, pers. com.).

### **Ozone**

Tri-atomic oxygen, or ozone, is a strong oxidant and useful tool for the treatment of water in large elasmobranch systems. When used correctly ozone can contribute to superior water quality, when used incorrectly it can result in the mortality of valuable specimens.

Throughout this section, it is important to remember the dual role of ozone as both a flocculent and disinfectant. When the ozone:organics ratio is low (i.e.,  $\text{g l}^{-1}$  ozone  $\ll$   $\text{g l}^{-1}$  dissolved organics), ozone will act as a flocculent, destabilizing dissolved organic molecules in suspension and bringing

about their coalescence. This process leads to the formation of a foamy mass at the water surface. As the ozone:organics ratio increases the flocculating properties of ozone are lost, giving way to direct oxidation and disinfection. There is a transition range where both effects can occur and thus, depending on the applied dose and organic load, ozone can function as a flocculent, a disinfectant, or both.

#### *Micro-flocculation*

When used in conjunction with a foam fractionator, flocculation removes unwanted dissolved organic molecules and other particles caught in the foam matrix. Removal of the foam matrix is important since dissolved organics will consume DO and depress pH. Thus, micro-flocculation results in higher ORP, higher pH, and clearer water.

It is important to tailor ozone dosing rates to achieve the intended function. Applying disinfection doses of ozone in foam fractionators will result in poor foam formation and possibly an unsafe concentration of ozone in the surrounding environment (e.g., the pump room). Micro-flocculation typically requires only  $0.03$ - $0.10$   $\text{mg l}^{-1}$  of ozone, although different systems may require slightly different ranges (Rozenblum, pers. com.).

When ozone dosing favors flocculation, foam will collect on the water surface inside ozone contact chambers, as well as the interior of foam fractionators. Unfortunately ozone contact chambers have no way to discharge the foam and early designs were outfitted (or retrofitted) with sprayers designed to liquefy the foam so that it would not accumulate. This process is not recommended since it effectively puts unwanted organic molecules back into solution.

#### *Disinfection*

While disinfection through the removal of microorganisms does occur in foam fractionators, not all pathogens, viruses, and bacteria are of suitable dimensions to be harvested in the foam matrix. More complete sterilization of water for large elasmobranch exhibits may be achieved through the use of high concentrations of ozone applied in ozone contact chambers. The disinfection capacity of ozone contact chambers is greater and more cost effective than sterilization with ultraviolet light (UV), especially for large elasmobranch systems (Aiken, 1995b).

Ozone contact chambers are designed specifically for water sterilization whereby system water is subjected to high doses of ozone and very high ORP (i.e., 800-900 mV) for several minutes. In this environment, a high kill ratio is achieved (i.e., >99%) as microorganisms are destroyed via cell lysis. This kind of disinfection is not possible in foam fractionators. Conversely, collection and removal of dissolved organic molecules through micro-flocculation are not effectively achieved in ozone contact chambers (Aiken, 2000).

Sterilizing concentrations of ozone typically start at ~1.0 mg l<sup>-1</sup>, with an application range of 0.3-1.5 mg l<sup>-1</sup> (Rozenblum and LaBonne, 2001). Contact chamber design has a significant impact on the dose required for sterilization. Disinfection should occur in sealed contact chambers preventing high concentrations of ozone from escaping into the surrounding environment. Ozone destruction units and proper ventilation are mandatory.

Microorganisms destroyed in ozone contact chambers are not removed from the LSS. The biomass remains in solution to be assimilated into biological films, consuming DO, and releasing carbon dioxide via degradation and microbial respiration. Incorporating foam fractionators into the LSS can mitigate these effects.

#### *ORP monitoring and control*

If ozone is used as a disinfectant in an ozone contact chamber (or for that matter as a micro-flocculent in a foam fractionator) it is recommended that ORP control be used. The higher dosages of ozone required for disinfection means that ORP readings can rapidly rise to dangerous levels. Poorly controlled ORP threatens fish health and can be responsible for elasmobranch mortality.

An informed understanding of the relationship between ORP, ozone, and residual oxidants is necessary for any curator, aquarist, or LSS operator who uses ozone on an elasmobranch system. When an operator is experiencing problems or is unfamiliar with an ozonation system, we recommended a cautious approach be applied until it is possible to operate the system correctly. Advice from experienced operators MUST be sought. Cohrs (2002) provides an excellent introduction and reference to ORP and how ozone affects ORP in aquarium exhibit waters.

#### *Residual oxidants*

Ozone acts as molecular O<sub>3</sub> at pH <7.0 and undergoes a decomposition reaction yielding hydroxyl radicals, the primary oxidants, at pH >9.0. Between a pH of 7.0 and 9.0, ozone exists in a transition range where both forms are present.

During seawater hyper-ozonation, and the associated elevation of ORP, a whole family of residual oxidants is produced. One of the better known residual oxidants is hyperbromous acid (HOBr), formed when bromide is oxidized by ozone. In natural seawater, there is a high concentration of bromide (~67.0 mg l<sup>-1</sup>) available to be oxidized. The germicidal activity of HOBr is analogous to that of hyperchlorous acid (HOCl) and it actively participates in disinfection (Johnson, 2001). Hyperbromous acid is extremely toxic to fishes and under no circumstances should it be allowed to enter an exhibit with elasmobranchs.

There are two general approaches to protecting elasmobranch exhibits from overexposure to ozone residuals or high ORP:

1. Mix high ORP water (e.g., from an ozone contact chamber) with sufficient non-oxidized water to give a final combined ORP of ~350 mV. Usually a 10:90% or 20:80% ozonated:non-ozonated ratio is used. In this case, residual oxidants are consumed by organic molecules in the untreated portion of the water, once the waters are re-mixed.
2. Pass ozone contact chamber effluent water through an activated carbon filter. Activated carbon will consume residual ozone (or other oxidant) molecules left in solution.

Both strategies have been successfully used in public aquariums for many years. While the latter method is much safer, it occupies more floor space and implies greater capital and operational expense.

ORP does not directly measure the concentration of ozone-related oxidants and should never replace direct testing for potentially toxic residual oxidants (e.g., ozone, bromine, hyperbromous acid, etc.). A total DPD chlorine test (Hach Company, Loveland, Colorado, USA) provides a good qualitative measure of residual oxidants and a modified indigo method, as described by Chiou et al. (1995), is often used for quantitative ozone analyses.

Should residual oxidants enter an elasmobranch exhibit it is possible to neutralize them quickly

using sodium thiosulfate. Approximately one milligram of sodium thiosulfate is capable of eliminating a similar amount of ozone oxidants in seawater prepared with natural levels of bromine (Hemdal, 1992). Emergency additions of sodium thiosulfate should be preceded by preparations for rapid pH changes and followed by continuous pH monitoring.

### **Effluent treatment**

Designers of elasmobranch exhibit LSSs should recognize that regulations governing the disposal of waste seawater vary widely. It is incumbent upon the designer to know the regulations governing their region and specify the LSS accordingly. In some cases, seawater disposal regulations can be extremely stringent. For example, SeaWorld Orlando is required to operate reverse osmosis plants to concentrate effluent from its closed systems and thus avoid the disposal of large volumes of wastewater from backwashes, etc.

### **Monitoring and record keeping**

A thorough water monitoring protocol should be implemented and exhibit water should be rigorously screened for critical parameters (e.g., DO, temperature, ORP, residual oxidants, etc.) on a regular basis. The importance of comprehensive water monitoring, record keeping, and a LSS evaluation program cannot be overemphasized.

## **LIFE SUPPORT SYSTEM PHILOSOPHIES**

A number of paradigms for the LSSs of large elasmobranch systems have been created over the years. Existing LSSs tend to fall into one of these basic models. The rapid sand + ozone model was used by most facilities constructed during the 1980's and early 1990's in the USA. In the past decade, however, alternative LSSs favoring foam fractionation, denitrification, and other technologies have been added to the mix.

### **Rapid sand filter + ozone contact chamber**

Typically rapid sand + ozone systems were designed to recycle exhibit water every 60-90 minutes, a rule of thumb that has been in use for at least 30 years (Hagen, 1970) (Figure 6.1). A number of newer designs use faster turnover

times. Ozone treatment of system water was accomplished in contact chambers. Early versions of these systems used air-stones as the ozone introduction device. Newer systems featured entrainment of smaller gas bubbles using venturis.

A portion of the rapid sand + ozone LSS must be devoted to the restoration of gas balance (LaBonne, pers. com.). This equilibration process includes off-gassing of carbon dioxide and nitrogen, and the restoration of oxygen levels lowered by animal and bacterial respiration. In some systems, degas towers are equipped with media to assist gas exchange and optimize gas balance (Johnson, pers. com.). Structurally these towers are akin to biotowers, without the biological function. Piedrahita and Grace (1991) describe a carbon dioxide removal system that consists of a packed column supplied with countercurrent aeration.

Although LSS design is moving away from systems dominated solely by rapid sand filtration, significant amounts of sand filtration may still be required for exhibits with long sight-lines, and exhibits where large quantities of particulates are produced such as ray feeding pools (Johnson, pers. com.).

While exhibits employing early versions of the rapid sand + ozone model tended to accumulate nitrogenous wastes, later versions avoided this problem through the addition of efficient foam fractionation and frequent backwashing (e.g., SeaWorld Ohio's shark exhibit's LSS incorporated foam fractionation on a 10% side-stream and backwashing on a 140 hour cycle).

Denitrification has been added, as a side-stream process, to a number of recently constructed rapid sand + ozone systems.

### **Foam fractionator-dominated**

Some designers now favor LSS configurations where-by foam fractionators comprise  $\geq 50\%$  of the total filtration capacity of the system (LaBonne, pers. com.). These systems are frequently referred to as fractionator-dominated (Figure 6.2). Rapid sand filters are relegated to a side-stream loop and ozone additions, where needed, are minimal. LaBonne (pers. com.) notes that the need for denitrification systems is reduced because organics are removed before the nitrification process, leading to nitrate accumulation, has taken place. LaBonne (pers. com.) considers the use of foam fractionators, to

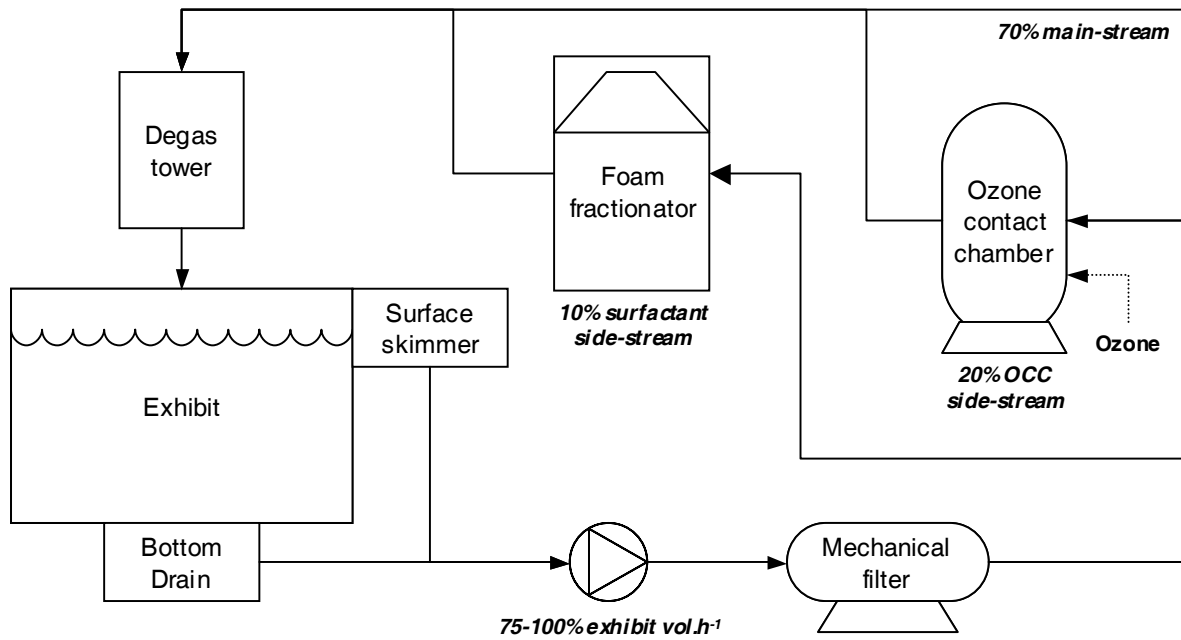


Figure 6.1. Basic process diagram of a rapid sand filter + ozone contact chamber LSS.

the exclusion of ozone contact chambers and traditional mechanical filtration systems, to be a design and operation goal for modern elasmobranch LSSs.

flocculation, and will remove some of the flocs before they reach the rapid sand filters.

Some LSS designers apply foam fractionators before rapid sand filters. In such applications, the foam fractionator will act as a site for micro-

**Parallel flow**

The term “parallel flow” is traditionally used to describe any LSS where parallel treatment

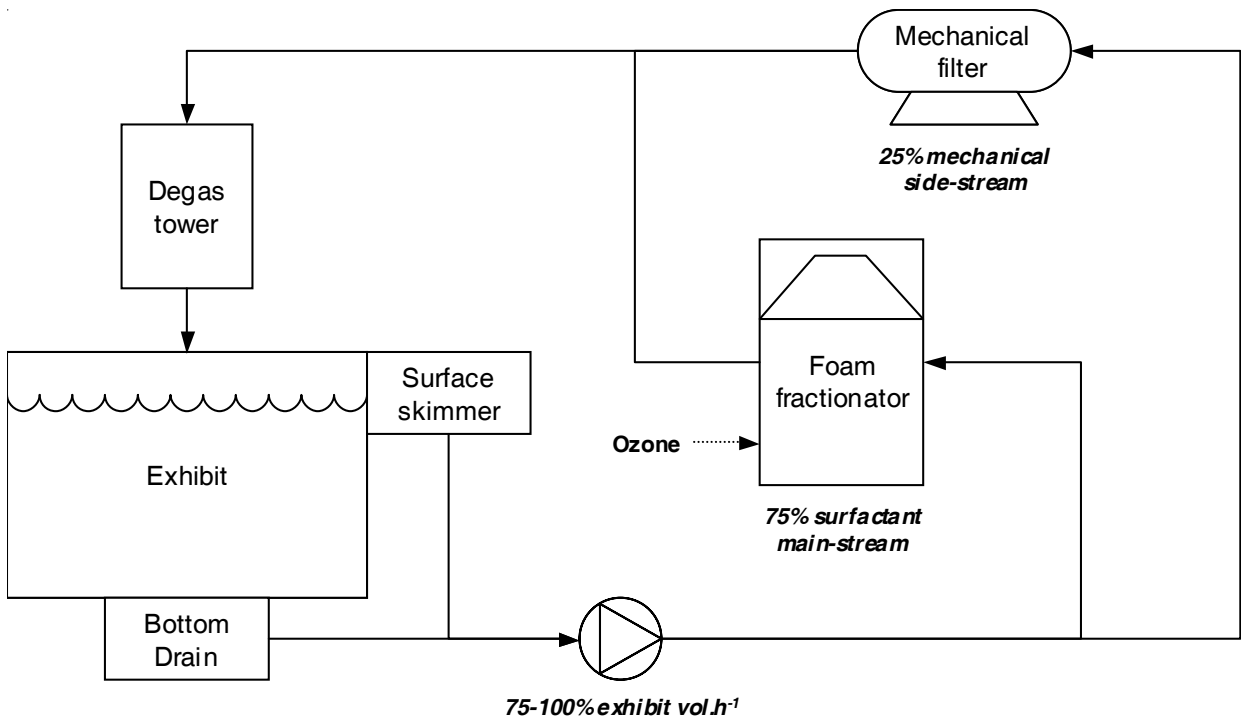


Figure 6.2. Basic process diagram of a fractionator-dominated LSS.

streams are employed. More recently the term has been used to denote a specific combination of separate rapid sand filter and foam fractionator streams (Johnson, pers. com.). This scheme uses less floor space and energy than the traditional rapid sand filter model. Parallel flow LSSs use particular water treatment processes where they are most needed: oils and surfactant-rich water from surface skimmers are directed to foam fractionators, while particulate-loaded bottom drain water is directed to rapid sand filters (Figure 6.3). Water flow is divided more or less equally between the two treatment streams and joins at a common degas chamber. Ozone can be added to a dedicated reaction chamber or foam fractionators as described above. A good example of this LSS philosophy may be found at Ripley's Aquarium of the Smokies, Gatlinburg, USA (Johnson, pers. com.).

particulate removal (Figure 6.4). Ozone acts as a micro-flocculent, improving mechanical filtration. An up-flow fluidized carbon bed is used to strip residual ozone and oxidants before water returns to the pool. A packed column degas chamber is employed to prevent supersaturation and to balance all dissolved gases (Johnson, pers. com.; Linn, pers. com.). It is believed that ozone reacting with carbon in the fluidized bed may be creating hydroxyl ions that enhance disinfection through a process known as advanced oxidation (Johnson, pers. com.).

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**Pre-ozonation**

Pre-ozonation has been applied at Discovery Cove and is intended to address bacteriological issues having regulatory significance for the immersion of guests within exhibit water. The entire process stream receives ozone, injected into an open contactor via venturis, prior to

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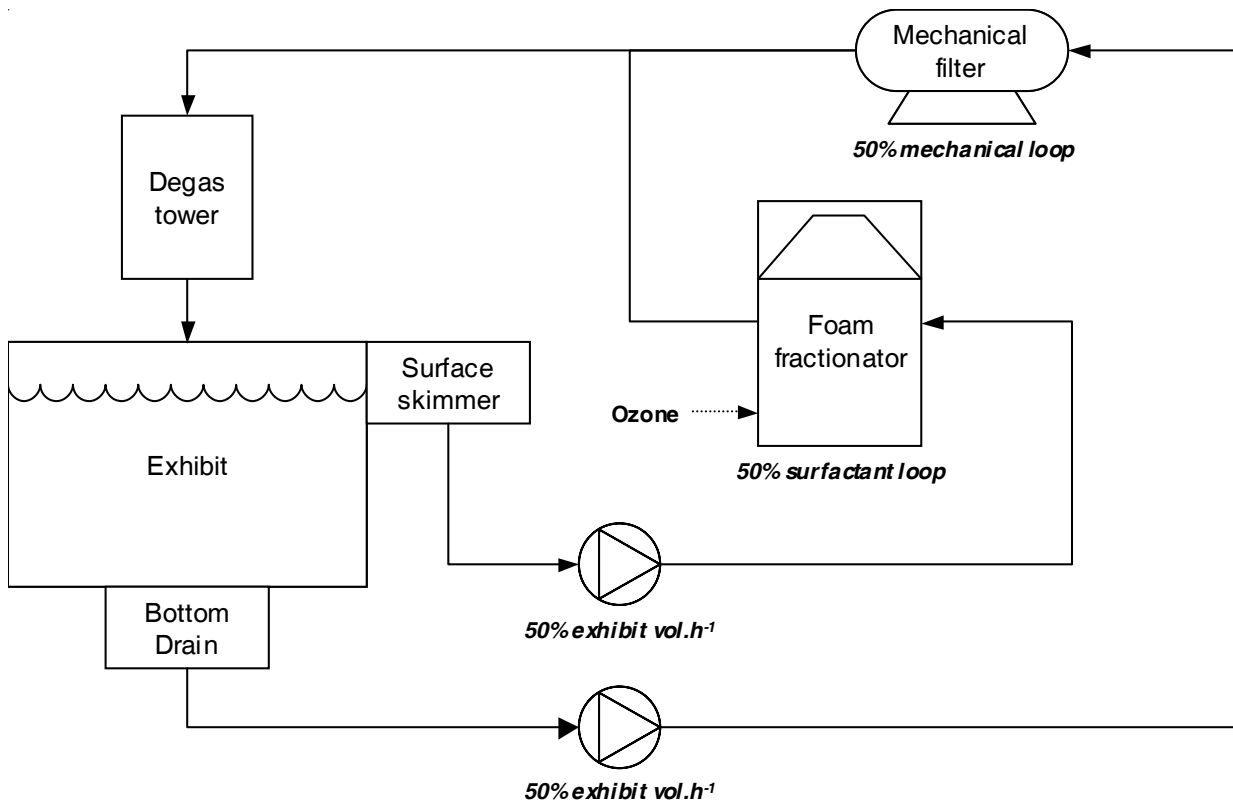
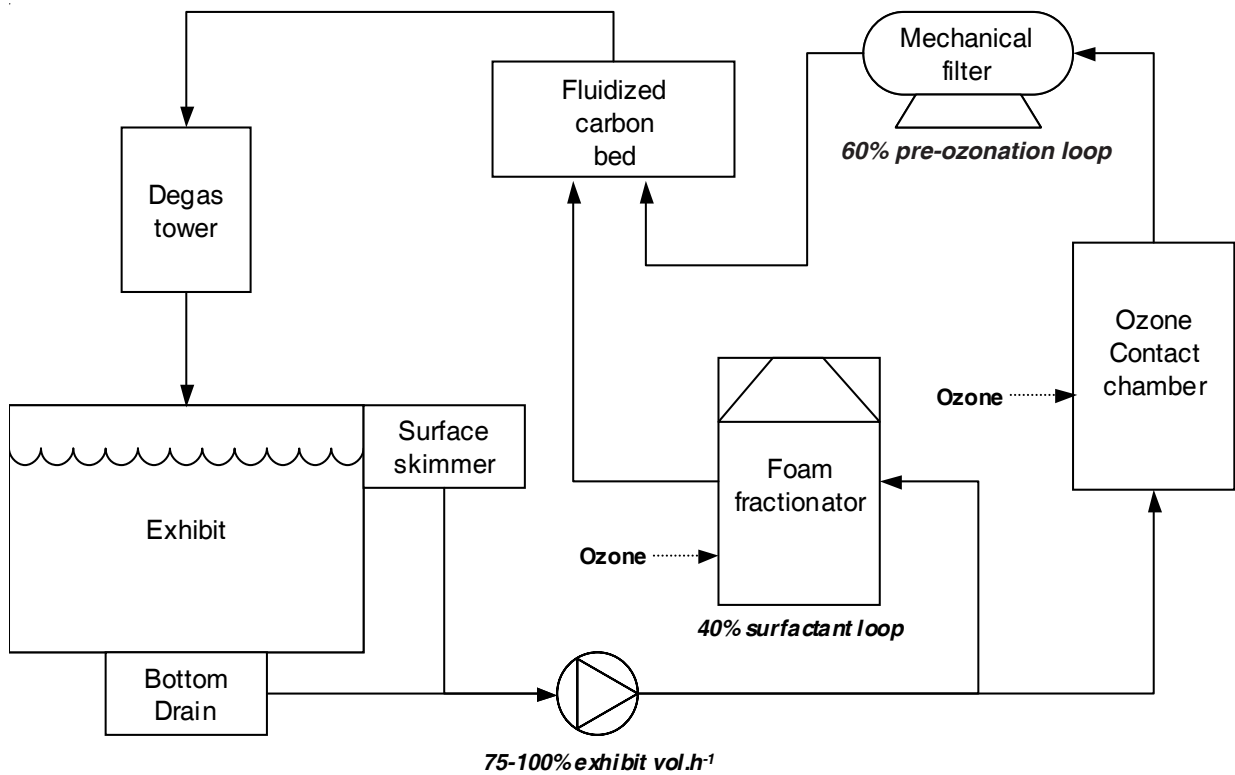


Figure 6.3. Basic process diagram of a parrallel flow LSS.



**Figure 6.4.** Basic process diagram of a pre-ozonation LSS.

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- www1 <http://wlapwww.gov.bc.ca/wat/wq/BCguidelines/tgp>
- www2 <http://www.fish-news.com/PH200.html>
- www3 [http://www.epa.gov/cgi-bin/ecotox\\_quick\\_search](http://www.epa.gov/cgi-bin/ecotox_quick_search)
- www4 <http://www.toxnet.nlm.nih.gov>
- www5 <http://www.reefball.com>
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