One Frog, Two Fish, Red Leg, Few Fish: Stress in the Aquatic Animal's Ecosystem
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“I don’t know what happened! Yesterday, this (insert your choice: frog, fish, xenole, lobster, sea urchin, etc.) looked fine. I walked in this morning and it was dead!”

How often has that statement occurred in your facility? “Aquatics” is a new realm for many research facilities and institutions. The husbandry is different. The species characteristics are different. Unlike laboratory mammalians, amphibians and fish require very specific microenvironments. Their basic life functions are influenced by and interrelated to one entity - the water. They eat, breathe, and eliminate waste through it. Many use gills for gas exchange, osmoregulation, acid-base balance, and nitrogenous waste excretion. Some can absorb and excrete directly through their skin. But one detail is not so different... like mammals, stress, and/or lack thereof, plays a major role in how aquatic species subsist.

It is essential to the scientific value of the research that stress and discomfort be minimized. Stress causes biochemical, physiological, and behavioral changes. It can affect growth, digestion, reproduction, osmoregualtion, healing, and disease immunity. 1

In the research setting, some stresses are unavoidable. Surgery, transport, handling, oocyte expression, anesthesia are just a few examples of inevitable stresses. With the help of the hormone cortisone, all animals can manage some stress. It is when several stressful situations occur simultaneously that the arbitrary threshold is crossed and the animal becomes compromised. (See Figure 1) And, given that animals are designed to hide stress, too often excess can quickly lead to disease, even death, before it is realized and a fix can be implemented. The objective is to reduce secondary stress to keep the animal levels manageable.

But the question remains: What is stress for an aquatic animal? Many stresses in the aquatic environment, especially water conditional factors, are invisible to the eye. To make matters more complex, many water parameters affect one another; and some stresses are simply misconstrued assessments from inaccurate human emotional interpretation of the situation.

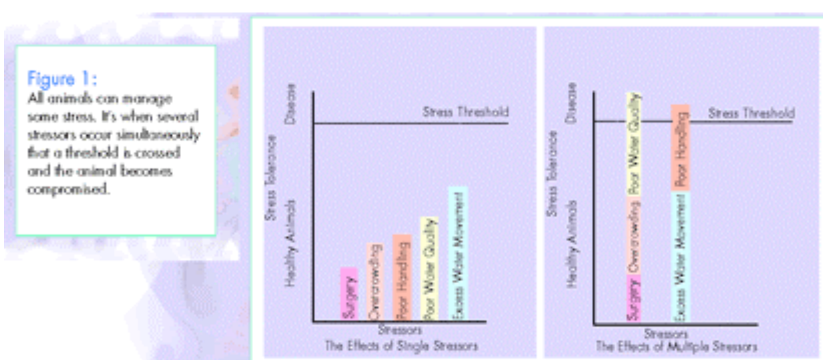

Figure 1: All animals can manage some stress. It's when several stressors occur simultaneously that a threshold is crossed and the animal becomes compromised.

Water Quality
In aquatics, stress is often the direct result of poor water quality. There are many aspects of water management that should be well understood and monitored at appropriate frequencies. Water quality parameters of ammonia, nitrite, nitrate, dissolved oxygen, carbon dioxide, pH, temperature, and salinity are the most common factors that affect the stress state of aquatic species.2 Unforeseen situations can occur at any time. If not prepared to handle a critical problem if it arises, losses to the aquatic population can be costly.

Chemicals and Heavy Metals
Undesirable substances, which cause stress, are often present in the initial water source. Most municipal sources (city water) have chlorine and/or chloramines added to make it safe for human consumption. If it is not removed before the water is used in the aquatic system, it can quickly kill even at doses as low as .006 mg/l.3

Heavy metals, such as copper, mercury, iron, lead, silver, zinc, vanadium, and nickel, can be introduced via certain types of piping and are often found in well water sources. While various metal ions, in the correct amounts, are necessary for proper enzyme function, when found in the wrong concentrations they can denature those same enzymes and lead to death. For example, very small amounts of copper, precisely those amounts normally found in natural sea water, are absolutely necessary for the correct functioning of the respiratory pigment, hemocyanin, in arthropods and mollusks. However, a slight increase in the amount of copper in the water surrounding the organisms will result in a similar increase in the internal cellular environment resulting in the denaturation of other cellular enzymes, killing these same organisms.4

Ammonia, Nitrite, and Nitrate
As the primary metabolite of aquatic species, ammonia is directly excreted from the animals. In addition, the proteins in excess foods, fecal material, the normal shed of body cells, and perhaps the undiscovered dead animal are broken down through a process called mineralization. The result of this decay process, among other things, is an excess of ammonia.

Ammonia is highly toxic if allowed to accumulate. It burns the gills of the animal causing hypertrophy, then hyperplasia of the gill lamellae. This reduces respiratory exchange and osmoregularity function, as well as sets the stage for bacterial infection. 2

Ammonia is present in the aquatic environment in two forms, un-ionized or “free” ammonia (NH3), and ionized ammonia (NH4+). The free form is more easily absorbed by the body tissue and is therefore more toxic than the ionized form. Free ammonia also prevents animals from excreting their own waste ammonia. Fortunately, in the normal pH range for most aquatics (6.8-8.2), most ammonia is in the ionized state. As pH increases, the lethal form of ammonia increases. Therefore, an ammonia level that may be tolerable at 7.6 can become lethal at 8.4.

Ammonia levels can be kept in check in two ways. They can be reduced by physical replacement of the water (as in flow-through type systems) or by allowing the water to pass through a biological filter (as in re-circulating systems). In the latter, ammonia is oxidized to a non-toxic form in a two-step nitrification process. In the first step, the aerobic bacteria Nitrosomonas sp. converts ammonia to nitrite. Nitrite is also toxic to aquatic animals, especially at lower pH levels, where it is converted to toxic nitrous acid. A second aerobic bacterium, Nitrobacter sp., converts the nitrite to nitrate. While ammonia and nitrite are potentially lethal stressors, nitrate is much more easily tolerated, yet, if levels are allowed to go unchecked, it too becomes toxic. By simply replacing 10-15% of the water within the system weekly, nitrate levels, and the potential stress, are kept in check.

pH
pH can be thought of as the abbreviation for the “power of hydrogen” and is the measure of the positively charged hydrogen ions (H+) and the negatively charged hydroxyl ions (OH-). Because it is on a logarithmic scale (1-14, with 7 being neutral) each point represents a 10x greater concentration than the previous point. A shift in pH is usually a sign of another issue, such as overcrowding, heavy detritus accumulation, an overabundance of dissolved organics, and poor aeration. In re-circulating systems, because of the nitrogen cycle and the presence of CO2, the pH naturally begins a downward shift. This can be corrected with proper aeration, weekly 10% water changes and, if needed, the addition of sodium bicarbonate. While most aquatics can tolerate a broad range of pH (marine species having the narrowest margin), sudden shifts in pH are stressful and can be dangerous.

Alkalinity and Hardness
Alkalinity refers to the buffering capacity of the water. It is determined by the proportionate concentrations of bicarbonate, carbonate, and hydroxide. Hardness is the direct measure of the total calcium and magnesium salts dissolved in the water. Since the carbonate that contributes to alkalinity is usually from the same source as that which creates hardness, the values are often equivalent. Excessiveness in either direction of these parameters is a particular stress. For example, aquatic organisms cannot survive in water lacking minerals, as in distilled water. And, exceptionally hard water can cause calcium crystals to form in the kidneys of fish. 2

Dissolved Gases
Improper gas concentrations can add stress. Dissolved Oxygen (DO) is one of the most critical elements within the aquatic system. Water does not hold oxygen well – in fact, it contains about 30 times less oxygen than air. 5 And, as temperature, altitude and salinity increase, dissolved oxygen saturation decreases. Most fish suffer severe stress when dissolved oxygen levels fall below 3.5 ppm.6

In re-circulating systems, DO levels are also critical for the health of the biological filter. While the animals in the system may be getting enough oxygen, if the levels are low at the filtration location, the aerobic bacteria will die and the ammonia and nitrite build-up will, in turn, kill the animals. Fortunately, in most of today's commercial systems, there is enough water circulation and airlift to eliminate insufficient DO problems, but it can be an issue with in-house built systems.

The degree of stress caused by low oxygen levels is also species specific. Some species are naturally found in very still water and have learned to compensate for low DO levels by coming to the surface to breath. Siamese fighting fish and Xenopus frogs are good examples of these.

Carbon-dioxide levels in excess of 20 mg L-1 has been reported harmful to fish.7 High concentrations of dissolved CO2 gas increases acidity (shifting pH down) because it is converted to carbonic acid in water. It is just as important to remove excess carbon dioxide as it is to replace oxygen.5 (Excess CO2 also promotes algae growth, which, while not necessarily a stress to the animals, certainly adds stress for the caretaker!)

Too much nitrogen causes "gas bubble" disease, or the human equivalent of "the bends " Supersaturation of nitrogen may be first noticeable as tiny bubbles that adhere to the surfaces of the aquarium. It can be caused by an air leak on a pressurized pump and is sometimes present in well water sources (especially in colder temperatures).

Hydrogen sulfide is a very poisonous gas with a strong, rotten-egg odor. It is formed by the activity of anaerobic bacteria on organic matter in areas where oxygen is low or absent. Trouble with this gas can arise if a container-type filter has been turned off for a day or two. If the power is restored and the gas is allowed to circulate through the system, the results can be fatal.

Temperature
Aquatic species are relying on external heat sources to thermoregulate. Aquatic reptiles require a heat lamp to bask under, but can overheat if they cannot retreat from it. Amphibians and fish are dependant on the temperature of the water around them. The majority of aquatic species can handle slow shifts in temperature (some, like the xenopus sp., even require it for oocyte production). Fish usually handle a rise in temperature better than a loss. But sudden changes, of more than a couple degrees, can induce shock and even death.

Lighting, Turbidity, and Visual Stimuli
Many aquatic species, fish in particular, are affected by sudden changes in light and by photoperiods. One facility, upon the installation of a 24-hour camera monitoring system, inadvertently discovered their perch were traumatized when the overhead lights automatically

clicked on or off. The fish would throw themselves violently around the tank in a wave of panic. This stress was easily eliminated with the installation of a gradual, light-dimming system. The lights came on and off slowly, replicating the natural effect of dawn and dusk.

Growth and reproduction cycles are also interdependent on light cycles in many aquatic species. Animals housed under incorrect intensities or durations may develop either acute or chronic problems in these areas.

Turbidity, the index of light absorption, is created by the amount of both particulate matter and dissolved solids in the water. It is not necessarily an indication of poor water quality. Some species, such as Xenopus, carp, catfish and tilapia, prefer a fair amount of turbidity. Others, like salmonids, prefer much clearer water.

Some species are from environments with crevices, sand, or plant materials which are used to conceal themselves. Xenopus laevis, crayfish, lobsters, clown fish, squid, and horseshoe crabs are a few examples of species that prefer to hide. Bright, clear tanks with no concealment options can be distressing. Just entering a room can send various species scrambling for cover, occasionally injuring themselves or others in the process. While some animals will eventually acclimate to visual stimuli, it is best to provide appropriate substrates and containers in which the animals can hide.

Turbulence

Excess water movement can be a stress for some species. Xenopus leavis, for example, are naturally found in very still, murky African deltas. An adult has approximately 180 lateral line organs distributed over its head, neck and trunk which detect the friction of the water movement.8 This can be indicative of the frog's next meal (an insect's flutter on the water's surface, the wiggle of a small fish) or it can alert the Xenopus to danger (the motion of a predator about to strike). Xenopus will often face the current to remain vigilant. I have often heard people confuse this with the animal "enjoying a water massage." Water movement is naturally a cause to react. Being on a constant "state of alert" is a stress, not a day at the spa.

For other species, lack of water movement is a stress. Sea urchins, oysters, mussels, starfish, and most marine invertebrates rely on powerful currents to bring food to and carry waste away from them. Some fish (salmon, trout, etc.) also prefer a higher current, as would be found in the swifter moving waters from which they originate.

Overcrowding and Isolation

Overcrowding can lead to increased aggression, reduced growth, and poor reproduction as well as both lack of feeding and cannibalism. In re-circulating systems, overcrowding can tax the processing capabilities of the biological filter and destroy the tank balance. Ammonia and nitrite levels will suddenly rise and the housed animals die. Just adding new animals to a re-circulating system can throw this balance off and threaten the health of the population.

Converse to overcrowding, some animals do poorly when housed in isolation. Many schooling species of fish require the company of others as their genetic make-up tells them there is safety in numbers. While the research may not always make it possible, danios, tetras, medaka, barbs, and loaches are all examples of fish that prefer to be in groups.

Handling

While many mammalian species adapt to handling, most aquatic species do not. Handling is both physically and physiologically hard on the animals. Many aquatic species have a slime coat that protects their skin from bacteria and parasites. Handling usually disrupts the integrity of the slime coat, leaving the animal susceptible to disease. It also induces the fight or flight response during which a quick release of hormones begins a rapid metabolism of sugars and a rise in blood sugar. Blood pressure, heart rate, and respiration all increase, and digestion may temporarily cease. These reactions normally initiate an inflammatory reaction which is suppressed in fish by an adrenalin release. Peculiar to fish is the regulation of water into and out of the body during flight or flight response. Under stress, freshwater fish absorb too much water and saltwater fish lose too much. The rapid metabolism of sugar reserves provides additional energy to overcome this fluid imbalance.9 In addition, most handling requires the animal to be removed from the water; for some, just the atmospheric pressure can be damaging.

Feeding Issues

Over feeding, underfeeding, and poor quality feeds are additional sources of stress. Many individuals tend to overfeed fish because the animals' natural behavior makes them appear to be ravenous. If you lived in a school of hundreds with no idea when the next meal may swim by, you'd tend to grab, too! But, the stress does not come from eating too much. On the contrary, fish will usually not overeat. Once satisfied, the animals will suddenly lose all interest in the meal. Instead, the excess food is left to decompose, causing the water quality to crash. While more rare, underfeeding and the use of poor quality feeds are additional forms of stress as they affect growth rates and compromise reproduction.

Fomites

Fomites are a usually unperceived stress. Introducing a toxic chemical or bacteria, without realizing, is all too easy. While most facilities have strict rules for wearing protective covering while working with the aquatics, almost everywhere someone dips an unprotected finger in without thinking. For some reason, the old "ten-second rule" seems to exist... "I'll just quickly re-adjust that drain cover"...or "Oops, didn't mean to drop that net in, I'll just fish out." One dip of a hand can introduce thousands of bacteria. Residues from hand lotions, hand soaps, net soak chemicals, and glove powders can also be devastating. And, the transfer of bacteria from one tank to another by re-using nets is a classic method of introducing a new stress. Typically, fomites cause the type of stress, then disease, in which "no one can figure out what's going on."

Conclusion

Typically, additional forms of stress can be from overuse, lack of quarantine period, shipping, parasites, and injuries. The list goes on and on. As you can see, the potential for stress in the aquatic world is always present. The importance of a staff well-trained in the basics of water husbandry is essential. Because of the vast variations in the animals, it is vital to know what the standard is for the particular species being housed. From there, it is simply a matter of recognizing the situations that can cause stress and eliminating as many as possible.

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