**SOP for In-situ Tests**

1. Using the water parameter, open the cover and remove the pH buffer from the device and screw a reading cage onto the reader.
2. Rinse the reader with water and switch on.
3. Soak it into the sample and press ‘measure’
4. Wait for about 4-5mins for the reading to stabilise then record down the results.
5. Once done, rinse with water and place it into the sample (if any) and remove the reading cage and place the cover back and perform the necessary housekeeping.

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| **Type of Test** | **Unit** |
| Total Dissolved Solids | parts per million (ppm) |
| Dissolved Oxygen | parts per million (ppm) |
| Salinity | part per thousand (ppt) |
| Temperature | Degree Celsius |
| pH | No unit measurement |

**SOP for Ex-situ Tests**

1. Ammonia - Rinse the sample cell and pour 5ml of sample into the cell.
2. Add the reagent 6 times using the 3 reagents given. Swirl and wait for 5 mins for the reaction to complete.
3. Using the reference chart, match the correct colour and record the concentration of the colour.

1. Nitrate - Rinse the sample cell and pour 10ml of sample into the cell.
2. Add the reagent 6 times using the 2 reagents given in order. Pour 1 full scoop of the dry chemical reagent and shake till most of it has been dissolved. Drop the last reagent 6 times.
3. Swirl and wait for 5 mins for the reaction to complete.
4. Using the reference chart, match the correct colour and record the concentration of the colour.

1. Nitrite - Rinse the sample cell and pour 5ml of sample into the cell.
2. Add the reagent 5 times using the 2 reagents given. Swirl and wait for 5 mins for the reaction to complete.
3. Using the reference chart, match the correct colour and record the concentration of the colour.

1. KH - Rinse the sample cell and pour 5ml of sample into the cell.
2. Add the reagent till colour change is seen. Final colour should be yellowish-orange.
3. Record the amount of times reagent has dropped.

1. GH - Rinse the sample cell and pour 5ml of sample into the cell.
2. Add the reagent till colour change is seen. Final colour should be yellowish-green.
3. Record the amount of times reagent has dropped.

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| **Type of Test** | **Units** |
| Ammonia | mg/L |
| Nitrate | mg/L |
| Nitrite | mg/L |
| KH | mg/L |
| GH | degree of General Hardness (dGH) |

**Theory on various tests**

Tests are performed to ensure husbandry are at optimal standard and not to have any mishaps and incidents happening to the fishes.

Total Dissolved Solids are performed to ensure that fishes are healthy and at optimal growth. For freshwater fishes, they are prone to fatal death as the dissolved solids are a prime cause as it would affect the stock density in the fish tanks. High TDS can cause an overgrowth of algae and how this works is since algae requires carbon dioxide, the production of algae increases which will then increase in alkalinity through the bicarbonate ions in water. A stable TDS level would be 350ppm - 400pm.

Henceforth, we introduce a water system that can aid in filtering the materials that cause such obstacles, the protein skimmer. The buildup of ammonia products and other bio wastes is collected through the filter to filter out the proteins. Clean water is then produced. Fresh and aquatic fishes normally do not produce as much as the seawater and marine fishes as not only does the fish increase protein products but from the water itself as its highly natural. We import seawater as we wish to receive most of its nutrients and try to keep it natural. If we were to recreate our own seawater, we would not be able to fully extort the full content and not only would it not be accurate, the cost production will be too high.

Testing of dissolved oxygen would be simply to indicate if there is enough aeration in the water. Since Singapore is a warm and humid country, warm water is not able to sustain high amounts of DO. Lack of oxygen would kill the fish through falling respiration and reduce good microbial activities in water however it varies from fish sizes. DO can also decrease overnight as when food is given in the afternoon and not consumed, the food will absorb the DO. Additionally, when aeration is introduced by the aerator, low production of oxygen is taken as plants would not be able to photosynthesise to produce the required amount of oxygen due to the lack of sunlight. Aerators are where pump natural air from the outside into the water through a gas inlet.

Two types of aeration can be found in the tank. A much smaller pore and one that is connected to the weighted sponge holder. These different pore sizes produce different pore air bubbles. The smaller the bubble, the better as it produces a more quantified amount, though covering less space, it has higher production of oxygen. Similar concept to a cube of sugar or broken sugar. Results will be faster from broken sugar as it dissolves faster.

Knowing how different fish react to different water properties, they are bound to be sensitive. For salinity, it differs as we categorise them under saltwater and seawater fishes. Lily’s are saltwater fishes and only require little amount whereas clown fishes are sea fishes. Salinity level is important as if there were to be out of the fish’s desired level, it can cause physical damage to their body. High salinity can also cause high stress and excrete mucus like liquid which is the hypotonic urine like humans to expel the excess. However, this case does not apply to marine fishes. Instead, they lose water and gain salt osmotic concentration to increase salt. A salinity level should be 35ppt.

Temperature wise is important, especially specific species. For example, shrimps at 23 degrees will be the most optimal range for their growth and this is  to prevent physical damage as they disintegrate easily with heat hence when collecting dead fishes, they will be noted as missing. Other fishes like Koi would be better under 24 degrees to reduce stress which can lower mucus produced by them and slows growth of other bacterias that are not needed. Low temperature can cause thermal stress behaviour which includes loss of equilibrium and sudden violent spasm. A cole shock that can slip the fish into coma when the central nervous system ceases to function.

pH is the most crucial part which can be quite dangerous to fishes. Not all fishes are adaptable and can result in death. Most fishes prefer a pH of 6-8. Measurement and monitoring of pH is difficult as a lot of factors fall on it like temperature, hardness of water, TDS and more. Gills, fins and tails are prone to temperature and pH. Hence, checking pH is important. If the water is too acidic, we can increase pH by dilution, however, the water change can inflict stress which is already stressful for the fishes as they are facing burns from the acidity of water. Instead, we use coral chips. These chips are made of calcium carbonate which acts as a buffer capacity to increase the pH.

Ammonia is the most common occurrence in a tank. The common test that is related to almost every other test. Ammonia is released from waste products of the fishes so when high density of fishes are found in a tank or when too much feed is given without proper diet, high ammonia levels will occur. When high ammonia levels are present, it can burn the fish's tail and when it is not treated, it can be chipped off causing more damage like lesions and abrasions to the tail, and in prolonged exposure, fatal death can take place by bacteria or fungus growing on it.

Additionally, fish waste breaks into nitrates which tend to lower pH. It burns with poison and choke and suffocate by the gills. All these result in an increase of respiration and stress in fish which can have a psychological effect on fish. Normally seawater would have a higher concentration of ammonia as more proteins are found than fresh water.

When an ammonia test is performed, it titrates the total concentration of TAN which means Total Ammonia Nitrogen. Hence, we will only be able to derive the concentration of ammonia through using the TAN calculator. To calculate, it needs reference to the amount of total ammonia, salinity, pH and temperature. TAN can also be inspected visually especially when there is an incoming shipment. Cloudy/Dark shade indicates high TAN. Possibility of this is caused by not fasting the fish for at least 2 days or late purchase order.

As many would have already known, nitrogen is very common and can be found in water. Nitrogen fixation will occur and produce the necessary reaction from nitrifying bacteria like nitrosomonas bacteria to form nitrite then denitrifying bacteria to form nitrobacter bacteria to form nitrate. To decrease the potential hazard of producing bad bacteria, we can introduce live rocks as they have bacterial properties that are beneficial and not just for aesthetics. They can culture good bacteria to mitigate the bad ones which can lower sickness in fish. This can create a good environment for the fishes to live in.

Nitrate has been found to be more toxic in salt water than in fresh water. It is reasonable to

assume that very high levels of nitrate cause stress and greater susceptibility to disease in marine aquariums since nitrate levels in the oceans are almost zero. Nitrite proves toxic because it breaks down the red blood cells and oxidises the iron in the haemoglobin into a stable state called methaemoglobin, which has no oxygen-carrying capacity. This process has the effect of turning the gills and blood brown.) The ability to convert methemoglobin back into haemoglobin determines how resistant a particular fish is to high nitrite levels.

Water hardness, generally referred to as analysis of water with part concerned on total dissolved salt content. KH refers to total hardness which relates well with TDS and water polarity. Temporary hardness is found as well and refers to total content of the major portion from calcium and magnesium salts. Hard water tends to have more salt than soft water. GH is the general hardness refers to total content of salts like hydroxides, bicarbonate and carbonates. KH and GH is generally to sit well with the environment to produce better growth.

Water hardness is dependent on alkalinity levels and amount of salt present. High salt content equates to hard water making non hard water fishes affected by it, resulting in decrease of blood calcium level however for hard water fishes have the ability to cope as they can excrete calcium hormone called calcitonin. Factors include osmosis pressure as non hard water fishes do not take in salt content very well. Hard water fish osmoregulatory systems can have reduced workload replacing ions from blood if in soft water.