The success of a commercial aquaculture enterprise depends on providing the optimum environment for rapid growth at the minimum cost of resources and capital. One of the major advantages of intensive recirculation systems is the ability to manage the aquatic environment and critical water quality parameters to optimize fish health and growth rates. Although the aquatic environment is a complex eco-system consisting of multiple water quality variables, it is fortunate that only a few of these parameters play decisive roles. These critical parameters are temperature, suspended solids, and pH and concentrations of dissolved oxygen, ammonia, nitrite, CO₂, and alkalinity. Each individual parameter is important, but it is the aggregate and interrelationship of all the parameters that influence the health and growth rate of the fish.

This unit reviews some of the most critical water quality parameters, recommended maximum or minimum concentrations, and how to measure them. Seceding chapters introduce engineering unit processes that are used to either remove, maintain or add to the system the limiting water quality factor.
Unless you’re a Fish, you can’t tell whether the water quality is optimal by just looking at the water or even the fish. The aquatic environment is totally alien to us air breathers and thus some form of water quality monitoring and measurement is extremely critical to any successful operation. The critical parameters, in order of importance, are the dissolved oxygen concentration, temperature, pH, un-ionized ammonia concentration, CO₂, nitrite, suspended solids, and alkalinity. Although each individual parameter is important, it is the aggregate and interrelationship of all the parameters that influence the health and growth rate of the fish. Each water quality parameter interacts with and influences other parameters, sometimes in complex ways. Concentrations of any one parameter that would be harmless in one situation can be toxic in another.
Each water quality parameter interacts with and influences other parameters, sometimes in complex ways. Concentrations of any one parameter that would be harmless in one situation can be toxic in another. For example, when aeration and degassing problems occur, carbon dioxide levels will generally become high while at the same time dissolved oxygen levels become low. The result of this particular situation is that not only is there less oxygen available to the fish, the fish are less able to use the oxygen that is available. The high carbon dioxide level of the water affects the fishes' blood capacity to transport oxygen, aggravating the stress imposed by low dissolved oxygen levels. Another excellent example of the complex interaction among water quality elements is the relationship between pH and the toxicity of ammonia. As will be discussed later, only the unionized fraction of the total ammonia concentration is toxic, and at low pH, most of the ammonia in the water is in the non-toxic ionized form. However, increasing the pH by only one unit, i.e., from 6.5 to 7.5, increases the concentration of the toxic unionized ammonia concentration by a factor of ten. Simply adding baking soda to a system to increase its alkalinity can inadvertently but easily create this extremely undesirable condition.

The relationship between water quality factors and their effect on fish growth rate and health is complicated. For example, fish lack the means to control their body temperature and maintain it independent of the environment. Environmental temperature changes affect the fishes' rate of biochemical reactions, which leads to different metabolic and oxygen consumption rates. At the lower ranges of the species tolerable temperature range, these rates decrease. As water temperatures increase, fish become more active and consume more dissolved oxygen, while simultaneously producing more carbon dioxide and other excretory products, such as ammonia. These increasing rates of consumption of necessary elements and production of detrimental elements can have a direct effect on overall fish health and survival if these parameters are allowed to exceed nominal values. If not corrected, the fish will become stressed to some degree. Even low levels of stress can have adverse long-term consequences in the form of reduced growth rates or mortality due to opportunistic organisms that take advantage of the stressed fish.
During the initial site selection process, one of the most critical factors to consider is the availability of an adequate water supply for both the initial facility and any planned (or imagined) expansion. When it comes to the availability of water, too much is definitely better than too little. After all, one is constructing an AQUAculture facility. The amount of water needed will depend on several factors such as species, density, management practices, production technology, and the degree of risk one is willing to accept. At a minimum, sufficient quantities of water are needed to routinely fill production tanks within a reasonable time (24–48 hrs), provide for routine and emergency flushing of tanks, filter backwashing, facility wash down and clean-up, and domestic requirements. A good rule of thumb is to have sufficient water available to provide a 100% water exchange of total system volume per day (Timmons, 2000). Thus for a total system volume of 379 m³ (100,000 gallons), a water supply is required that is able to provide 379 m³ per day or 0.26 m³/min (70 gpm). This may be reduced some for a warm water species.

Beyond the minimum exchange volume, the additional quantity of new water required for any given system is directly dependent on the degree of reuse or recycling of the "old" water that is already available in the system. There are numerous reasons to reuse the supply water as much as possible, besides the obvious decrease in water demand and reduced discharge water that must be treated. These include the reduction of water heating or cooling requirements, which are major factors in the economics of many warm-water species production systems. Additionally, and becoming increasingly important, recycling of system waters results in the reduction in wastewater discharge and the corresponding costs involved in the treatment of large discharge flow streams.
There are three categories of reuse systems: serial-reuse systems, partial-reuse systems, and fully recirculating systems. Partial-reuse systems reuse a greater percentage of total system volume than do serial-reuse systems, and the fully recirculating systems reuse a greater percentage of total system volume than do the partial-reuse systems. The degree of water reuse affects the depletion/accumulation rate of important water quality parameters. The greater the reuse rate, the more conditioning the reused water must have in order to restore the water quality to target parameters. Typically, the most important and first encountered limiting factor that governs the density of fish reared in a system is the concentration of dissolved oxygen that is available to the fish. The next most important factors are the amounts of un-ionized ammonia and dissolved carbon dioxide levels. These two parameters are interconnected. This is due to the direct effect that dissolved carbon dioxide has on pH and the relationship of pH to the toxicity of ammonia-nitrogen. As the dissolved carbon dioxide levels decrease, the pH increases, which in turn increases the toxicity of the total ammonia-nitrogen in the system. For example, if salmonids are the species being grown, the maximum upper safe limit for chronic exposure to carbon dioxide is from <9 to 30 mg/L, and for un-ionized ammonia-nitrogen <0.0125 to 0.03 mg/L. The chosen reuse system must be able to sustain the necessary levels of dissolved oxygen while also keeping the amounts of dissolved carbon dioxide and pH below their respective limiting levels.
Serial-reuse systems have been used extensively in trout and salmonid raceway production systems, where the limiting water quality factor is usually the dissolved oxygen concentration between raceway sections. By adding oxygen, the water can be used over again in the next section of the raceway, until ultimately the accumulated ammonia levels become too high. This simple concept has significantly increased raceway production, but at a higher system cost due to the requirements for more sophisticated oxygenation systems and monitoring systems. Economic risk is also higher, because there are more fish in the raceway that are subject to a catastrophic event.
Partial-reuse systems are an alternative culture system that can sustain high production densities on less than 20% of the total flow that would be required to grow the same quantity of fish in a serial-reuse system. Partial-reuse systems separate the solid wastes from the main water recirculation loop by using a dual-drain system. In this system, the circular production tank is used as a “swirl” separator to concentrate the solids into the center drain. From there, they are removed with a relatively small discharge flow stream (15–20% of the quantity of water in the tank). The majority of the water flow leaving the tank, i.e., the remaining 80–85%, is discharged from the tank through a fish-excluding port located approximately midway on the tank sidewall. This flow stream is relatively free of settleable solids and can be easily treated with a high-capacity microscreen filter, carbon dioxide stripping system, and an oxygenation system. The ammonia levels are controlled by dilution with make-up water, typically 10–20% of the flow (a volume approximately equal to the solids waste discharge flow from the center drain), and by controlling the system pH. The key operating water quality parameter in this type of system is the amount of dissolved carbon dioxide. Adjusting the amount of carbon dioxide stripped by the degassing system will control the system’s pH. When the system is operated so that the dissolved carbon dioxide level is the limiting water quality parameter, the water will have a low pH, and thus the corresponding maximum total ammonia-nitrogen level will also be well below the level at which it would become critical.
The Freshwater Institute’s pilot-scale partial-reuse system consists of three 3.66 m by 1 m deep circular ‘Cornell-type’ dual-drain culture tanks (Summerfelt, et al., 2000). The ‘Cornell-type’ dual-drain tank provides for efficient and effective solids removal by concentrating and flushing the suspended solids through the tank’s bottom-center drain. In a recent study, the total suspended solids concentrated discharge through the three culture tanks’ bottom-center drains average $26.2 \pm 2.1$ mg/L, compared to $2.5 \pm 0.2$ mg/L through the elevated side-wall drain (Summerfelt, et al., 2000). In the current system, approximately 5 - 20% of the flow is discharged through a bottom-center drain and the remaining flow exists through an elevated side-wall drain located at the water surface. The flow from the bottom-center drain is continuously discharged directly to the treatment system located in the greenhouse. The remaining discharge, 80-95% of the recirculating flow, is collected and filtered through a rotating drum filter (Model RFM 3236, PRA Manufacturing, Ltd., Nanaimo, British Columbia, Canada) equipped with 90 μm mesh screens, before it enters a pump sump. The water is then pumped by several 1.2 kW centrifugal pumps through a packed aeration column for aeration and carbon dioxide stripping. The water exits the aeration column and flows through a low head oxygenator (Model MS-LHO-400 gpm, aluminum construction, PRA Manufacturing Ltd., Nanaimo, British Columbia, Canada) installed within a cone-bottom sump. The water then flows by gravity back to the three production tanks and is discharge at multiple sidewall ports to provide for water circulation. The system’s water’s pH is controlled by adjusting the amount of carbon dioxide stripped in the aeration column by turning a forced air fan “on and off”. The fan is controlled by a pH controller (GLI International, Milwaukee, Wisconsin). Monitoring systems track the dissolved oxygen, pH, temperature, make-up water, and total flow rates through the system. In addition, supplemental or emergency oxygen is available by in-tank oxygen diffusers, controlled both manually and by the dissolved oxygen monitoring system. Currently the system is operating at a total flow rate of from 1200 to 1900 lpm (300-475 gpm), with a bottom-center discharge rate of 170 to 220 lpm (45-60 gpm), and a make-up water flow rate of from 200 to 300 lpm (50 – 75 gpm).
In a fully recirculating system, engineering unit processes are used to treat the water so that only a small percentage is discharged daily. This can range from 10% total system volume per day to as little as 1%. This closure of the systems is critical when extensive and expensive water quality parameters are required, such as with high or low temperature production and marine systems. This course will introduce you to the numerous engineering subsystems required to obtain a high degree of system closure.
The Freshwater Institute’s Recirculating Growout System is constructed around a 150 m³ production tank, 9 m in diameter and 2.5 m deep circular ‘Cornell-type’ dual-drain culture tanks, currently growing artic char. As with the partial-reuse system, the ‘Cornell-type’ dual-drain tank provides for efficient and effective solids removal by concentrating and flushing the suspended solids through the tank’s bottom-center drain. Under the current operating parameters, approximately 3-7% of the flow is discharged through the tank’s bottom-center drain and the remaining 93-97% of the flow through an elevated side-wall discharge. The total flowrate is 4,750 L/min or a Hydraulic Retention Time (HRT) in the production tank of 31.5 minutes. The solids laden flow from the bottom-center drain is discharged into a swirl separator and then combined with the side-wall stream and filtered through a rotating drum filter (Model RFM 4848, PRA Manufacturing, Ltd., Nanaimo, British Columbia, Canada) equipped with 60 µm mesh screens, before it enters a pump sump. The discharge from the swirl separator flows is combined with the overflow from the sump and flows to the greenhouse sump. Three 3.75 kW pumps (one dedicated backup) provide a flow rate of approximately 4,750 Lpm to a fluidized sand filter, (Cyclo Biofilter™), 2.7 m in diameter and 6.1 m tall. The static sand capacity is approximately 8.5 m³ or a depth of 1.5 m. The Cyclo Biofilter™ had a design TAN assimilation capacity of 200 kg of feed/day or 0.7 kg TAN/m³/day. After leaving the biofilter, the water flows through a stripping column to remove excess CO2 and into a Low Head Oxygenator (LHO), which increases the water DO concentration to approximately 14 mg/L. The LHO can be used either for oxygen supplementation or ozone can be added for disinfection purposes. Finally an inline Horizontal channel UV filter is used to reduce the heterotrophic plat count and in addition, destroys any residual dissolved ozone. The system is currently stocked with Artic char at close 100 kg/m³ or a biomass of 13,500 kg. Approximately, 100 kg of feed is currently fed per day.
One of the most important requirements for a successful aquaculture facility site is a high quality water source, with sufficient capacity to provide for initial needs and contemplated future expansions. Thus, when choosing an aquaculture site, the water supply must be thoroughly investigated and quantified in terms of both quality and quantity. Aquaculture is unlike other agriculture activities, in that it requires a continuous supply of high quality water. Most wells operate only periodically, so a traditional well pump test is usually conducted for only a short time, to measure the well's ability to deliver a certain quantity of water within a certain time. However, under normal aquaculture operations, the well is pumped continuously. The result can be that the actual productivity of the well is much less than indicated by the short duration test, and that the originally expected quantities of water will not be available. This will result in serious difficulties down the road.

There are several sources of water for aquaculture operations, each with distinct advantages and disadvantages. Since the whole point of recirculating aquaculture systems is to minimize water usage, the two most often used sources are groundwater and municipal water supplies. Both normally have the quality, quantity and reliability required, and the actual choice between them is based on availability and economics. The use of surface waters is not recommended due to the higher risks of contamination by pollutants, fish eggs and larval, disease microorganisms, and the wide seasonal temperature variations.
One of the major advantages of groundwater sources is their constant temperature throughout the year. Shallow sources of groundwater approximate the mean air temperature of the area. The chemistry of groundwater is directly dependent on the geology of the area surrounding the source. In limestone areas, groundwater is hard, and high in calcium and carbon dioxide. In areas of granite formation, the groundwater tends to be soft, low in dissolved minerals and carbon dioxide. As will be discussed later, there are advantages and disadvantages to both, emphasizing the need for early extensive water quality testing.

The disadvantages of groundwater, especially from deep wells, are the high concentrations of dissolved toxic gases such as hydrogen sulfide (H2S), methane (CH4), and carbon dioxide (CO2). Most groundwater contains little or no dissolved oxygen because of the biological processes occurring in the aquifer recharge zone. In addition, once pumped to the surface, groundwater often is supersaturated with nitrogen gas. Thus, the groundwater must be initially conditioned by some form of aeration and degassing to remove the excess nitrogen gas and add oxygen. High concentrations of dissolved iron can also be a problem. Once aerated, the iron precipitates out of solution and often must be removed by some form of granular filtration or settling basin.
Another potential source of water is from a municipal water supply. However, it must be kept in mind that these sources are designed and treated to safeguard the health of humans, i.e., chemicals such as chlorine (1.0 mg/L) and fluorine are added. To be useable in RAS; these chemicals must be neutralized either chemically (sodium thiosulfate to remove chlorine and chloramines) or by filtration (activated carbon). Chlorine is very detrimental to fish and levels as low as 0.02 mg/L are stressful.
The very nature of aquaculture makes it almost impossible to formulate a definitive "one size fits all" list of water quality standards. The wide range of species, temperature regimes, and production techniques makes any such list only "recommendations", at best. In intensive recirculation systems, the species of choice is raised in a kind of chemical soup consisting of numerous physical, chemical, and biological factors that are interrelated in a complex series of physico-bio-chemical reactions. These reactions affect every aspect of the culture from fish survival and growth rates to biofilter performance and solids removal. A basic understanding of water chemistry is critical for the success of any intensive system.

The next few slides list the most critical water quality parameters required to fully characterize the potential water supply and provides very general recommendations of water quality criteria for each parameter.
### Water Quality Standards

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness, Total (as CaCO₃)</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>&lt;0.15</td>
</tr>
<tr>
<td>Nitrogen (N₂)</td>
<td>&lt;110% total gas pressure</td>
</tr>
<tr>
<td></td>
<td>&lt;103% as nitrogen gas</td>
</tr>
<tr>
<td>Nitrite (NO₂⁻)</td>
<td>&lt;1, 0.1 in soft water</td>
</tr>
<tr>
<td>Nitrate (NO₃⁻)</td>
<td>0-400 or higher</td>
</tr>
</tbody>
</table>

Water quality standards.
Water quality standards.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen Dissolved (DO)</td>
<td>&gt;5</td>
</tr>
<tr>
<td></td>
<td>&gt; 90 mm Hg partial pressure</td>
</tr>
<tr>
<td>Ozone (O₃)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>pH</td>
<td>6.5-8.5</td>
</tr>
<tr>
<td>Salinity</td>
<td>&lt;0.5 to 1</td>
</tr>
<tr>
<td>Total dissolved solids (TDS)</td>
<td>&lt;400</td>
</tr>
<tr>
<td>Total suspended solids (TSS)</td>
<td>&lt;80</td>
</tr>
</tbody>
</table>
The following slides review the most important water quality parameters. Depending upon your source of water and species being raised, there may be others just as important or even more so!
Of all the water quality parameters, dissolved oxygen is the most important and most critical parameter, requiring continuous monitoring in intensive production systems. Nature played a cruel joke on aquaculture when she decided that the saturation concentration of dissolved oxygen would be highest at low temperature and lowest at high temperatures. This condition is exactly the opposite of what fish require for basic metabolism and food conversion, which is highest at high temperatures and lowest at low temperatures. Although the air we breathe contains 21% oxygen, oxygen is only slightly soluble in water. As a result, aquatic species must spend a great deal of energy to remove the dissolved oxygen from water, as compared to the energy that land dwelling species expend to obtain oxygen from the air. As previously mentioned, oxygen solubility decreases as temperature and salinity increase. Both barometric pressure and altitude directly affect oxygen concentration.

It is difficult to specify critical dissolved oxygen concentrations, because the response to low dissolved oxygen is not life-or-death, but a continuum of physiological effects. Also, these effects are influenced by the exposure time, the size and health of the fish, water temperature, concentration of carbon dioxide, and other environmental conditions. In general though, warm water fish feed best, grow fastest, and are healthiest when dissolved oxygen concentrations are above about 5 mg/L. However, concentrations of dissolved oxygen greater than this level of saturation appear to provide no additional benefit to fish. The gills can only transfer so much oxygen to the blood, and are very near or at the maximum transfer capability when the environmental dissolved oxygen concentrations are at the recommended concentrations. Higher oxygen concentrations in the water do not result in any additional oxygen carried by the blood stream.

For salmonids, as a group, the rearing unit effluent should contain from 6.0 to 8.0 mg/L dissolved oxygen (DO). For catfish and tilapia, allowable minimum levels are much lower than for salmonids, e.g., 2 or 3 mg/L, while it is certainly recommended to stay much closer to 5 or 6 mg/L. This variation in what the allowable minimums are has to do with the fact that partial oxygen pressure (pO2) appears to be a more valid way to determine the lower limits. A pO2 of 90 mm Hg seems to be a reasonable target for salmonids (Downey and Klontz, 1981). The atmosphere contains 21% oxygen, and at standard pressure of 760 mm Hg, this represents a pO2 of 0.21 • 760 or 160 mm Hg or 56% of saturation. Where dissolved oxygen saturation represents 9.0 mg/L, 90 mm Hg represents 5.1 mg/L DO, and where saturation represents 12.5 mg/L DO, 7.0 mg/L represents a pO2 of 90 mm Hg. These ranges represent temperatures from 20°C down to 5°C at sea level elevation (760 mm Hg). As noticed, the warmer the water the lower the effluent DO in mg/L can go, while still representing a pO2 of 90 mm Hg. Applying 90mm pO2 @ 30°C for tilapia would set a target value of 4.2 mg/L for culture tank oxygen levels.
Temperature

Three Classifications:

- cold-water species below 15 ° C (60° F)
- cool-water species between 15 °- 20° C (60°- 68° F)
- warm-water species above 20° C (68° F)

Water temperature is second only to dissolved oxygen in importance and effect on the economic viability of a commercial aquaculture venture. Temperature directly affects the physiological processes, such as respiration rate, efficiency of feeding and assimilation, growth, behavior and reproduction. Fish have traditionally been grouped into three classifications depending on their temperature preference: cold-water, cool-water and warm-water. Cold-water species prefer water temperatures below approximately 15 ° C (60° F), cool-water species between 15 °- 20° C (60°- 68° F), and warm-water species above 20° C (68° F). These are not exact definitions and factors involved in the tolerance of fish to different temperatures; include species, age, size and past thermal history.

Fish are classified as poikilothermic or cold-blooded, which means that their body temperature is approximately the same as their surrounding environment. Therefore, each species has an optimum temperature range that maximizes growth and an upper and lower limit beyond which they cannot survive. Within the species tolerable temperature range, growth rates increase as the water temperature increases, until the optimum temperature is reached. Above the optimum temperature, the increased energy requirements for food conversion and other metabolic processes ensure that the law of diminishing returns applies. Also, at higher than optimum temperatures, the fish food conversion ratios are lower. Further temperature increases beyond optimum are of no benefit, and may in fact approach lethal levels. Thus to insure maximum growth and minimize stress, system temperatures need to be maintained as close as possible to optimum value.
Nitrogen is an essential nutrient for all living organisms, and is found in proteins, nucleic acids, adenosine phosphates, pyridine nucleotides, and pigments. However, nitrogen is required in relatively small quantities, and physiological needs are easily satisfied. Excess quantities then become nitrogenous wastes, and removal is necessary. The fish create and expel various nitrogenous waste products through gill diffusion, gill cation exchange, and urine and feces excretion. In addition to the urea, uric acid, and amino acid excreted by the fish, nitrogenous wastes accumulate from the organic debris of dead and dying organisms, uneaten feed, and from nitrogen gas in the atmosphere. Decomposing these nitrogenous compounds is particularly important in intensive RAS because of the toxicity of ammonia, nitrite, and to some extent, nitrate.

Ammonia, nitrite, and nitrate are all highly soluble in water. Ammonia exists in two forms: un-ionized NH₃, and ionized NH₄⁺. The relative concentration of ammonia is primarily a function of water pH, salinity, and temperature. The sum of the two (NH₄⁺ + NH₃) is called total ammonia or simply ammonia. It is common in chemistry to express inorganic nitrogen compounds in terms of the nitrogen they contain, i.e., NH₄⁺–N (ionized ammonia nitrogen), NH₃–N (un-ionized ammonia nitrogen), NO₂–N (nitrite nitrogen) and NO₃–N (nitrate nitrogen). This allows for easier computation of total ammonia-nitrogen (TAN = NH₄⁺–N + NH₃–N) and easy conversion between the various stages of nitrification.
Un-ionized ammonia (NH₃–N) is the most toxic form of ammonia because of its ability to move across cell membranes, so the toxicity of TAN is dependent on the percentage of the un-ionized. An increase in pH, temperature, or salinity increases the proportion of the un-ionized form of ammonia nitrogen. The concentration of NH₃-N can be computed knowing the pH from the following equation:

\[
[TAN] = \frac{\frac{[NH_3-N]}{pK_a} + \frac{1}{pH-pK_a}}{1 + \frac{[NH_3-N]}{pK_a} + \frac{1}{pH-pK_a}}
\]

where \([TAN]\) is the measured concentration of total ammonia nitrogen (mg/L); \(pK_a\), the acidity constant for the reaction (9.40 at 20 °C); \(pH\), the measured pH of the solution; \([NH_3-N]\), computed concentration in (mg/L).

For example, given a TAN = 5.0 mg/L at 20ºC and a pH of 7.0, the mole fraction of un-ionized ammonia is only 0.020, negligible impact on most fish. However, at a pH of 9.0, the un-ionized ammonia increases to 1.43 mg/L, killing most typical fish in hours. The fraction of un-ionized ammonia at different temperatures and pH are included in the Appendix.

Ammonia appears to have a direct effect on the growth of aquatic animals. Un-ionized ammonia is toxic to fish at low concentrations, with 96-hour LC50's varying widely by species from as low as 0.08 mg/L NH₃-N for pink salmon to 2.2 mg/L NH₃-N for common carp. In general, warm-water fish are more tolerant to ammonia toxicity than cold-water fish, and freshwater fish are more tolerant than saltwater fish. In general, for commercial production, un-ionized ammonia concentrations should be held below 0.05 mg/L and TAN concentrations below 1.0 mg/L for long-term exposure.
Percent unionized ammonia-nitrogen as a function of pH and temperature.

<table>
<thead>
<tr>
<th>Temp</th>
<th>6.0</th>
<th>6.5</th>
<th>7.0</th>
<th>7.5</th>
<th>8.0</th>
<th>9.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>-</td>
<td>0.1</td>
<td>0.2</td>
<td>0.6</td>
<td>1.8</td>
<td>15.7</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>0.1</td>
<td>0.3</td>
<td>0.9</td>
<td>2.7</td>
<td>21.5</td>
</tr>
<tr>
<td>20</td>
<td>-</td>
<td>0.1</td>
<td>0.4</td>
<td>1.2</td>
<td>3.8</td>
<td>28.4</td>
</tr>
<tr>
<td>25</td>
<td>0.1</td>
<td>0.2</td>
<td>0.6</td>
<td>1.8</td>
<td>5.4</td>
<td>36.3</td>
</tr>
<tr>
<td>30</td>
<td>0.1</td>
<td>0.3</td>
<td>0.8</td>
<td>2.5</td>
<td>7.5</td>
<td>44.6</td>
</tr>
</tbody>
</table>
Nitrite is the ionized form of the relatively strong acid, nitrous acid and is the intermediate product in the process of nitrification of ammonia to nitrate. Although nitrite is converted to nitrate relatively quickly by ozone and the nitrifying bacteria in a properly balanced biofilter, it is a problem in recirculating systems because it is being created on a constant basis, so the fish are continually exposed to certain concentrations. Therefore, it is an important water quality parameter to monitor and correct if acceptable limits are exceeded. Nitrite is toxic because it affects the blood hemoglobin's ability to carry oxygen. When nitrite enters the bloodstream, it oxidizes the iron in the hemoglobin molecule from the ferrous state to the ferric state. The resulting product is called methemoglobin, which has a characteristic brown color, hence the common name “brown-blood disease”. The amount of nitrite entering the blood depends on the ratio of nitrite to chloride in the water, in that increased levels of chloride reduce the amount of nitrite absorption. Chloride levels can be increased by adding ordinary salt (sodium chloride) or calcium chloride. At least a 20:1 ratio of chloride to nitrite-nitrogen (Cl: NO₂⁻N) is recommended for channel catfish in ponds, tilapia, and rainbow trout.
Nitrate is the end product of nitrification and is the least toxic of the nitrogen compounds, with a 96-h LC values usually exceeding 1000 mg NO₃-N /L. In recirculation systems, nitrate levels are usually controlled by daily water exchanges. In systems with low water exchange or high hydraulic retention times, denitrification has become increasingly important.
The pH value expresses the intensity of the acidic or basic characteristic of water. In chemical terms, pH is the negative logarithm of the hydrogen ion concentration. The pH scale ranges from 0 to 14, with a pH of 7.0 corresponding to the neutral point. Values of pH below 7.0 are acidic (the H+ ion predominates), and above 7.0, values are basic or alkaline (the OH+ ion predominates). The pH of most groundwater's and surface waters are buffered by the bicarbonate-carbonate system and have pH values between 5 and 9. Exceptions to this are groundwater with high concentrations of dissolved carbon dioxide, and water draining from some mines (acid mine drainage (AMD)). AMD is the result of the oxidation of mineral sulfides, which becomes sulfuric acid. Seawater is buffered by the bicarbonate-borate system and has a relatively stable pH between 8.0 and 8.5. The optimum pH for the growth and health of most freshwater aquatic animals is in the range of 6.5 to 9.0.

Exposure to extreme pH can be stressful or lethal, but it is the indirect effects resulting from the interactions of pH with other variables that are more important in aquaculture. pH controls a wide variety of solubility and equilibria reactions, the most important of which is the relationship between the un-ionized and the ionized form of ammonia and nitrite. pH also affects the toxicity of hydrogen sulfide and metals such as copper, cadmium, zinc and aluminum.
In general terms, alkalinity is a measure of the pH-buffering capacity or the acid-neutralizing capacity of water. In chemical terms, alkalinity is defined as the total amount of titratable bases in water expressed as mg/L equivalent calcium carbonate (CaCO₃). Sometimes alkalinity is expressed as milliequivalents/liter, where 1 meq/L equals 50 mg/L as CaCO₃. The principle ions that contribute to alkalinity are carbonate (CO₃²⁻) and bicarbonate (HCO₃⁻). In practical terms, alkalinity is measured by titration with sulfuric or hydrochloric acid to the methyl orange endpoint (pH of 4.5).

Alkalinity ranges in freshwater from less than 5 mg/L in soft water to over 500 mg/L, and is determined by the geology of the aquifer or watershed. The alkalinity of seawater is about 120 mg/L CaCO₃. Required alkalinity concentrations are directly linked to system pH and carbon dioxide concentrations. To maintain carbon dioxide concentrations at less than 15 mg/L and pH between 7.0 and 7.4 requires an alkalinity concentration less than 70 (high pH condition) to 190 mg/L CaCO₃ (low pH condition).
The relationship between pH, alkalinity, and CO₂ concentrations.

In recent years, as aquaculture system stocking density and hydraulic retention time has increased, the relationship between pH and alkalinity has become a significant issue. This relationship requires careful monitoring and adjustment of both alkalinity and carbon dioxide levels to maintain optimum pH for both the aquatic species being grown and the biofilters. Alkalinity is easily adjusted through the addition of sodium bicarbonate (NaHCO₃), common baking soda. Other materials can be used, but sodium bicarbonate is commercially available in 50 to 100 lb (23 to 45 kg) bags, safe, inexpensive, and easy to apply. It has very high water solubility and rapidly dissolves in water at ambient temperature. A general rule of thumb is that for every pound of feed, approximately 0.25 lbs (113 g) of sodium bicarbonate should be added to the water. Carbon dioxide concentrations are routinely controlled through degasification systems, such as counter-flow gas stripping towers.
Hardness is the term used to describe the ability of water to precipitate soap. The harder the water, the greater the amount of soap that must be added to a given volume of water to get the same cleaning action. In chemical terms, hardness is defined as the total concentration of primarily calcium (Ca$^{2+}$) and magnesium (Mg$^{2+}$), iron, and manganese in terms of mg/L equivalent calcium carbonate (CaCO$_3$). In practical terms, hardness is measured by chemical titration. The total hardness of natural waters ranges from less than 5 to over 10,000 mg/L CaCO$_3$. Waters have traditionally been classified as soft (0–75 mg/L), moderately hard (75–150 mg/L), hard (150–300 mg/L), or very hard (> 300 mg/L).

Hardness is often confused with alkalinity, probably because both are commonly defined in terms of mg/L CaCO$_3$. In fact, if the alkalinity of the water originates from limestone, the concentrations of hardness and alkalinity can be similar if not identical. Conversely, many coastal-plain area groundwaters have high alkalinity and very low hardness concentrations. Aquifers in regions of basalt and granite often have waters of low total hardness and low alkalinity, because of the relative low solubility of these minerals. If this low hardness and low alkalinity water is used for aquaculture, the water must be "hardened" by adding dissolved calcium to support newly fertilized freshwater fish eggs and for calcification of larval skeletal structures. Calcium and magnesium also decrease the toxicity of dissolved metals. Recommendations for total hardness range from 20 to 300 mg/L.
Carbon Dioxide is highly soluble in water, but concentrations in pure water are low (0.54 mg/L at 20°C), due to its low concentration in the atmosphere (about 0.035% by volume). Most of the carbon dioxide in an aquaculture water column is produced by animal respiration and the decomposition of organic matter, with a small percentage coming from atmospheric diffusion. The concentration of carbon dioxide in groundwater can range from 0 to 100 mg/L, depending on the biological activity in the aquifer recharge zone. The concentration of dissolved carbon dioxide in surface waters depends on the rate of respiration, photosynthesis, and gas exchange with the atmosphere.

Exposure to high carbon dioxide concentrations reduces respiration efficiency and decreases the tolerance to low dissolved oxygen concentrations. High levels of carbon dioxide in the water reduce carbon dioxide excretion at fish gills. This, in turn, causes the CO₂ concentration in the fish blood to increase, lowering the blood plasma pH, which creates a condition called respiratory acidosis. When a fish is in this condition, the amount of oxygen that the blood hemoglobin can carry is reduced and respiration distress can occur, even with high concentrations of dissolved oxygen in the water. This is referred to as the Bohr-Root effect. An upper limit of 15–20 mg/L carbon dioxide is recommended as steady state maximum for finfish, although this recommendation is poorly supported by research. However, as a management technique, higher concentrations (60–80 mg/L) have a narcotic effect on aquatic animals, and are sometimes used on a temporary basis as an anesthetic to reduce handling stress and during treatment procedures.

Carbon dioxide differs from oxygen, nitrogen, and other gases, because its concentration in water is determined both by a gas-liquid equilibrium relationship and also by a series of acid-base reactions. Gas-liquid equilibrium influences the transfer of carbon dioxide between air and water and the acid-base reactions determine the chemical form in which dissolved inorganic carbon is present in water.
Solids – settleable, suspended, dissolved

Three categories:
- settleable
- suspended
- fine or dissolved solids

• upper limit: 25 mg TSS/L
• normal operation (species dependent)
  • 10 mg/L for cold water species
  • 20 – 30 mg/L for warm water species

Rule of Thumb
Solids produced by fish:
0.3 to 0.4 kg TSS for every 1 kg of feed fed

Waste solids accumulating in an aquaculture system come from uneaten feed, feed fines, fish fecal matter, algae, and biofilm cell mass sloughed from biological filters. Studies indicate that fish produce between 0.3 to 0.4 kg total suspended solids (TSS) for every 1 kg of feed fed. Waste solids influence the efficiency of all other unit processes in a recirculating system. They are a major source of carbonaceous oxygen demand and nutrient input into the water, and can directly affect fish health within recirculating systems by damaging fish gills and harboring pathogens. The tentative upper limit for freshwater fish is 25 mg TSS/L, with 10 mg TSS/L usually recommended for cold water species and 20 to 30 mg/L for warm water species like tilapia. Therefore, solids removal is one of the most critical processes in aquaculture systems. Optimally, solids need to be removed from the fish culture tank as soon as possible, while creating as little turbulence and mechanical shearing as possible.

Solids are generally classified into three categories: settleable, suspended, and fine or dissolved solids. The difference between settleable and suspended solids is simply the time it takes for them to settle to the bottom of an Imhoff cone. Settleable solids settle out in less than an hour. Suspended solids do not, and therefore require a treatment process other than conventional gravity settling basins. Fine and dissolved solids are by their very nature difficult to remove.
Salinity refers to the total concentration of dissolved ions in water, the major contributors being calcium, sodium, potassium, bicarbonate, chloride and sulfate. Salinity is usually reported as parts per thousand (ppt, grams of salt per kilogram of water). The salinity of natural waters tends to reflect the climate, geography and hydrological conditions of the immediate surroundings. Each aquatic species has an optimum range of salinity for reproduction and growth, although the salinity tolerance of most aquaculture species is rather broad. For example, rainbow trout fingerlings are produced in freshwater, acclimated to saltwater and then grown out to marketable size in sea cages at salinities as high as 20 ppt. Most freshwater fish of importance in aquaculture reproduce and grow well at salinities up to a least 4-5 ppt.

Osmoregulation is the process of maintaining the concentration of dissolved salts in the body fluids by regulating the uptake of ions from the environment and through the restriction of ion loss. Freshwater fish for example tend to accumulate water because they have body fluids more concentrated in ions than the surrounding water. When exposed to salinity values outside of their optimum range, aquatic species must spend considerable energy for osmoregulation at the expense of other functions, such as growth. If salinity deviates too far from optimum, the animal cannot maintain homeostasis and dies. The blood of freshwater fishes has an osmotic pressure approximately equal to the osmotic pressure of a 7 ppt sodium chloride solution. Often times to reduce stress and reduce energy required for osmoregulation, freshwater aquaculture systems are maintained at 2-3 ppt salinity.
Although dissolved oxygen (DO) can be measured analytically by using the Winkler Method and a simple titration, the dissolved oxygen meters available today allow for rapid and accurate analysis over a range from zero to supersaturation. Two types of electrodes are commonly used: polarographic and galvanic. In simple terms, both of these meters consists of an electrode, which produces a signal proportional to the concentration of the oxygen in the water, and instrumentation to convert the signal reading to a visual display or recording device. A typical polarographic DO electrode or probe consists of a gold electrode and a silver-silver oxide reference electrode. The electrodes are bathed in 4 M KCL and separated from the sample by a membrane, usually made of Teflon, polyethylene, or fluorocarbon. The membrane is permeable to gases and the rate at which oxygen crosses the membrane is directly proportional to the dissolved oxygen concentration in the sample. When an electrical voltage is applied to the probe, molecular oxygen diffusing across the membrane reacts with the cathode (gold ring) and a small current flows to the anode electrode (silver). In a galvanic system, the electrode generates a small voltage (mV range) proportional to the dissolved oxygen concentration. Both systems require temperature, atmospheric pressure, and salinity compensation, usually accomplished by a combination of calibration and instrumentation hardware and software. Currently there are numerous DO meters available, over a wide range of price scales. As usual, you usually will “get what you pay for”, so invest in a good oxygen meter (~ $500 minimum).
Traditionally, temperature was measured with a simple mercury thermometer. Recently, these are becoming harder and harder to obtain, due to the significant environmental harm (and potential economic hardship) it will do if broken and mercury is released into the environment (or in some cases, the production tank!). Thankfully, there are a host of alternative measurement devices, and most of them are more convenient and flexible in their use. To start with, most DO meters and pH meters have some form of built-in temperature measurement for calibration and temperature compensation. Second, simple handheld temperature meters are relatively cheap, accurate, and easy to use. Finally, these types of temperature measurements allow for easy data logging and control.
Like the dissolved oxygen meters, easy to read pH meters are available over a wide range of specifications and price. In addition to pH, most pH meters are also capable of measuring other ion specific electrodes, which include such parameters as ammonia, nitrate, ORP, dissolved oxygen, conductivity, among others. However, please keep in mind that most of these ion specific electrodes were designed to be used under laboratory conditions and require some sample pretreatment, and they are limited to specific ranges of concentration. It is recommended that a combination, gel-filled pH probe be used. This instrument is inexpensive, less susceptible to clogging, needs no refilling with electrolytes, and requires little or no maintenance.
Several types of dissolved carbon dioxide meters have recently become available. These work by first measuring the pH of the solution, and then calculating the CO₂ concentration based on the alkalinity (which must be known) and the carbonate equilibrium relationships. If the alkalinity is relatively constant, then CO₂ levels can be easily monitored and controlled by simply measuring the pH.

Carbon dioxide is usually measured by titration of a water sample to the phenolphthaleine endpoint (pH 8.3) with a standard base. Approximate CO₂ concentrations can be determined from known values of pH, temperature, and alkalinity using either a formula or table values.
Salinity can be indirectly measured using the measurement of a physical property such as refractive index, conductivity or density. Of these, the two most commonly used in aquaculture are the refractive index and conductivity measurements. A refractometer utilizes a change in the refractive index as function of the salt concentration to directly display the salinity level. It is very simple to use. Simply place a drop of water on the window and look through the eyepiece to directly read the salinity in parts per thousand. Refractometers provide fast, accurate salinity measurements, but some form of temperature-compensation is recommended when used outside their normal operating range of 20°C. One problem they do have is that most commercially available models have a wide salinity range of 0–100 ppt, making accurate measurements at low salinities difficult.

Electrical conductivity measurements can also provide a simple and quick measurement of salinity. Conductivity meters measure the flow of electrical current between two electrodes submersed in water, which is directly proportional to the concentration of ions, i.e., salts, in the water. They can be very accurate over a wide range of concentrations; these meters are direct reading and very portable.

Hydrometers measure salinity based on changes in water density (specific gravity) as a function of salinity. However, accurate and reliable hydrometers are more expensive than refractometers or electrical conductivity meters that provide the same degree of accuracy and ease of use, so they are typically not used. Inexpensive hydrometers are not recommended at all except for very gross estimates.
Almost all of the following chemical analyses are easily performed colorimetrically with off-the-shelf analysis kits manufactured by several companies. In most cases, using these kits is an easy process. Reagents come premixed in small vials, capsules, or bags, which requires only opening, mixing, and waiting for the specified period of time. Then, the mixture is measured, usually with a spectrophotometer. Analysis is quick, cheap, requires very little expensive laboratory equipment, and does not generate large quantities of hazardous wastes.
In the basic Winkler method, a sample of water is treated with manganous sulfate, potassium iodide, and sodium hydroxide. Under these highly alkaline conditions, the manganous ion is oxidized by the dissolved oxygen to manganous dioxide. Sulfuric acid is then added to dissolve the precipitate and produce acid conditions for the oxidation of iodide to iodine by manganous dioxide. The quantity of iodine released is proportional to the amount of dissolved oxygen (DO), and is estimated by titration with standard sodium thiosulfate with a starch indicator as the endpoint. This sounds difficult, but in practice, all of the reagents are available in a premixed form in many commercially available test kits and thus DO measurement is a fairly routine activity. With the availability of relatively low cost dissolved oxygen meters, this method is usually used only for cross checking and calibration purposes.
Chemical Analysis – CO₂

4500-CO₂ Carbon Dioxide

Free CO₂ reacts with sodium hydroxide (0.0227 N) to form sodium bicarbonate; completion indicated using a pH meter (8.3) or phenolphthalin indicator.

1 ml of NaOH equals 1 mg/L CO₂.

The simplest method of determining carbon dioxide concentration in the water column is to use a nomograph when the pH and total alkalinity are known. Then, these values can be compared to the nomographic data to determine the CO₂ concentration of the sample.

If pH and alkalinity are not known, they must first be determined. Then, CO₂ can be determined by adding a titrating solution of sodium hydroxide (0.0227 Normality or N) to a specified volume of sample water (normally, 250 mL). Since water at a pH over 8.34 does not contain appreciable amounts of carbon dioxide, the amount of a base (normally, sodium hydroxide) needed to raise the sample's pH to the 8.34 pH endpoint is approximately equivalent to the carbon dioxide content of the sample. Free carbon dioxide reacts with sodium hydroxide (0.0227 N read strength titrating solution) to form sodium bicarbonate, which raises the sample's pH. When sufficient amounts of the titrating solution of sodium hydroxide have been added to raise the sample's pH to 8.34, and then the quantity of sodium hydroxide that was required to reach this endpoint is comparable to the amount of CO₂ in the sample, at a relationship of 1 mL of sodium hydroxide (NaOH) equaling 1 mg/L CO₂ concentration of the sample.
Alkalinity is a measure of the acid neutralizing capacity of the water. Alkalinity can be measured by titration with 0.02 N sulfuric acid and titrating to a pH end point or using a phenolphalein indicator to show the color change as the indicator to stop adding titrant. With a 0.02 N sulfuric acid, one mL of titrant equals 10 mg/L of alkalinity (defined as calcium carbonate, CaCO₃). Only a few items are required to perform these measurements: a 125 mL beaker, a 50 mL buret, 0.02 N sulfuric acid (available premixed), methyl orange indicator, and a dropper.
### Chemical Analysis – Ammonia, Nitrite and Nitrate

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia</td>
<td>colorimetric Nesslerization, ion specific electrodes</td>
</tr>
<tr>
<td>Nitrite</td>
<td>colorimetric</td>
</tr>
<tr>
<td>Nitrate</td>
<td>reducing to nitrite with cadmium catalyst, measure nitrite, ion specific electrode</td>
</tr>
</tbody>
</table>

The easiest method for measuring ammonia, nitrite, and nitrate is to use a colorimetric technique and either a color wheel comparison or a spectrophotometer.

For ammonia, the colorimetric Nesslerization method is the most popular. In this method, a nessler-ammonia reaction produces a yellow to brown color that is proportional to the ammonia-nitrogen concentration. This is a simple process to use, requiring only the addition of one commercially available reagent to the water sample, and then taking a measurement of the ammonia-nitrogen concentration with a spectrophotometer at 425 nm.

Nitrite is usually measured using a Colorimetric method. Diazotized sulfanilamide and NED dihydrochloride are added to the water sample, which creates a reddish purple azo dye, which is then measured with a spectrophotometer at 543 nm. The degree of transmittance in the spectrophotometer correlates to nitrite concentration data as presented in the form of standardized curves.

Finally, nitrate is measured by reducing it to nitrite with a Cadmium catalyst and then measuring nitrite concentration.

Reagents for all of these analysis methods are readily available in premixed or packet form. There are also several manufacturers of spectrophotometers, including Hach, YSI, and LaMotte. Although the use of a color wheel comparison is applicable in some cases, for commercial systems the cost of a spectrophotometer and the flexibility and accuracy it provides is worth the expense.

Ion Specific Electrodes (ISE) allows measurement of ammonia, nitrate, and several other parameters using the pH/mV input of a standard pH meter. Although these have been used in the wastewater treatment industry and in a laboratory setting, they are not popular in the aquaculture industry. This is probably because of the difficulty in maintaining calibration, the need to pre-treat the water sample, and high equipment costs associated with this type of measurement method.
Total solids (TS) are the weight of the material residue left after evaporation of a sample at a temperature of 105°C. Total suspended solids (TSS) is the portion of material retained by a filter, and total dissolved solids (TDS) is the portion of material that passes through the filter. Total volatile solids (TVS) is the difference in weight of the total solids after burning them to ashes, and settleable solids (SS) is the material that settles out of suspension within a defined period of time.

The most commonly used measurement of solids is the total suspended solids and the settleable solids. The amount of total suspended solids is simple to determine, although a fair amount of laboratory equipment is required. The equipment requirements are: a drying oven (105°C), a filtration setup including vacuum pump, an analytical balance (0.1 mg), and glass fiber filters (Whatman GF/C). To take a TSS measurement, first weigh the glass fiber filter, and then filter a known volume of sample through it. Next, dry the filter at 105°C for at least one hour. When dry, weigh the filter again and divide the difference in weight by the volume of sample. The result is the total suspended solids concentration (mg/L).

Settleable solids are measured with an Imhoff cone, which is a cone shaped container with volume gradations at its base. A sample of 1 L is poured into the Imhoff cone and allowed to settle for one hour, after which the volume of settleable solids in the base of the cone are read in mL/L.
Phosphorus is measured by using a colorimetric technique similar to ammonia-nitrogen. In this method, ammonium molybdate and potassium antimonyl tartrate are added to the sample, and they react to form a heteropoly acid. Then, ascorbic acid is added, which reduces the heteropoly acid to intensely colored molybdenum blue. The reactive phosphorus concentration (orthophosphorus) is then measured with a spectrophotometer at 880 nm.
A water quality lab doesn’t have to be large, but it should be dedicated only to that task.