A Description and Operations Manual for a Water Column Simulator Which is Used to Study the Behavior of Organisms in Vertically Stratified Waters

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TABLE OF CONTENTS

ABSTRACT

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An aquarium system is described, which may be operated to simulate vertically stratified fresh, estuarine or marine waters. The Water Column Simulator (WCS) provides the opportunity to examine the behavior of organisms under uniform or well-defined gradients in the vertical plane. The apparatus comprises a 4500 L aquarium provided with a regulated water delivery and circulation system, vacuum degasser apparatus, seasonal photoperiod lighting, continuous physico-chemical monitoring via a computerized data acquisition system, and time-lapse video recording in conjunction with a computerized digital image analysis program. A stable, vertically stratified water column may be established by provision of water of different densities. Dissolved oxygen, salinity, temperature, total gas pressure, water velocity, and light intensity are manipulated and controlled during operation under continuous-flow conditions.

A physical description of the WCS is provided which includes dimensions, construction materials, equipment manufacturers and specifications plus detailed schematic diagrams. Instructions for operation, calibration and maintenance of the WCS apparatus and associated equipment are supplied.

RÉSUMÉ

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Le présent article décrit un système d'aquarium qui peut servir à simuler la stratification verticale des eaux douces, estuariennes ou marines. Le simulateur à colonne d'eau offre la possibilité d'examiner le comportement des organismes dans des conditions uniformes ou en présence de gradients bien définis dans le plan vertical. L'appareil comporte un aquarium de 4500 L doté d'un système d'alimentation et de circulation de l'eau, un dégazeur sous vide, un éclairage simulant la photopériode saisonnière, un dispositif de suivi physicochimique en continu grâce à un système informatisé de collecte des données, et un magnétoscope à enregistrement séquentiel associé à un programme d'analyse des images numériques. On peut créer une colonne d'eau stable, à stratification verticale, par un apport d'eau à différentes densités. On peut modifier et contrôler la teneur en oxygène dissous, la salinité, la température, la pression totale des gaz, la vitesse de l'eau et l'éclairement au cours de l'opération, dans des conditions de circulation continue de l'eau.

L'article donne une description physique du simulateur, avec ses dimensions, les matériaux employés, le nom des fabricants et les spécifications, ainsi que des diagrammes détaillés. On fournit aussi les instructions pour le fonctionnement, l'étalonnage et l'entretien du simulateur et de l'équipement connexe.

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The concept for the Water Column Simulator (WCS) originated from in-situ studies on the vertical distribution of fish (Birtwell 1977), and the requirement for laboratory apparatus that could control some of the variables affecting fish behavior. The basic WCS was constructed in 1984, at the Department of Fisheries and Oceans, West Vancouver Laboratory, British Columbia, Canada, by Western Canada Hydraulics Ltd., and has subsequently been modified and improved. The apparatus is housed in a self-contained, sound-insulated, temperature and humidity controlled building which reduces visual and acoustic interference with the experimental organisms. A plan showing the arrangement of the major apparatus components is presented in Figure 1.

An important feature of the WCS apparatus is its' ability to maintain a vertically stratified water column which mimics that of an estuary with a well-defined halocline and/or thermocline between adjacent horizontally-flowing water masses. The WCS aquarium provides an opportunity to study the behavior of organisms in the vertical plane under controlled laboratory conditions. This contrasts with the majority of other gradient systems that have been employed to study animal behavior in the horizontal plane (McCauley 1977; Larrick et al. 1978; Giattina and Garton 1983; Rand 1985). In addition to vertical stratification, the apparatus provides the flexibility of controlled manipulation of several physico-chemical conditions such as salinity, dissolved oxygen, total gas pressure, water velocity, light intensity, and temperature within three distinct layers of water. The system is designed to recirculate water in each horizontally flowing layer, with continuous partial replacement. This replenishment facilitates the maintenance of dissolved gas levels and the vertical stratification of salinity or temperature.

The apparatus comprises ten major components: a 4500 L Plexiglas® aquarium, water delivery system, water circulation loops, water heating and cooling systems, an instrument package for water quality monitoring, adjustable photoperiod lighting, a computerized data acquisition system, and video recording equipment integrated with a digital image analysis program. The objective of this manual is to describe the function and operation of the WCS apparatus and its' associated components.

WATER DELIVERY SYSTEM

PLUMBING MATERIAL

The function of the water delivery system is to supply fresh and seawater to the WCS apparatus for filling and for the continuous replacement of water during its operation. The water delivery system plumbing incorporates thermoplastic polyvinyl chloride (PVC) pipes, which are schedule 40 unless otherwise noted, and described in the conventional unit of inches. The principal reason for selecting PVC pipes is the unique combination of chemical resistance and physical properties it makes available at a reasonable cost. Plastic piping has resistance to nearly all acids, caustics, salt solutions, and other corrosive liquids, however it is dissolved by polar solvents such as ketones, some chlorinated hydrocarbons and aromatics (Celanese Piping Systems 1979). It does not corrode, rust, scale, pit, or rot, and resists the growth of algae, bacteria and fungi that could cause offensive odors or health concerns to animals in the receiving waters. Due to the smooth inner wall, plastic piping facilitates maximum flow rates, abrasion resistance and minimum build-up of sludge and slime. Because most plastics are non-conductive, PVC piping is not subject to galvanic or electrolytic corrosion, a major cause of failure when metal pipe is installed underground. Plastic pipe can be buried in acid or alkaline, wet or dry soil without painting or other special protective coatings. Plastic pipe is tough and strong with tensile and burst strength sufficient to handle operating pressures encountered in most moderate-service processes. Plastic piping is versatile in that it can be fabricated by a variety of methods: solvent welding, fusion welding, threading and flanging. PVC pipe weighs one-sixth as much as metal pipe, which makes it easier to handle, join and install, especially in cramped spaces.

SEAWATER SUPPLY

Seawater is pumped (Figure 2, No. 5) directly from Burrard Inlet adjacent to the West Vancouver Laboratory; the seawater intake is situated 109 m offshore at a depth of 18 m below the low tide level. Incoming seawater tends to be high in particulate and organic matter, which reduces water clarity and impedes observation of the organisms within the waters of the WCS aquarium. Accordingly, the seawater travels in an 8" PVC pipe from the pumphouse through a set of 4 seamless, wound fiberglass, high-rate pressure sand filters (Stark Aquatic Systems Inc., Seattle WA) (Figure 2, No. 6). The filter medium is #20 sieve grade crystal silica sand which has the capacity to remove particles of 20 μm diameter. Subsequently, seawater travels through a 4" pipe for approximately 175 m to the WCS building where the line is reduced to 2". Seawater flow to the WCS aquarium is regulated by two 2" ball valves (Figure 2, No. 29 and No. 17) on a 2" pipe located near the base of an aeration tower (Figure 1, and Figure 2, No. 24) which is used to ensure that the gases in solution are at air saturated levels.

The seawater drawn from Burrard Inlet varies seasonally in terms of salinity (26-30 ‰) and temperature (6.7-14.2 °C). This seawater can also be low in dissolved oxygen, especially in the summer, and levels as low as 60% of air saturation have been recorded. To compensate for possible reductions, oxygen is injected, post-filter, into the main 8" seawater supply line via porous stainless steel spargers, which are perforated-plate distributors used to even out liquid-flow fluctuations. Oxygen flow is manipulated by a motorized control valve regulated by a dissolved oxygen monitor and controller (PT4 Multi-channel Oxygen Monitor and Oxyguard Probe, Point Four Systems Inc., Port Moody, BC; accurate to ± 2% of reading, 0 to 51 $mg \cdot L^{-1}$). An oxygen probe downstream of the injection point measures the dissolved oxygen in the seawater and sends a signal to the O_2 monitor/controller system. Because oxygen is being injected into water which is under pressure in the seawater lines (approx. 30 psi), it readily dissolves without nitrogen and other dissolved gases being removed, or "stripped", from the water. This means that the amount of oxygen that can be added to the seawater will be limited by the criteria set for Total Dissolved Gas Pressure (TGP). TGP at the West Vancouver Laboratory is not to exceed 101% of ambient pressure and dissolved oxygen is not to exceed 93% of air saturation.

When the sand filter system is unavailable, seawater can be filtered at the WCS building through two consecutive filter manifolds (Figure 2, No. 8) before entering the apparatus. The filtration manifold is downstream of valve no. 29 (Figure 2); the 2" seawater supply pipe divides into four parallel pipes, three of which are fitted with a 50 µm pleated polyester fabric cartridge filter (R50-BB, Amatek, Pittsburgh, PA) in a flow through polypropylene plastic housing (No. 10, Blue-Cold, Fabricated Plastics, Ltd., Toronto, ON). Seawater can be diverted through the fourth pipe when filtration is not required. True union ball valves, located upstream and downstream of each housing, permit isolation of the filters during replacement without interruption of water flow or a significant change in operating pressure. Pressure gauges (0-60 psi, Marsh Bellofram Corp., Newell, WV) mounted upstream and downstream of the filters indicate operating pressures; with a minimum pressure of 14 psi required for normal operation. Further downstream this plumbing pattern is repeated with a 30 µm filter cartridge (R30-BB, Amatek, Pittsburgh, PA) housed in a polypropylene plastic manifold (No.20 Blue-Cold, Fabricated Plastics Ltd., Toronto, ON). Empirical testing has indicated that, depending on turbidity and suspended solids concentrations in incoming seawater from Burrard Inlet, the filters should be replaced after 12 h of continuous operation. If filters become clogged while the WCS is operating in the continuous replacement mode, the incoming water flow rate decreases and the water level in the aquarium drops proportionately. This results in substantial air entrainment in the water circulation system, which could jeopardize the vertical stratification in the aquarium and cause damage to the centrifugal recirculation pumps (Figure 3, No. 5). The water may then be supersaturated with gases and the ability to regulate gas levels will be hampered. Operation of valve No. 18 (Figure 2) diverts the flow of filtered seawater directly to the WCS aquarium or through gas equilibration columns (Figure 2, No. 19 and No. 2) prior to admission into the aquarium.

FRESH WATER SUPPLY

Well water is pumped from a 22 m deep aquifer on the West Vancouver Laboratory (WVL) site. The water is fed through approximately 175 m ft of 4" piping from the well to the base of an aeration tower adjoining the WCS building. A 2" pipe diverts well water from the main supply line directly to the WCS aquarium and is regulated at valve No. 27 and No. 9 (Figure 2). A second 2" pipe delivers well water from the main supply line to an aeration tower (Figure 2, No. 24) and the flow of aerated well water to the WCS is regulated at valve No. 26 and No. 9 (Figure 2). Therefore, well water conducted to valve No. 9 (Figure 2) has been either shunted through the aeration tower (Figure 2, No. 24) or has bypassed the tower and remains unaerated. The water temperature varies seasonally, ranging from 9.5 °C to 12.0 °C. The water is typically of consistent quality, i.e. low in dissolved oxygen (23 ± 1% saturation), high in nitrogen

(127 ± 0.3% saturation) and has a total gas pressure of 103 ± 0.5%. Therefore, once well water is delivered to the WCS building, gas-equilibration is required to render it suitable for fish culture and maintenance.

AERATION OF INCOMING WATER

Aeration Tower

Adjacent to the WCS building, is the aeration tower, which is a structure with a platform 12 m above ground level (Figure 2, No. 24). On the platform are 10 buckets, suspended in a vertical series, above a 4,900 L fiberglass header tank reservoir. Each bucket contains a perforated plate on the bottom and is filled with bio-rings (2.5 cm flexi-rings, Koch Engineering Ltd., Calgary, AB) which fracture and disperse incoming water (MacKinlay 1991) into droplets and thin films, providing a large surface area for the exchange of gas. Water is then discharged from the lowest bucket into the reservoir. Layers of Styrofoam™ insulation, float on the water surface and also cover the reservoir, and act in addition to the exterior foil backed bubble reflectex insulation, to minimize heat loss or gain. The reservoir is equipped with a float switch coupled to both audible and visual low water level alarm systems.

Gas Equilibration Columns

There are four gas equilibration columns (Figure 2, No. 2) through which water may pass before supplying the WCS. Depending on requirements, the columns can be used to equilibrate salt or fresh water with gasses and to vary and control dissolved oxygen levels.

Operation of valves No. 10, 11, 19 and 20 (Figure 2) can divert the flow of water into the gas equilibration columns (Figure 2, No. 2) or directly into the WCS aquarium. Therefore, the WCS aquarium can receive water which has been either unequilibrated, equilibrated in an aeration tower (Figure 2, No. 24 and Figure 1), a gas equilibration column (Figure 2, No. 2 and Figure 1) or consecutively through both. The columns equilibrate the dissolved gases in solution, in the incoming water, with those in the atmosphere. Oxygen saturation increases and nitrogen plus argon saturation decreases while only minor changes occur in total gas pressure. For example, 32 $L \cdot min^{-1}$ of 10.4 °C pre-column fresh water of DO_2 23.4%, TGP 102.8% and N_2 + Ar 127.3% will have post-column values of DO_2 83.2%, TGP 101.2% and N_2 + Ar 108.9%.

As each gas equilibration column is identical only one column will be described. A wooden framework adjoining the WCS building supports the 2.4 m column whose top is 6.8 m above ground level. The column consists of a 3" acrylonitrile butadine styrene (ABS) pipe insulated on the exterior with 2 cm of polystyrene foam and coated with fiberglass for weather protection. The column is filled with biorings (2.5 cm flexi-rings, Koch Engineering Ltd., Calgary, AB) which fracture and disperse incoming water. Water flow to the column is regulated through two identical delivery systems each containing a flow meter (CalQFlo®, Model CF-41000LN, Blue and White Industries, Westminster, CA) (Figure 2, No. 1) and two valves (Figure 2, No. 11 and No. 16). A 2" supply pipe feeds water to the top of the column, which is partially sealed to restrict gas emission and raise equilibration efficiency.

Gas equilibrated water from the column flows into a polyethylene constant head reservoir (Figure 2, No. 3) of 140 L capacity. A 0.68 m high, 2" central overflow standpipe (Figure 2, No. 31) connected to a drain pipe maintains the constant volume. The constant head reservoir is covered with a Plexiglas® lid. The column is mounted into an opening in the Plexiglas® lid and the connection is sealed with a neoprene collar. Operation of valve No. 12 (well water) or No. 21 (seawater) directs water through a side outlet connected to a 2" pipe located near the reservoir's base and into a supply loop for the WCS aquarium (Figure 2).

Seawater, which has passed through the gas equilibration columns and constant head reservoir, flows into the WCS water recirculation system (Figure 3) through a 2" pipe, downstream of valve No. 21 (Figure 2). If required, either fresh or salt water can bypass the columns and flow directly into the WCS by means of valve No. 10 for well water and valve No. 19 for seawater (Figure 2). The pipes, which bypass the columns, rejoin the main supply pipe downstream of valve No. 12 (Figure 2, well water) and valve No. 21 (Figure 2, seawater).

Once past the gas equilibration columns the water delivery lines divide into the three PVC loops, which comprise the WCS aquarium water recirculation system. The division occurs downstream of valve No. 12 and 21 (Figure 2), where the well water and seawater pipes each divide via a series of tee-fittings and bushings into three ¾" lines. Each of the three ¾" seawater lines and three ¾" well water lines is fitted with a ball valve (Figure 2, No. 13 and No. 22), check valve (Figure 2, No. 14 and No. 25) pressure regulator and flow meter (Catalog No. FC-FI-A-¾, Fliterchem Corp., Alhambra, CA) (Figure 2, No. 4). The true union ball valves regulate flow and are threaded to facilitate disassembly for cleaning. The six pipes provide either well water or seawater to each of the three water recirculation loops in the apparatus, which supply each of the 3 zones of the WCS aquarium. Downstream of the ball valve on each of the six ¾" pipes, the line converges with a 2" pipe by means of a tee-fitting and a bushing prior to entering a heat exchanger (Figure 2, No. 30 and Figure 3, No. 4).

DEAERATION OF INCOMING WATER

Nitrogen Gas Injection

The WCS may be applied to examine fish behavior in relation to hypoxic conditions (low dissolved oxygen levels). Low dissolved oxygen (DO_2) levels can be obtained in both well water and seawater by using a predominantly parallel current system, in which both water and nitrogen gas enter the top of the gas equilibration column and flow downward (Figure 2, No. 2 and Figure 1). This arrangement was found to be more efficient than a counterflow system in this application. A water trap (Figure 2, No. 33) on the drain pipe leading from the constant head reservoir prevents the escape of nitrogen from the equilibration column during deaeration events.

For deaeration purposes nitrogen is injected into the column 0.8 m from its top through gas impermeable butyl rubber tubing (¼" internal diameter) at the end of which is a 0.5 m diffuser of Micropore™ tubing (Figure 2, No. 28). Nitrogen cylinders, controlled by glass bead gas flowmeters (Catalog No. 36-541-31, Manostat, New York, NY) and regulators, located inside the WCS building (Figure 1), supply N_2 to the gas equilibration column (Table 1). Two nitrogen cylinders are connected in parallel by a ¼" tee-fitting and butyl rubber tubing connected to the flow meters. This configuration minimizes the interruption to nitrogen flow during cylinder replacement.

The optimum flow of nitrogen required for efficient deaeration of incoming water was determined empirically over 40 min trials; the oxygen stripping rate was highest during this period (Table 2). Determination of dissolved oxygen (Model 7932 dissolved oxygen meter, Leeds & Northrup, Covington, GA; and Model 57 dissolved oxygen meter, YSI Inc., Yellow Springs, OH), salinity (Model 33 salinity meter, YSI Inc., Yellow Springs, OH; accurate to ± 1.1%, range of 0-40 ‰), temperature (calibrated mercury thermometer) and TGP (Model 300C tensionometer, Alpha Designs Ltd., Victoria, BC; accurate to ± 1 mm Hg, range of 73% to 190% TGP) were established downstream of the gas equilibration column.

Vacuum Degasser Apparatus

A vacuum degasser apparatus (prototype, Point Four Systems Inc., Port Moody, BC) (Figure 1) provides an alternative approach, to gas stripping with nitrogen, for generation of hypoxic water for use in the WCS aquarium. Degassing is accomplished by exposing the incoming fresh or seawater to a partial vacuum within a 25 x 350 cm PVC column (Figure 6, No. 1). The magnitude of the vacuum regulates the degree of degassing that occurs and hence the level of dissolved oxygen and other gases in effluent water. The control of the water level in the degasser column is maintained by a dynamic equilibrium between water flow and vacuum pressure. Water supplied through a PVC ball valve and flowmeter (Figure 6, No. 14) enters the top of and is dispersed down the packed (2.5 cm flexi-rings, Koch Engineering Ltd., Calgary, AB) column. Effluent water passes via a ¾ h.p. single stage impeller pump (Grundfos Pumps Corp., Clovis, CA) (Figure 6, No. 7) through a PVC ball valve and into a 40 L polyethylene header tank before distribution to the WCS aquarium. Excess water, which is not required in the aquarium, is discharged to waste using a stainless steel bypass solenoid valve (Figure 6, No. 17) controlled by a float activated switch (Figure 6, No. 6) located in the degasser column at the air-water interface.

Measurement and control of dissolved oxygen concentration in the effluent water from the degasser column is achieved through a feedback control loop using a temperature compensated galvanic cell oxygen probe (Figure 6, No. 16) with a multichannel oxygen monitoring unit (Model 1W, Oxyguard, Point Four Systems Inc., Port Moody, BC; accuracy ± 2.0% saturation, 0-100% saturation range). Information is supplied from the oxygen probe to the oxygen controller (Figure 6, No. 11), which in combination with a vacuum gauge (Figure 6, No. 10) and a solenoid valve (Figure 6, No. 12) located on the vacuum line to an aspirator, regulate the absolute pressure within the degasser column. An aspirator (Figure 6, No. 2) is used to evacuate from the column, the gases which accumulate when generating hypoxic water, and to maintain the desired vacuum pressure. In performance tests, a lower operating limit of 14.5% (1.9 $mg \cdot L^{-1}$) dissolved oxygen saturation level in column effluent was maintained with a water input of 38 $L \cdot min^{-1}$ (salinity 0 ‰, temperature 1 °C) and a column pressure of 50 mm Hg absolute.

The apparatus is equipped with various alarms, fail safe interlocks and other features which are described below. Power to the pump ceases automatically, should the water level in the column fall below a set point, thus preventing pump damage from dry operation. Control of power to the pump is via a timer on the alarm panel (Figure 6, No. 3) in conjunction with the level control switch (Figure 6, No. 6). Once activated, the timer will disengage the pump after a time delay unless it is deactivated by the level control switch. To prevent the column from flooding and overflowing in the event of a pump malfunction or power failure, a solenoid valve (Figure 6, No. 15) on the water supply line is interlocked with the pump and is closed when the pump or power is off. A check valve (Figure 6, No. 19) on the pump discharge line prevents water backflow and loss of vacuum in the column, in the event of an interruption in normal pump operation. A dual purpose check valve (Figure 6, No. 8) releases air through the top of the column, during filling with water, prior to pump start up and also allows water to escape if the column is inadvertently flooded, preventing pressure damage. Although it is unlikely that water would be drawn into the vacuum line under normal operating conditions, a water trap (Figure 6, No. 13) collects any water which may accidentally enter the vacuum line from the top of the column during start up procedures. The collected water is purged from the vacuum line through ball valves thereby preventing entry into the solenoid valve as damage to this valve through corrosion and plugging of internal parts could result in faulty vacuum control.

DISSOLVED GAS SUPERSATURATION OF INCOMING WATER

The apparatus that is used to generate dissolved gas supersaturated water with a total gas pressure (TGP) up to 145% saturation is, with a few modifications, the previously described vacuum degasser column (Point Four Systems Ltd., Port Moody, BC) (Figure 6) operated in a pressurized mode. Supersaturation of dissolved gases is accomplished by exposing incoming water at ambient atmospheric pressure to elevated pressure within the 25 x 350 cm sealed PVC column. Water and compressed air are introduced into the top of the column and allowed to pass through the exchange medium (2.5 cm flexi-rings, Koch Engineering Ltd., Calgary, AB) under pressure. The magnitude of the pressure and adjustment of water flow rates regulates the degree of dissolved gas supersaturation that occurs. When water is pressurized inside the column, there exists a dissolved gas deficit relative to saturated values at a specific pressure. Therefore, water that is saturated at atmospheric pressure has the capacity to absorb more gas before becoming fully saturated at the elevated pressure.

The control of seawater level in the pressurized column is maintained through dynamic equilibrium between inflowing water and pressurized air. Seawater supplied from the heated water reservoir by a magnetic drive pump ($^1/_3$ h.p., max capacity 35.6 gallons·min^{-1}, max head 39 ft, Iwaki Co. Ltd., Tokyo, Japan) through a PVC ball valve (20 L·min^{-1}), enters the top of, and is dispersed down the air pressurized column through a bed of plastic packing material (2.5 cm flexi-rings). The packing fractures the incoming water into droplets and thin films, providing a large surface area for the absorption of gas. The level of dissolved gas supersaturation is controlled by adjusting the supply of compressed gas (air) to the column via a flowmeter. If gas addition rate is increased, the water level in the column is lowered, exposing a greater depth of packing, which results in a higher absorption capacity of the column. If gas addition rate is reduced, the water level rises as gas in the headspace is absorbed, and both the depth of exposed packing and absorption capacity is reduced.

Water level in the column will change until a steady state position is reached commensurate with compressed gas addition rate. Water level is controlled automatically via a solenoid valve on the compressed air supply line and an adjustable water level sensor that is attached to a sight glass on the side of the column. The water level sensor is moved to the desired height on the sight glass, which corresponds with the water level to be maintained. If the water level falls to the level of the sensor, the sensor will turn the compressed air supply off via the solenoid valve. With no air entering the column, the water level will start to rise until the level sensor turns the air supply on which results in the water level dropping again. As the solenoid valve cycles on and off the water level is maintained between the upper and lower limits of the sensor's dead band (also referred to as hysteresis). The hysteresis is approximately ¼" and is the difference in the meter indication on the sensor between the value at which the relay energizes (the set point) and de-energizes.

Water exits the bottom of the column by gravity and from there is then distributed to the WCS aquarium. When supersaturating the column water with dissolved gases, no attempt is made to alter the O_2/N_2 ratios, while maintaining a constant total gas pressure in the water that exits the column. It is important to know the relationship of nitrogen and oxygen when elevating total gas pressure in water as studies with juvenile coho salmon showed that increases in the ratio of O_2 to N_2 resulted in increased tolerance to the total gas supersaturation (Rucker 1976) and in studies with juvenile steelhead trout, fish died more rapidly as the ratio of O_2 and N_2 decreased (Nebeker et al 1979). When oxygen is removed from water by metabolic and chemical action, or when oxygen is added to the water by photosynthesis, there is a definite change in the ratio of oxygen and the inert gases. Experimentally using seawater, DO_2 concentration in the water exiting the column was documented and the % nitrogen (+ argon) saturation values and O_2/N_2 ratios were calculated according to the equations of Colt

(1984). Constant TGP levels of 115%, 120% and 130% in 20.7 °C salt water exiting the column corresponded with calculated mean O_2/N_2 ratios of 106.8%/117.6%, 111.7%/122.85% and 122.0%/132.0% respectively. These O_2/N_2 ratios did not differ appreciably from those prevailing in the summer of 1997 in the surface waters ($\leq$ 1.5 m) of Port Moody Arm, BC (Birtwell et al. 2001). Control water of 100.6% TGP at 11.4 °C was associated with an O_2/N_2 ratio of 97.5%/101.5%.

WATER CIRCULATION

GENERAL OVERVIEW

After the aeration or deaeration and filtration of the water in the water delivery system, water enters into a circulation loop of the WCS. Water from each of three loops enters separately controlled layers which constitute the "top", "middle" and "bottom" zones of the aquarium (Figure 3, No. 33). The 4,500 L aquarium measures 244 cm in height, 244 cm in width and 80 cm in breadth and is constructed of clear, 19 mm thick acrylic panels. A bed of sand isolates the aquarium from pump vibration. Four horizontal steel bars reinforce the aquarium at distances of 50, 91, 168 and 234 cm from the floor. The water circulation loop provides a current at an appropriate velocity for aquatic organisms in the water column and supplies a continuous and regulated flow of replacement water to each of the three zones in the water column (Figure 3, No. 41). The water circulation loops provide for the maintenance of a well defined halocline or thermocline between adjacent, horizontally flowing water masses, when a stratified water column is required.

There are three identical water circulation "loops" and each can be independently supplied with either well water or seawater or a combination of these. Each loop is supplied with water, from valve numbers 14 or 25 (Figure 2), and this water is admitted to a corresponding zone of the WCS aquarium (Figure 3, No. 33). For simplicity, only one loop and its components will be described in this section. Upon entering the loop from the 2" water delivery pipe, the water flows through a bushing and into a 4" pipe before entering a double-walled aluminum heat exchanger (Figure 3, No. 4). Municipal cooling water flows through the heat exchanger counter-current to the flow of the experimental water for increased thermal exchange efficiency. The municipal water may be chilled by a refrigeration system and thereby used to control and reduce the temperature of experimental water in the WCS.

A 3" pipe carries water from the heat exchanger into a centrifugal pump (Model 80-200MC, Ingersoll-Rand Co., Gateshead, England) (Figure 3, No. 5), electrically powered by a three-phase, 7.5 h.p. induction motor (Toshiba International Corp., Houston, TX). The pump impellers and surfaces that are in contact with water were coated with epoxy (Intergard EXA 008, International Marine Coatings Ltd., North Vancouver, BC) to prevent corrosion and contamination of experimental waters. The pump has a ½" valve at its base to permit draining (Figure 3, No. 6). Water exits the pump via a 3" diameter vertical pipe. A 3" tee-fitting allows water to flow directly into the aquarium or to be partially redirected through the heat exchangers by means of a bypass loop (Figure 3, No. 7). This bypass loop may be isolated from the system by closing a 3" ball valve (Figure 3, No. 8) downstream of the tee-fitting. Two valves (Figure 3, No. 9) direct water in the bypass loop through two polyethylene filter housings (Figure 3, No. 10). The bypass loop is equipped with two valves which are ½" and $^{3}/_{8}$" in size respectively (Figure 3, No. 11 and No. 12) which are connected by flexible tubing to a drain. These valves function as air bleeds and are manually opened and closed as required, to purge air from the system, when the centrifugal pump for that loop is started.

Water that does not pass through the bypass loop flows directly to the aquarium through a 1 m vertical 3" pipe (Figure 3, No. 14) at the top of which is a 90 degree elbow. The elbow is connected by means of a bushing to a 6" horizontal pipe, which is 4.5 m in length (Figure 3, No. 15). A ½" valve (Figure 3, No. 16) which is connected to a drain by flexible tubing, is located on the upper surface of the elbow and functions as an air bleed.

A stainless steel orifice plate (Figure 3, No. 17) is mounted in the 6" pipe approximately 3.2 m downstream from the elbow. A pair of tappings, located upstream and downstream of the orifice plate, are connected by flexible tubing to a differential pressure indicator gauge (Model 227, ITT Barton Instruments, City of Industry, CA) (Figure 3, No. 17). A second pair of tappings is located upstream and downstream of this pressure indicator and is connected by flexible tubing to a $^{3}/_{8}$" valve and to a drain. The gauge measures the pressure difference across the stainless steel orifice and, utilizing a relationship between mass flow rate and pressure difference, gives a reading which is related to water flow (velocity) through the WCS aquarium. The pressure indicator is equipped with air bleed valves (Figure 3, No. 18) which are connected by flexible tubing to a drain.

A 6" butterfly valve (Model WA-20-20, Chemtrol Division, Santa Barbara Control Systems, Santa Barbara, CA) immediately downstream of the orifice plate is used to regulate the flow to the aquarium (Figure 3, No. 19). Downstream of the 6" butterfly valve, the 6" pipe turns 90 degrees downward and into the vertical plane and then 90 degrees and into the horizontal plane, to its entry into the aquarium (Figure 3, No. 25). Three separate tee-fittings, upstream of the entry location, connect the 6" pipe to a vertical standpipe (Figure 3, No. 20), a level control standpipe (Figure 3, No. 21) and temperature and conductivity sensors (Figure 3, No. 24), respectively. A ½" valve is tapped into the 6" pipe near the sensors (Figure 3, No. 23) which allows for the withdrawal and sampling of circulating water, just before it enters the aquarium. An open 3" diameter standpipe rising vertically to a height of 0.5 m above the aquarium is used for insertion of the oxygen probe into waters entering the aquarium, the introduction of materials (e.g., dyes or toxicants), and the removal of samples for analysis.

The level control standpipes (Figure 3, No. 21) are 2" inside diameter and are connected to the main 6" pipe by flexible tubing. The 2" pipe is attached, to a 2 m, ½" diameter, threaded rod which is fixed but can rotate freely when a handle at its base is turned, thereby determining the standpipe height. A scale beside the level control standpipe denotes its relative height and enables the standpipe elevations to be reproduced during subsequent experiments. The standpipes continually overflow to a drain while the WCS is operating and they regulate the total depth of water in the aquarium, the depth of each zone, and the position of the interfaces between each zone. Flow meters (CalQFlo® Model CF-40750LN, Blue and White Industries Ltd., Westminster, CA) are installed in the level control standpipes (Figure 3, No. 22) to facilitate the balancing of inflow and outflow waters. Conductivity and temperature sensors are mounted on an acrylic plate (Figure 3, No. 24) which is bolted to the flange of a 6" tee-fitting and they extend 23 and 19 cm respectively into the waterflow, to monitor waters just before they enter the aquarium. The plate is removable for probe cleaning and service.

Water enters the left side of the aquarium 0.5 m downstream of the conductivity and temperature sensors (Figure 3, No. 24) through a 6" pipe centrally positioned in the zone (Figure 3, No. 25). Water entering the aquarium impinges upon a 12.7 mm thick acrylic water dispersion plate, mounted perpendicular to the flow (Figure 3, No. 42), which is perforated with an asymmetrical pattern of 6 mm diameter holes. A dispersion cone (Figure 3, No. 26) is mounted centrally on the perforated plate 8.5 cm from the aquarium wall and distributes incoming water over the perforated plate to facilitate the production of laminar flow in each zone. Downstream of the water dispersion plate a Nitex® screen (mesh size 1 mm) is mounted on a Plexiglas® frame and this prevents small organisms from swimming through the dispersion plate.

Under vertically stratified conditions wherein fresh water overlay salt water, flow pattern tests utilizing dyes indicated turbulent mixing of fresh water and seawater at the halocline. The halocline is at the boundary of adjacent horizontally moving water masses (zones). To minimize this turbulence, 6 mm acrylic wings, which project 14 cm horizontally into the aquarium water, were attached to the aquarium wall at the top left side of each zone. To remove air trapped beneath the wings, capillary air bleeds (1.57 mm i.d., Figure 3, No. 28) were inserted through the wings and between the aquarium wall and the dispersion plates.

Water flows from the left side of the aquarium, and exits the right side, through outlets located in the center of each zone (Figure 3, No. 41) and Nitex® screens identical to those on the water entry side. Each outlet is connected to a 6" pipe, which conveys water from the aquarium, and into the heat exchangers (Figure 3, No. 4). Water, which re-enters the heat exchangers, has completed one circulation loop through the WCS. The aquarium may be drained by the operation of a 2" ball valve (Figure 3, No. 34), located in the lower left corner.

VERTICAL STRATIFICATION

A stable and reproducible vertical stratification of fresh water above seawater is essential for studying the behavior of aquatic organisms, for example, under simulated estuarine conditions. Previously, the water column has been stratified in the WCS aquarium by the continuous replacement (19.0 or 26.5 $L \cdot min^{-1}$) with air-equilibrated well water (12.0 ± 0.4 °C) to the top zone and seawater (11.0 ± 0.5 °C) to the middle and bottom zones. A distinct well-defined halocline resulted and a stable stratification was maintained throughout several days with mean salinities of 0.4 ± 0.2 ‰, 27.6 ± 0.8 ‰ and 27.1 ± 0.7 ‰ in the top, middle and bottom zones respectively. The maintenance of this stable vertical stratification in the aquarium required the initial adjustment of the level control standpipe position (Figure 3, No. 21), a continuous replacement water flow and a water velocity of at least 3 $cm \cdot sec^{-1}$ (Figure 3, No. 17, differential pressure indicator gauge setting of 3.0).

The time required for 90% replacement of water in each zone of the WCS aquarium was examined empirically and compared to the theoretical values calculated using the equations provided by Sprague (1969). For example, salinity was measured while fresh water in the middle and bottom layers was replaced by seawater at 23 $L \cdot min^{-1}$. The measured times required for 90% replacement were 2 h 35 min and 2 h 30 min respectively, very close to the theoretical value of 2 h 40 min. Similarly, aerated fresh water (10.5 $mg \cdot L^{-1}$ dissolved oxygen) in the top zone, was replaced by deaerated well water (2.4 $mg \cdot L^{-1}$ dissolved oxygen) at 28 $L \cdot min^{-1}$ and after a 2 h 30 min replacement time, dissolved oxygen levels were 2.7 $mg \cdot L^{-1}$.

WATER VELOCITY

Water velocity is controlled by the Chemtrol™ 6" butterfly valves (Figure 3, No. 19) and measured by differential pressure indicator gauges (Model 224, ITT Barton Instruments, City of Industry, CA) (Figure 3, No. 17). The gauge manufacturer's manual provides a table of gauge display vs. velocity but since the original dispersion plates (Figure 3, No. 42) in the WCS have been slightly modified, tests were performed to empirically measure water velocities through each zone of the aquarium relative to the expected gauge readings.

Conventional instruments such as the propeller driven current meter (Ott Messtechnick, GmbH & Co., Germany) and electromagnetic current meters (Model 201D, Marsh-McBirney Inc., Gaithersburg, MD) were not accurate to determine velocities less than 10 $cm \cdot sec^{-1}$ and therefore timed dye injections were conducted to determine the speed of water movement across the aquarium. Rhodamine dye was injected through capillary air bleeds (Figure 3, No. 28) and the time required for the center of the dye cloud to move from the inlet to the middle the aquarium was observed. The dye became diffused as it moved across the aquarium and visual determination of the time at which the center of the cloud reached the outlet was difficult. Accordingly, a light sensor (Model LI 193SB Spherical Quantum Sensor, LI-

Cor Inc., Lincoln, NE; ± 5% accuracy) was placed at the outlet of the aquarium, at the bottom of each zone, and the time was recorded from the introduction of the dye to the lowest light intensity (the time at which the center of the dye cloud reached the outlet). Linear regression equations describing gauge setting vs. velocity in each zone were derived to provide information on flow rates for future operators of the system.

The velocities at each gauge setting are similar across each zone. The differential pressure indicator gauge setting ranges from 0.1 (0.6 $\text{cm}\cdot\text{sec}^{-1}$) to 10.0 (5.79$\cdot$cm sec^{-1}) however when operating under vertically stratified and simulated estuarine conditions the halocline becomes diffused as velocity is increased past 3.2 $\text{cm}\cdot\text{sec}^{-1}$ (3.0 on the gauge). Velocities of up to 3.7 $\text{cm}\cdot\text{sec}^{-1}$ (4.0 on the gauge) can be achieved under isohaline conditions. The upper velocity limit for isohaline conditions is imposed by water being forced out of the vertical standpipe (Figure 3, No. 20) of the top zone. If warranted, these standpipes could be extended or capped to allow for higher velocities in the aquarium.

TEMPERATURE CONTROL OF AQUARIUM WATER

COOLING

A refrigeration system reduces and controls the temperature of the experimental water and removes heat, which has been produced by the three centrifugal water recirculation loop pumps (Figure 3, No. 5). The refrigeration system is comprised of a Freon™ based chiller system and heat exchangers. Heat can be removed from experimental water as required, for each zone of the WCS separately, by directing flow for that zone through one of three double-walled, aluminum heat exchangers (Figure 3, No. 4 and Figure 4, No. 1). The heat exchangers conduct chilled municipal water between the outer walls and transport experimental water in a counter-current flow through the central cylinders.

The municipal water is cooled before entering the heat exchangers by the Freon™ chiller system, which is located in the annex adjoining the WCS building (Figure 1). The chiller system removes heat from the municipal water, which, subsequently, removes heat from the experimental water over the length of the heat exchangers. Thus a two coolant (i.e. Freon™ and municipal water) system cools the experimental water. A schematic showing circulation of water and Freon™ as well as the location of the associated components is presented in Figure 4. The major components of the chiller system are as follows: a 10 h.p. compressor (Model 06DA537, Carlyle Compressor Co, Syracuse, NY) (Figure 4, No. 2), a water-cooled condenser (Model CSTC 848, Dunham Bush Inc., Hartford, CN) (Figure 4, No. 3), two custom designed 5 ton Freon™ filled chillers (General Refrigeration Engineering, Vancouver, BC) (Figure 4, No. 5) and a 2 h.p. centrifugal water pump (KNM Begemann, Guelph ON) (Figure 4, No. 11). The Freon™ chiller system is provided with a dryer (Figure 4, No. 4) and a sight glass indicates the moisture level and all components of this system are interconnected with copper piping.

The Freon™ system (Figure 4, No. 24) cools the municipal water in the chillers. Municipal water is recirculated through a loop as follows: a 2 h.p. centrifugal pump (Figure 4, No. 11) supplied with water through a ball valve (Figure 4, No. 22) propels municipal water into the horizontal plane through a 90 degree elbow and a ball valve (Figure 4, No. 12), where it is divided by a tee-fitting before entering two chillers (Figure 4, No. 5). Municipal water flows out of the chillers in two 1 ½" PVC pipes which recombine into one 3" pipe by means of a tee-fitting and a 90 degree elbow and ball valve (Figure 4, No. 25). Two ¼" valves tapped into the pipes upstream (Figure 4, No. 16) and downstream (Figure 4, No. 15) of each chiller function as air bleeds. Municipal water supplied by a ball valve (Figure 4, No. 13) to the single 3" pipe, is then divided by a series of tee-fittings, reducer bushings and an elbow into three 2" pipes; each fitted with a ball valve (Figure 4, No. 6). Water in each of the three pipes then divides by a tee-fitting and either enters or bypasses the aluminum heat exchangers (Figure 4, No. 1; Figure 2, No. 30 and Figure 3, No. 4). Each heat exchanger cools experimental water from a single circulation loop of the WCS (Figure 3) and therefore corresponds to a discrete zone of the aquarium (Figure 3, No. 41).

Depending on experimental requirements, the presence or absence of chilled municipal water in the heat exchangers will determine whether or not cooling of experimental water will occur. There is a 2" manual ball valve (Figure 4, No. 7) in each of the three 2" pipes, which allows water to bypass the heat exchangers. Three-way control valves mounted with self-adjusting, motorized, integrated valve actuators (ML-7984, Honeywell Ltd., North York, ON) (Figure 4, No. 17) are downstream of the ball valves. They are used with electronic modulating signal controllers which are mounted on an instrument panel in the WCS building (T775E Remote Temperature Controller, Honeywell Ltd., North York, ON, accuracy ± 1 °C at 25 °C.) (Figure 4, No. 19), to regulate the chilled water supply to the three heat exchangers. The control valves open and close automatically to determine whether chilled municipal water will enter the heat exchangers or bypass them. The temperature controllers are connected to temperature sensors (Figure 4, No. 20) which are immersed in the experimental water through an aperture in the pipe, 30 cm upstream of the heat exchangers. The temperature set point on the three controllers may be adjusted independently and determines the temperature of water circulating in each zone of the WCS. The temperature controllers operate with an ambient temperature range of –22 °C to 60 °C and a setpoint adjustment range of –40 °C to 104 °C. When an aquarium water temperature of 11.7 °C is required at a replacement water flow of 20 $L \cdot min^{-1}$, the cooling capacity is limited to 27 °C below replacement water temperature and can be controlled to ± 0.47 °C.

Downstream of the three-way control valves (Figure 4, No. 17), water enters three 2" pipes, each of which is equipped with a manual ball valve (Figure 4, No. 21). These pipes rejoin the main 3" pipe through a series of tee-fittings, a bushing and an elbow. One-half meter downstream of the ball valves (Figure 4, No. 21), the 3" pipe is connected by means of a tee-fitting, bushing and ball valve (Figure 4, No. 23) to a 1 ½" pipe which conveys municipal water to a drain. This valve remains closed while the refrigeration system is operating.

Municipal water used to fill the condenser, is controlled at valve No. 14 and to fill the Freon™ chiller system is controlled at valve No. 8 (Figure 4) and also enters an open, 9 L, rectangular expansion tank (Figure 4, No. 18). The expansion tank allows for changes in volume caused by water temperature changes in the chiller system. Water flows from the expansion tank into a 1 ½" pipe which is inserted into the 3" pipe 0.2 m downstream of the drain valve (Figure 4, No. 23). The expansion tank is mounted on a bracket, 6 cm above the chillers (Figure 4, No. 5). A ½" pipe is inserted into the right side of the expansion tank, for overflow to the drain, at a distance of 6 cm below the tank top (Figure 4, No. 9) and the tank is drained by valve No. 10 (Figure 4). While the refrigeration system is operating, approximately 0.5 – 1.0 $L \cdot min^{-1}$ of water is required to be continuously supplied to the expansion tank to compensate for water lost through the packing gland of the chiller system water pump.

HEATING

In addition to the refrigeration system, the WCS apparatus is equipped with a 2-stage boiler system, which may be used to heat water prior to delivery to the aquarium (Figure 2, No. 32). The main components of the heating system are municipal (Model 0 00, cartridge circulator, Taco Inc., Cranston, RI) and experimental water pumps (Model 5MD, 1/8 h.p. magnetic drive pump, Little Giant Pump Co., Oklahoma City, OK), and a Superchanger titanium heat exchanger (Tranter Inc., Whichita Falls, TX), a Super Hot electric hydronic boiler (Model E, Allied Engineering Company, North Vancouver, BC), and a Mini-Therm II gas-fired hydronic boiler (Model JV, Teledyne Laars, Oakville, ON) which are all located in the annex adjoining the WCS building (Figure 1). In addition, an electronic modulating signal controller (T775E Remote Temperature Controller, Honeywell Ltd., North York, ON; accuracy ± 1 °C at 25 °C.) is mounted on an instrument panel in the WCS building (Figure 1).

The water is circulated, by pump, between the reservoir for heated water on the aeration tower platform, the electric boiler and heat exchanger (Figure 5). Municipal water is heated by an electric element in the boiler (Figure 5) and pumped in a continuously circulating loop through a titanium heat exchanger (Figure 5) where heat is transferred from the municipal water to the experimental water.

The heat exchanger unit is of plate and frame design. The heat transfer plates are equipped with gaskets which have holes pierced in the corners. The gaskets seal the structure and in conjunction with the holes allow fluid to flow counter-current in alternate channels. The thin fluid interspace coupled with the corrugated titanium plate design induces turbulence that produces extremely high heat transfer coefficients.

The benefits of a titanium heat exchanger are that elemental titanium is a very strong lustrous white metal, which is less than half as heavy as an equivalent volume of steel. Titanium and titanium alloys readily form stable protective surface layers, which give them excellent corrosion resistance in many environments, including seawater, oxidizing acids, alkalis, and chlorides. Titanium is nonmagnetic, has a heat tolerance of up to 440 °C, and exhibits bactericidal properties due to its inherent surface oxide film.

A remote sensor in the aeration tower reservoir monitors the water temperature and relays the information to the temperature controller unit. The controller unit operates with one temperature input supplied by the remote sensor and is capable of providing 2 stages of relay output for on/off control, one stage each for the gas-fired and electric boiler units. Each stage of the controller has an independent temperature set point, which can be configured to operate in the heating mode. Relay outputs are energized at temperature set point minus differential value (usually 1°C for WCS experiments) and are de-energized at set point temperatures. The controller unit utilizes a liquid crystal display for interactive prompting during user programming and display of sensed and assigned setpoint and differential values.

In a situation where the first stage electric boiler is incapable of attaining the set point temperature the second stage auxiliary gas-fired boiler will be activated through the energized relay. The gas-fired boiler raises the temperature of the municipal water flowing in the heat exchanger thereby increasing the rate of heat transfer to the experimental water. Water in the aeration tower reservoir (Figure 2, No. 24) at 10 °C can be heated up to a maximum of approximately 32 °C at a replacement flow rate of 23 $L{\cdot}min^{-1}$, when both boiler units are activated simultaneously.

The copper tube gas-fired hydronic boiler has a thermocouple/continuous burning pilot configuration. Propane is supplied from cylinders outside the WCS building (Figure 1) to this boiler through an automatic changeover regulator (Type R966, Fisher Controls International Inc, MicKinney, TX). The regulator is designed to operate at low pressure vapor service (usually factory set to deliver 11 inches of water column outlet pressure at 100 psig inlet pressure) and withdraws gas from one cylinder until the pressure drops to 6 psig and then automatically switches to an alternate cylinder. A built-in indicator on the regulator signals with a red flag when the changeover has occurred. Overpressure protection against excessive build-up is provided by an internal relief valve on the regulator, which opens when downstream pressure reaches 1 psig on regulators set at 11 inches water column outlet pressure.

WATER QUALITY MONITORING

Temperature, dissolved oxygen, and conductivity are measured by probes located in the water lines entering each of the three zones of the WCS and at precise locations in the aquarium water column. Information from the probes is displayed on meters which are centrally located on an instrument panel in the WCS building (Figure 3, No. 35 and Figure 1). Output from the meters is relayed to a data acquisition system.

COMPUTERIZED DATA ACQUISITION SYSTEM

The Data Acquisition System (Figure 1) comprises a computer (AST 386 AT compatible with math coprocessor and a Opto-22 AC24AT RS 485 Interface Card), color monitor (EGA\VGA color adapter card), data acquisition panel (Opto-22 16 Point Analog I/O Rack with Optomux Brain Board, 9 Opto-22 AD3 4-20 ma I/O modules, and 450W UPS Battery Backup Power Supply), water quality instrumentation and a customized monitoring software package (Bentek Systems Ltd., Vancouver, BC; Genesis Control and Monitoring Software, 1992, Iconics Inc. Foxborough, MA). The data acquisition system permits the real time logging, trending and display of data from 9 probes (3 zones of the WCS aquarium, each supplied with a salinity, dissolved oxygen and temperature meter) in graphical or tabular format and is expandable for additional instrument inputs.

Signals from the meters are automatically captured at pre-selected time intervals while manual data capture is triggered any time the computer keyboard space bar is pressed. The data is archived in a simple ASCII file format, which ensures compatibility, easy manipulation and importation into various data analysis programs, plotting routines and spreadsheet packages. The operator can bypass or activate the audible system alarm and determine acceptable water quality limits for each sensor's output. If the measurements deviate from these output limits, an alarm will be tripped, sounding off the computer horn.

WATER QUALITY MANIFOLD AND SAMPLING PORT SYSTEM

Depending on experimental requirements, a vertical manifold system (Figure 3, No. 32) can be installed on the right side of the WCS aquarium to allow the abstraction of water samples at discrete depth intervals. The manifold consists of a 5 x 183 cm perforated PVC strip mounted on the right rear wall of the aquarium. Intramedic polyethylene capillary tubing (PE-205, 1.57 mm i.d., Clay Adams, Division of Becton, Dickinson & Company, Parsippany, NJ.) is inserted through the manifold and projects 3 mm into the water column. Samples are extracted through the tubing at eight ports corresponding to the middle of each zone of the aquarium and 5 depths at 2 cm intervals above and below the halocline, thermocline or oxycline which is typically set at a depth of 0.74 m from the water surface in the WCS aquarium. A hypodermic needle (16G 1½, 5198, Becton, Dickinson & Co., Rutherford, NJ.) fitted with a 3-way stopcock is inserted into the end of the capillary tubing which is insulated to prevent heat gain or loss. The point of the needle is sanded to prevent tube puncture. A plastic 1 ml syringe (No. 2A 370 14, Becton, Dickinson & Co., Rutherford, NJ.) is used to extract the sample through the stopcock.

The manifold system has been used to define small scale hydrographic structure around the halocline or thermocline and the vertical distribution of other factors such as dissolved oxygen. Water drips continuously through the tubing during measurement of oxygen partial pressure (PO_2). To measure oxygen partial pressure (mm Hg) each water sample is withdrawn through 6.5 m of capillary tubing and then injected into the measuring chamber of an oxygen meter (Radiometer, Model PHM71b Acid-Base Analyzer with Probe Model E5046, Copenhagen, Denmark; range 0-800 mm Hg, accuracy ± 1.2 mm Hg, precision 1 mm Hg) (Figure 3, No. 36). The measuring chamber is located in a stainless steel housing within a glass jacket. The measuring chamber, housing and jacket comprise a thermostatted cell (Model D616, Radiometer, Copenhagen, Denmark). The temperature of the cell is regulated to ± 0.2 °C by a water bath (Model FK10 70092 with heat controller Model F4921, Haake Buchler Instruments Inc., Saddlebrook, NJ) which recirculates deionized distilled water between the bath and the cell.

When the aquarium is vertically stratified, determinations of salinity in samples, withdrawn at each port by syringe, through the capillary tubing, is measured with a temperature compensated refractometer (Aquafauna, Argent Laboratories, Redmond, WA). The temperature of the water at the five sampling ports around the thermocline is measured by a telethermometer (Model 43TD, YSI Inc., Yellow Springs, OH; range 0-50 °C, accuracy ± 0.5 °C, precision 1.0 °C) (Figure 3, No. 47) with a switcher (Model 4002, YSI Inc., Yellow Springs, OH) and temperature probes (Model 401, YSI Inc., Yellow Springs, OH). The probes are coated with silicon as a precaution against corrosion and inserted into ports in the vertical sampling manifold (Figure 3, No. 32) from which they project 5 mm into the water column.

DISSOLVED OXYGEN

Dissolved oxygen sensors are inserted into the water flowing into the 3 zones of the WCS aquarium through standpipes, which rise vertically from the 6" horizontal pipe at the left side of the aquarium (Figure 3, No. 20). Dissolved oxygen is monitored with polarographic Oxyguard probes connected to a PT4 multichannel oxygen monitoring system (Point Four Systems Inc., Port Moody BC; range 0-51 $mg \cdot L^{-1}$, accuracy ± 2%, resolution 0.1 $mg \cdot L^{-1}$).

Each probe is a temperature-compensated self-polarizing galvanic measuring element, which produces a millivolt output proportional to the oxygen present in the medium surrounding it. The galvanic oxygen probe can be considered a low impedance millivolt generator, primary battery or fuel cell. The probe is divided into an upper part with a silver cathode, zinc anode and cable, and a cap with membrane and electrolyte. Oxygen diffuses through the membrane onto the cathode, where it reacts chemically before combining with the anode. This chemical process develops an electrical current within the probe, which flows through a built-in resistor. The resistor externally connects the anode and cathode converting the current (microamps) generated into a millivolt output (approximately 5 mV per ppm dissolved oxygen). This millivolt signal is led to the monitor via a two-core cable. The probe uses very little oxygen for its measurement, however to offset this demand, a minimum water velocity at the sensor of 3 $cm \cdot sec^{-1}$ is required. This velocity is exceeded in the water circulation loop, when the WCS is in operation.

The components of the PT4 monitor are housed in a waterproof plastic junction box with a clear Plexiglas® front cover (Figure 3, No. 35 and Figure 1). The PT4 monitor utilizes an internal microprocessor and a multichannel 10-bit analogue-to-digital conversion subsystem. The microprocessor gathers information from the sensors via A/D converters, performs unit conversion and relays the formatted data to the programmable, dot matrix, liquid crystal display.

CONDUCTIVITY

Conductivity sensors compensated to 25.0 °C (Model 112-09, Rosemount Analytical, Uniloc Division, Irvine, CA; 0-500 and 0-200,000 µmhos range) are mounted on an acrylic Plexiglas® end plate in the 6" pipes at the left hand side of the WCS aquarium. A sensor projects into the water flowing into each of the 3 zones, upstream of and adjacent to, the aquarium (Figure 3, No. 24). The sensors are connected to meters (Model 750C-0304 Conductivity Analyzer Transmitters, Rosemount Analytical, Uniloc Division, Irvine, CA; range 0-50,000 µmhos, accuracy ± 2% full scale, precision 1 µmhos) which are located on an instrument panel in the WCS building (Figure 3, No. 35 and Figure 1). The computerized data acquisition system software converts input conductivity (at 25.0 °C) from the meters to salinity using the equation described by Accerbone and Mosetti (1967).

TEMPERATURE

Temperature sensors (Model RTP 120730120061, Action Instruments Inc., San Diego, CA), which are mounted on the same acrylic plates (Figure 3, No. 24) that support the conductivity sensors, register the temperature of water entering each zone of the aquarium. The display is an input digital temperature indicator (Model VIP501 RTD, Action Instruments Inc., San Diego, CA; range 0-60 °C, accuracy 0.1 °C) located on an instrument panel in the WCS building (Figure 3, No. 35 and Figure 1) and the sensors are 100 A platinum 3-wire bulbs.

The temperature of water exiting each zone of the aquarium is measured by sensors immersed in the experimental water through an aperture in the pipe 30 cm upstream of the heat exchangers (Figure 1). The temperature is digitally displayed on electronic modulating signal controllers (T775E Remote Temperature Controller, Honeywell Ltd., North York, ON; accuracy ± 1 °C at 25 °C) which are located on the instrument panel (Figure 3, No. 35 and Figure 1) in the WCS building.

Temperature is also monitored in the aquarium by 12 remote, wireless data loggers (Onset Stowaway Tidbit waterproof temperature logger, Onset Computer Corporation, Pocasset, MA; range -5 to 37 °C, accuracy ± 0.2 °C) fastened vertically to a Velcro® strip spanning the height of the water column. Typically, the loggers are positioned evenly throughout the 3 zones of the aquarium and when the WCS is vertically stratified, consecutively along the gradient in the thermocline. The loggers are deployed and the information is later retrieved via a connection to a computer with an interface cable. The data is exported from the Logbook® software program (Onset Computer Corporation, Pocasset, MA) into standard text files. This ensures easy importation of the data into various data analysis programs, plotting routines and spreadsheet packages.

TOTAL GAS PRESSURE

Total gas pressure (TGP) determinations are made using tensionometers (Model 300C, Alpha Designs Ltd., Victoria, BC; range -200 to 700 mm Hg or 73% to 190% TGP, accuracy ± 1 mm Hg) and by applying the calculations provided by Colt (1984). Tensionometers are instruments that determine if dissolved gas supersaturated conditions exist, by measuring the total gas pressure of dissolved gases in the water i.e., nitrogen, oxygen, carbon dioxide and argon. Dissolved gas supersaturation occurs when the total dissolved gas pressure in the water is greater than the ambient atmospheric pressure at the surface of the water.

The tensionometer consists of a meter which houses the electronics and batteries and a submersible probe connected by a cable. The tensionometer probe contains a precision pressure transducer, conditioning electronics, and a gas permeable membrane. The sensing membrane consists of approximately 4 m of very small-bore silicone tubing. One end of the tube is closed off; the other end is connected to the pressure transducer, which converts the internal tube pressure to an electrical signal. The tube is permeable to all gases. When immersed in water, gases diffuse through the tubing wall until the gas pressure inside and outside the tube reach equilibrium. At this point the tensionometer amplifies the transducer output and displays the differential gas pressure (ΔP) on a 3-digit liquid crystal display.

A tensionometer probe is mounted in the center of each zone of the WCS and oriented vertically on the downstream side of the aquarium against the Nitex® screens. To minimize erroneous readings caused by bubble accumulation on the probe membrane, the meter cables are attached to a pulley system. This allows the bubbles to be dislodged, by manually agitating the probes prior to obtaining readings.

PHOTOPERIOD LIGHTING

The lighting system for the WCS aquarium is designed to provide a simulation of diffuse daylight for biological experiments. The system consists of a Light Pipe™(TIR Systems Ltd., Vancouver, BC) (Figure 3, No. 37) illuminated by a 400 W metal halide luminaire (HQI-T400 W/DH, Osram, Germany) (Figure 3, No. 39) and incorporates an adjustable shutter mechanism (Figure 3, No. 38) and a red filter illumination system for night videotaping (Figure 3, No. 40).

The Light Pipe™ consists of long (3.0 x 0.2 x 0.2 m) square pipes with one downward emitting surface, which illuminates the WCS from above. The Light Pipe™ is lined with patented prismatic film that allows it to act as a conduit and an even diffuser of light. The metal halide luminaire supplies a light spectrum very similar to natural daylight, which is emitted as a collimated beam into the acrylic Light Pipe™ and transmitted horizontally along its length by total internal reflection. The Light Pipe™ and luminaire are suspended horizontally, 0.3 m above the aquarium, by stainless steel flat rod brackets. A small electric fan is mounted on a bracket adjacent to the lamp to dissipate the heat that the lamp generates. The luminaire is connected to a timer which turns it off at night (i.e. 0.5 h after the start of full dark and on again 0.5 h before the start of dawn) to minimize heat build-up and conserve bulb life.

Lighting conditions in the WCS were measured to reveal how the light intensity and extinction within the aquarium compared to natural light in fresh water, estuarine and marine environments. Light energy levels were recorded using a light meter (Model LI 188Bm LI-Cor, Lincoln, NE) and spherical quantum sensor (Model SPQA 0380, LI-Cor, Lincoln, NE) which measures photosynthetically active radiation (400-700 nm) in microEinsteins per second per square meter ($\mu E \cdot s^{-1} \cdot m^{-2}$). Although the degree of light penetration in the aquarium varies with turbidity, measurements in clear well water ranged between 50 $\mu E \cdot s^{-1} \cdot m^{-2}$ at the surface to 12 $\mu E \cdot s^{-1} \cdot m^{-2}$ at 1.9 m depth. These values are similar to those measured on a cloudy, rainy day (May 30, 1984) in the estuary of the Campbell River, BC where values ranged from 56 $\mu E \cdot s^{-1} \cdot m^{-2}$ at the surface to 24 $\mu E \cdot s^{-1} \cdot m^{-2}$ at 1.8 m depth (Birtwell and Kruzynski 1987, Piercey et al. 1985). Light intensities in the aquarium were lower in seawater due to the higher concentration of particulate matter and ranged between 45 $\mu E \cdot s^{-1} \cdot m^{-2}$ at the surface to 10 $\mu E \cdot s^{-1} \cdot m^{-2}$ at 1.9 m depth. These values are similar to those measured on a cloudy, rainy day (July 8, 1997) in the marine waters of Port Moody Arm, Burrard Inlet, BC where values ranged from 37 $\mu E \cdot s^{-1} \cdot m^{-2}$ at the surface to 11 $\mu E \cdot s^{-1} \cdot m^{-2}$ at 1.5 m depth (Birtwell et al. 1998).

A circular shutter mechanism (Figure 3, No. 38) installed between the light source and the acrylic prism light guide, at a distance of 1.5 cm from the luminaire, provides seasonal photoperiod control. The shutter consists of a 26" diameter, light weight alloy rim fitted with four spokes to a hub. The shutter is rotated once every 24 h by a precision-geared synchronous motor (Model BK 13-5-6, 100-60, Synchron Ltd. USA). Aluminum foil occludes a sector of the circular shutter, with the proportion of opaque area (or angle of occlusion) determining the number of hours of darkness. Light is progressively occluded (15 degrees per hour) from the Light Pipe™ as the opaque sector of the shutter rotates past and eclipses the luminaire. This movement creates an artificial illumination cycle of full dark and daylight, including two 4.5 h periods of "twilight" representing dawn and dusk. Due to limitations of the lighting system, the duration of dawn and dusk are longer than that found in nature (i.e. 4.5 h vs. 45 min each respectively). However, the duration of full daylight (i.e. number of hours after the end of dawn and before the start of dusk) in the WCS aquarium is equivalent to the number of daylight hours provided in the tables of standard times of sunrise, local noon, sunset and civil and nautical twilight and local sidereal time, which are distributed by the National Research Council of Canada, Dominion Astrophysical Observation. This is accomplished by reducing the length of the full darkness period in the WCS, in terms of the number of hours of a certain photosynthetically active radiation level ($\mu E \cdot s^{-1} \cdot m^{-2}$) penetrating the aquarium water column, to less than that which occurs in nature. Although the full darkness period is reduced, the end of the dusk period and the beginning of the dawn period are almost equivalent to the full dark light intensity, due to the gradual progression of the shutter mechanism. When the WCS is operating, the photoperiod is adjusted every two weeks by changing the angle of occlusion on the shutter, using the nautical twilight tables as a reference.

Filming of experimental animals in the WCS aquarium at night is facilitated by the use of a red filter illumination system. This component is mounted at the left side of the Light Pipe™ (Figure 3, No. 40) and contains a halide lamp (Model ENH 250 W, Phillips Lighting Co., Somerset, NJ) with a far-red dichroic filter (Model RG 695, Schott-Fostec, LLC., Auburn, New York). Dichroic filters will not darken or alter spectral response over time regardless of light intensity or duration and reflect heat rather than absorbing it, eliminating the need for a thermal expansion split. The filter emits wavelengths between 680 - 760 nm, peak transmission occurs at 705 nm and 50% transmission at 695 and 722 nm. These wavelengths are considered to be outside the light spectrum visible to the dark-adapted salmonid retina as most fish do not have a photoreceptive visual pigment which is sensitive to red (705 nm) light (Duncan 1956), and therefore, minimal disturbance will be experienced by the animals in the WCS aquarium at night. Sufficient illumination is provided for night filming as the high-resolution video camera used in conjunction with the red filter illumination system that is sensitive to the far-red end of the spectrum. The red filter illumination system is cooled by a fan, to dissipate any generated heat. Operation of this system is controlled by a timer, which turns the light on 1 h before the start of "dusk" and off 1 h after the start of "dawn".

VIDEOTAPE RECORDING AND DIGITAL IMAGE ANALYSIS

VIDEOTAPE RECORDING

A high resolution, closed circuit, black and white video system is used to record the behavior of organisms in the aquarium. Vinyl curtains, which enclose the aquarium, lighting systems, and camera, occlude extraneous light and minimize external disturbance to the experimental organisms. Adaptations to the aquarium, such as the application of rolls of black MACtac® (which is a colored, self-adhesive pressure sensitive paper) to the exterior surface of the back wall and painting the floor of the aquarium flat black, were necessary to optimize video recording by reducing the light reflection. The use of a dehumidifier and electric fans have eliminated condensation from the Plexiglas® background of the aquarium, which also reflected light during filming.

A high-resolution video camera (Model WV1850, 800 lines, Panasonic, Secaucus, NJ) fitted with an automatic iris lens (12.5 mm, wide angle APC, Computar, New York, NY) and an extended-red Newvicon® tube enhances observation over a broad range of lighting conditions. Generally, the type of camera tube selected is based on the need to balance resolution, integration (lag) and contrast. The Newvicon® tube offers good resolution, moderate lag and low image distortion. Newvicon® tubes are characterized by a high light sensitivity and the spectral response of the photoconductive target extends into the near infrared range. The camera is mounted onto a vertical support bar, approximately 2 m from the front of the aquarium (Figure 1). The camera lens automatically adjusts to changing light intensities and is very sensitive to low light conditions (i.e. minimum 0.1 lux). The camera can be used in nocturnal conditions under low level red light illumination as it has a high sensitivity in the near-infrared region of the spectrum (< 940 nm) and a peak sensitivity at 780 nm. This is close to the peak transmission wavelength of the red filter illumination system.

The aquarium is observed on a high-resolution closed circuit, black and white video monitor (Model WV-5470, 850 lines, Panasonic, Secaucus, NJ), located outside the light occluded area. Images may also be recorded for playback, albeit at a reduced resolution (300 lines), on a time-lapse SVHS video recorder (Model AG-6750, 400 lines, Panasonic, Secaucus, NJ). A single high-grade 120 mm tape can record information for up to 480 h, although typically experiments require 120 h of recording (1 frame per second). A built-in, time-date generator (Model NV-F85, Panasonic, Secaucus, NJ) and microprocessor featuring a calendar function display time and date on the monitor and imprints this information on the videotape.

In some instances, organisms are behind four horizontal steel girders, which span the height of, reinforce and encircle the aquarium, making accurate determinations of position difficult during the playback of filmed images. As a result a remote controller (Model AG-A670, Panasonic, Secaucus, NJ) equipped with a still, frame forward and reverse jog-shuttle function is required to facilitate videotape analysis and interpretation.

DIGITAL IMAGE ANALYSIS

The precise location of individual and groups of, organisms in the WCS is quantified from an analysis of videotapes, using a computerized image analysis system. The analog video images are captured by a video processor (PIP 640B Video Digitizer Board, Matrox Electronic Systems Ltd., Dorval, Que.) which is a "plug in card" that allows an IBM PC microcomputer to perform frame grabbing operations on a video signal from an external source. When filming schools of fish, the computer software program SNAP (Sci Tech Consultants Inc., Vancouver, BC) is used to digitize the image of an organism on the video frame by transforming the x, y co-ordinates of each organism into graphical images that depict the school distribution and precise location in the aquarium. Concomitantly, the program calculates statistical parameters for each image which include the central tendency or mean of the distribution, the median value which gives some indication of the normality of the distribution and standard deviational ellipse values which give a statistical indication of the shape of the school.

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Table 1. Gas saturation levels measured in water exiting gas equilibration columns injected with a steady flow of nitrogen gas

Water Flow (Lmin^{-1})	Nitrogen Flow (Lmin^{-1})	Gas Saturation (%)		
		Total	N_2 and Argon	O_2
SEA WATER (8.2 °C / 26.6 ‰ Salinity)				
60	0	101	105	100
60	4	101	111	75
60	8	101	116	59
60	12	101	119	45
60	20	100	121	23
60	27	100	122	15
90	12	102	119	48
FRESH WATER (10.2 °C / 0.4 ‰ Salinity)				
32	0	101	109	83
32	4	101	122	33
32	8	101	128	10
32	12	101	130	2

Table 2. Water quality determinations measured in water exiting gas equilibration columns injected with a steady flow of nitrogen gas.

Water Flow ($Lmin^{-1}$)	Nitrogen Flow ($Lmin^{-1}$)	Elapsed Time (min)	Salinity (‰)	Temperature (°C)	Oxygen (% saturation)	Oxygen (mgL^{-1})
5	1.35	40	27.0	14.0	41	3.6
5	6.50	40	27.0	15.2	20	1.7
5	10.35	40	26.5	17.0	19	1.6
5	14.75	40	27.0	16.5	13	1.1
7	6.50	40	26.0	18.0	16	1.3
10	1.35	40	27.0	12.4	78	7.0
10	2.40	40	27.5	13.3	69	6.1
10	3.55	70	27.0	12.4	50	4.5
10	4.80	40	27.5	13.1	48	4.3
10	6.50	40	25.0	17.0	34	2.9
10	10.35	40	27.5	13.0	27	2.4
20	14.75	30	22.5	10.0	66	6.4

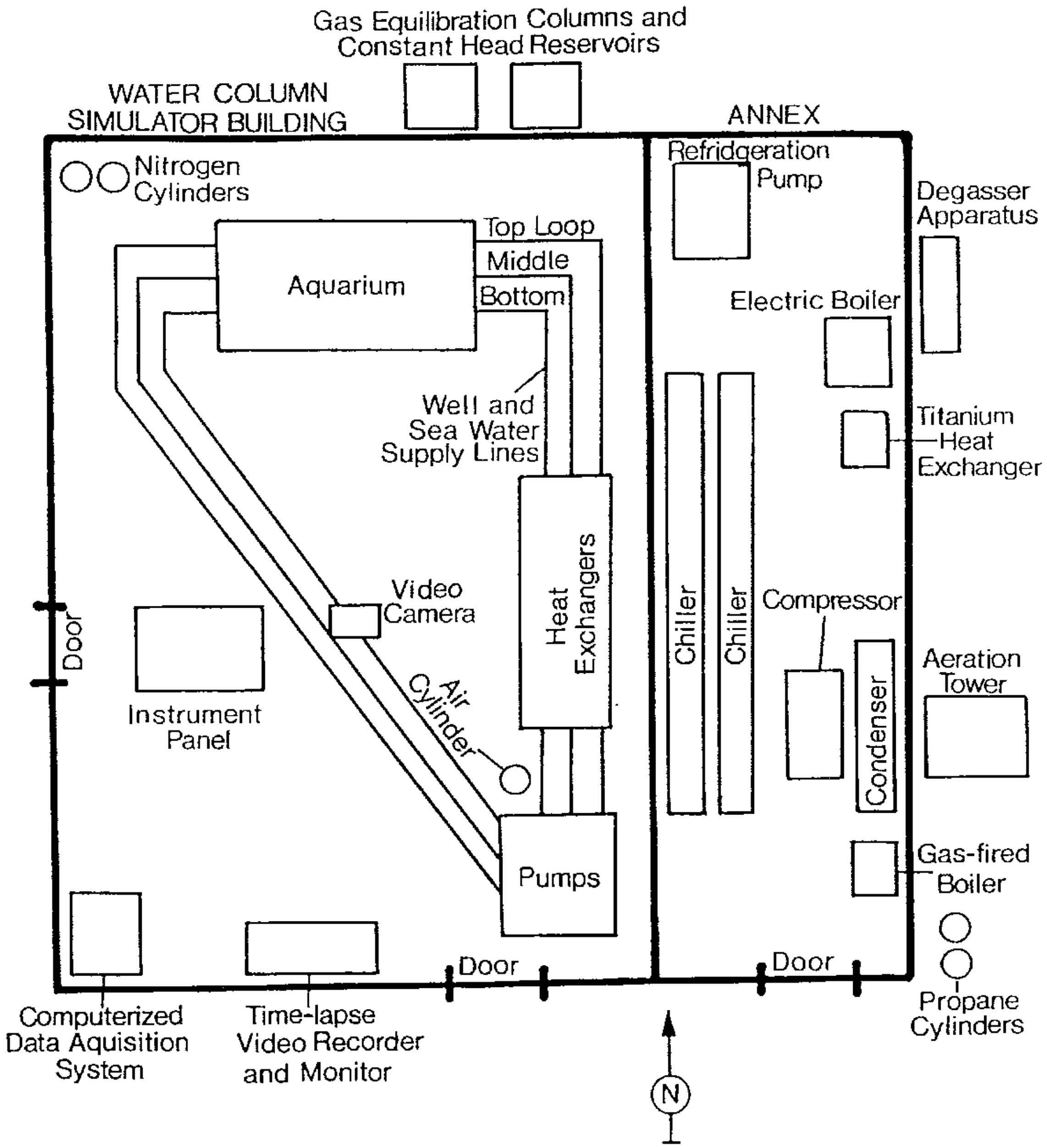

Figure 1. Water Column Simulator site plan

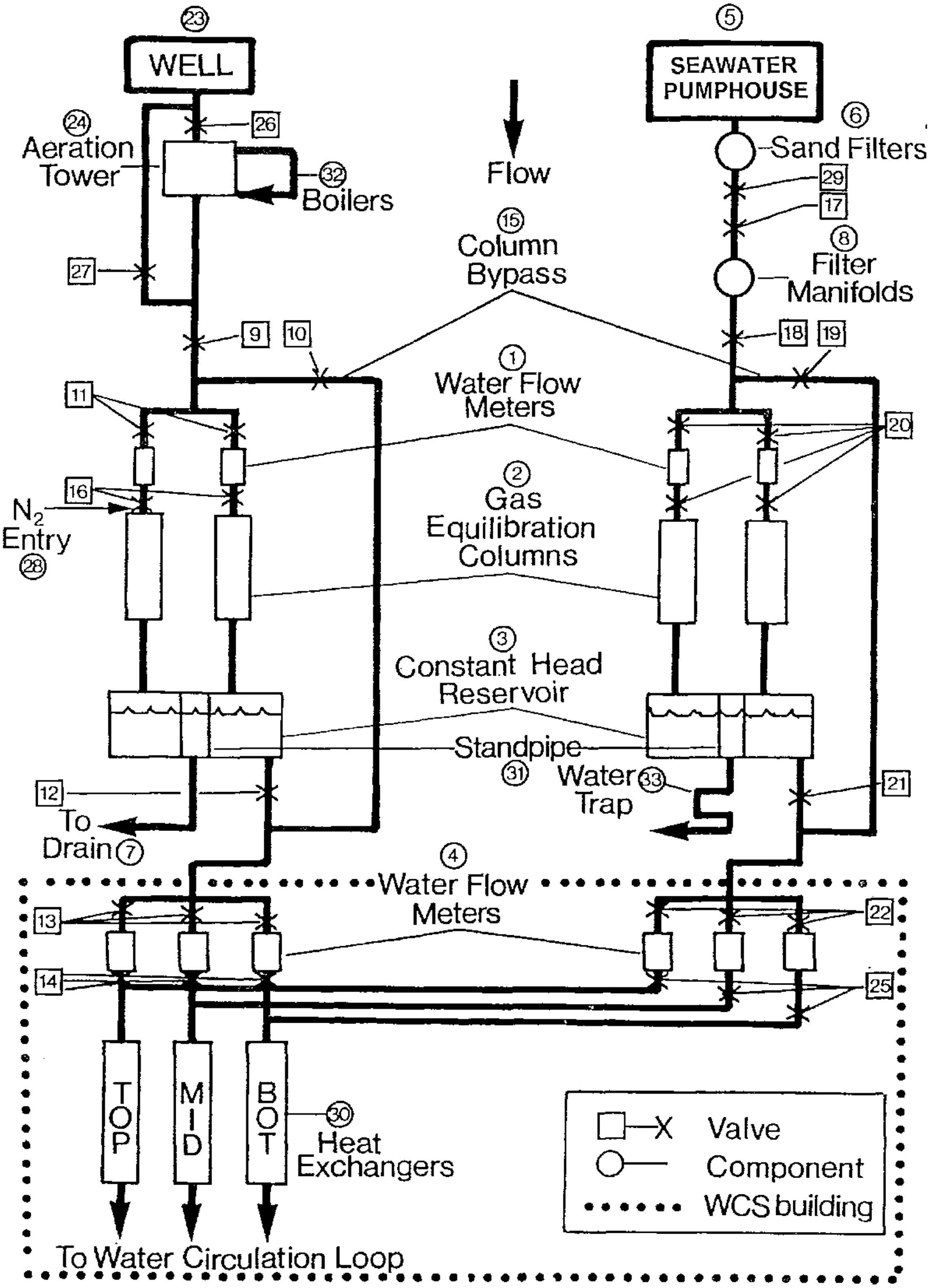

Figure 2. Schematic of the Water Column Simulator water delivery system

Figure 3 Legend

1. Valve (drain)
2. Valve (water supply)
3. Flow meter
4. Heat exchanger
5. Centrifugal Pump
6. Valve (pump drain)
7. Bypass – recirculation loop
8. Valve (bypass)
9. Valve (bypass filters)
10. Polyethylene filter housings
11. Valve (air bleed)
12. Valve (air bleed)
13. Valve (air bleed)
14. Vertical pipe
15. Main water supply to aquarium
16. Valve (air bleed)
17. Orifice plate and differential pressure indicator gauge
18. Valves (air bleeds)
19. 6" Butterfly valve
20. Vertical standpipe
21. Water level control standpipe
22. Flow meter
23. Valve (water sampling)
24. Conductivity and temperature sensor location
25. Entry location for main water supply
26. Water dispersion cone
27. Flexible hose
28. Capillary air bleeds
29. Acrylic horizontal wings
30. Nitex® screen (entry side)
31. Nitex ®screen (exit side)
32. Water sampling manifold with extracting ports
33. Aquarium – lateral view
34. Drain valve
35. Instrument panel and chart recorder
36. Oxygen meter
37. Light Pipe™
38. Adjustable shutter mechanism
39. Metal halide lamp (luminaire)
40. Red filter illumination system
41. Exit location of main water supply (or zone)
42. Water dispersion plate
43. Syringe manifold
44. 3 way stopcock
45. Thermometer manifold
46. Peristaltic pump
47. Telethermometer
48. Air injection from cylinder

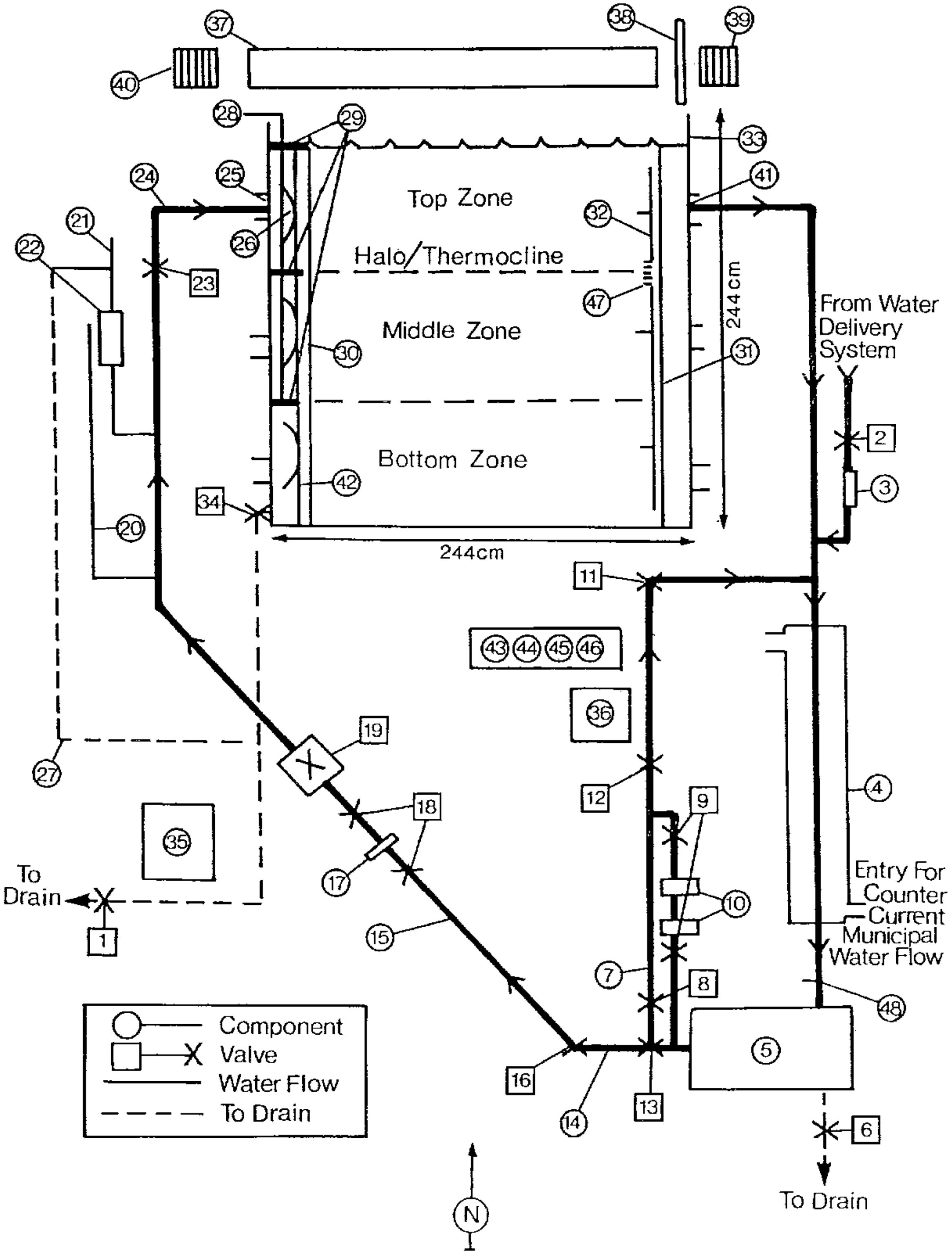

Figure 3. Schematic of the Water Column Simulator water circulation loop

Figure 4 Legend

1. Aluminum heat exchangers
2. Compressor (10 h.p.)
3. Condensor
4. Dryer and sight glass
5. Freon™ filled chillers
6. Ball valve (2")
7. Ball valve (2")
8. Gate valve (½")
9. Expansion tank overflow to drain
10. Expansioni tank drain valve (½")
11. Centrifugal water pump (2 h.p.)
12. Ball valve (3")
13. Ball valve (2")
14. Ball valve (½")
15. Valve (air bleed downstream of chiller)
16. Valve (air bleed upstream of chiller)
17. Three-way control valvues mounted with motorized valve actuators
18. Expansion tank (9L)
19. Electronic modulating temperature signal controllers
20. Temperature sensor
21. Ball valve (2")
22. Ball valve (3")
23. Ball valve (1½") on municipal water drain line
24. Solenoid valves on Freon™ circulation system
25. Ball valve (3")

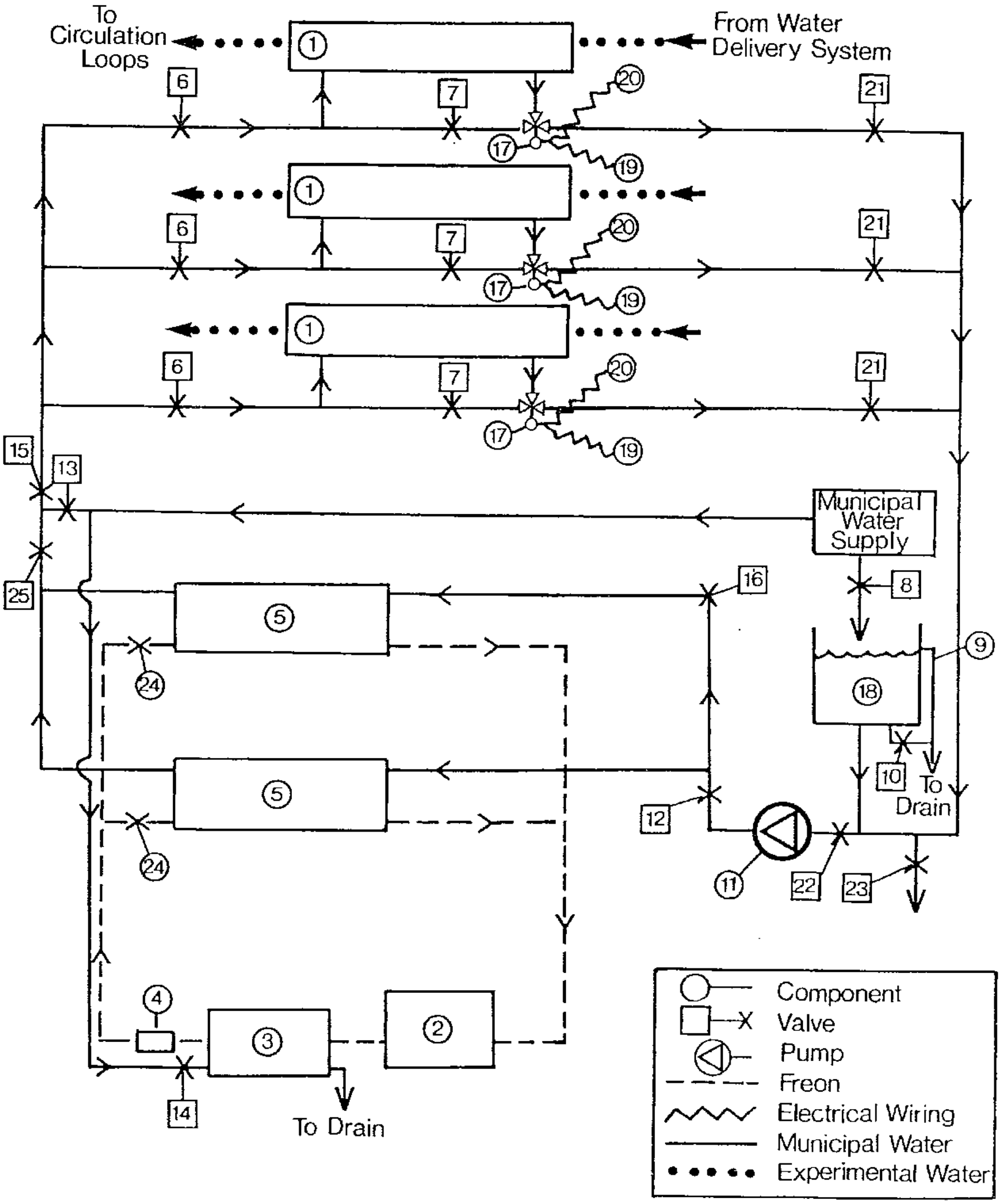

Figure 4. Schematic of the water refrigeration system

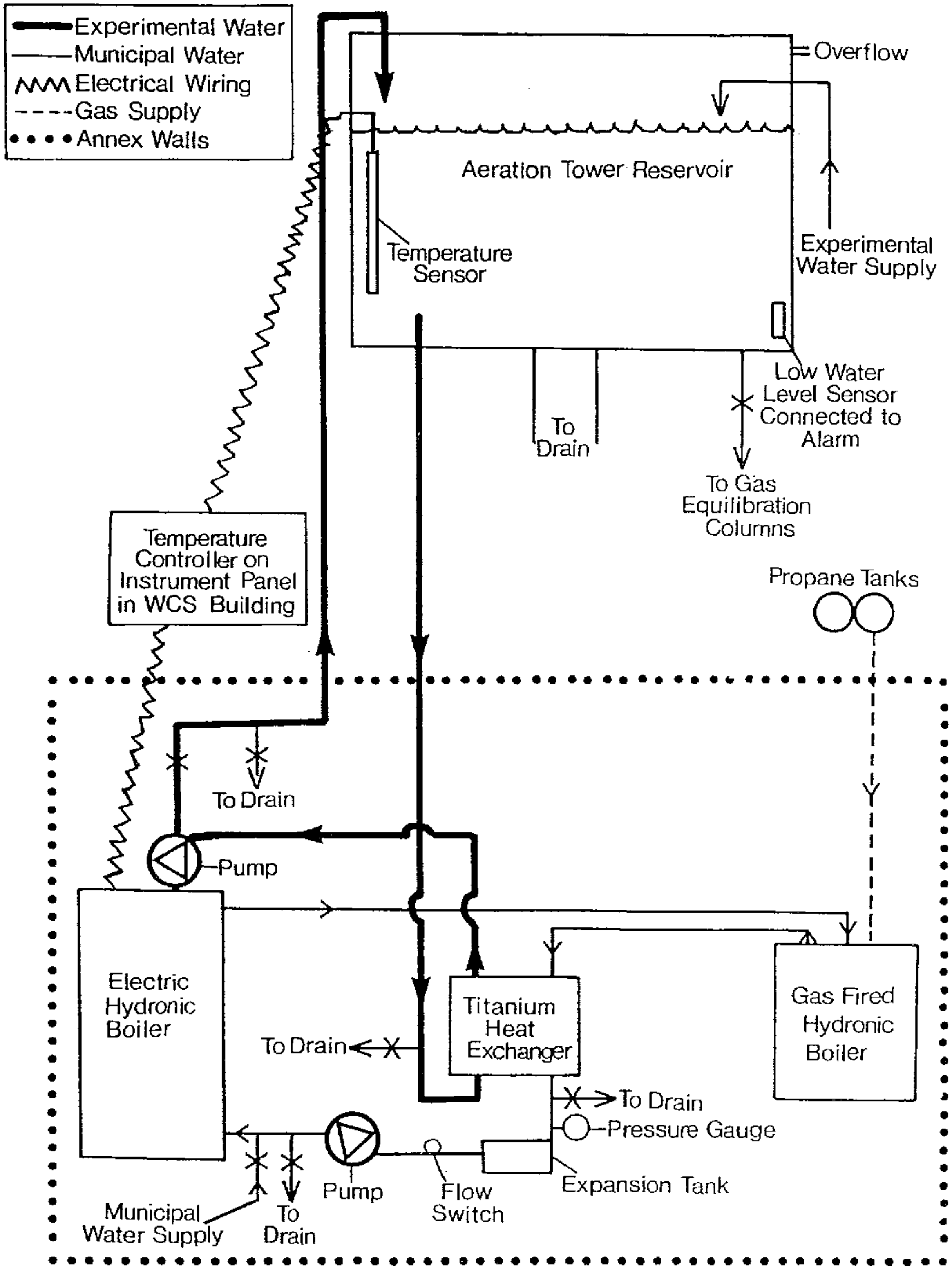

Figure 5. Schematic of the water heating system

Figure 6 Legend

1. Packed PVC column
2. Aspirator
3. Control/Alarm panel
4. Flow control valve
5. Flow control valve
6. Float activated level control switch
7. Single stage impeller pump (¾ h.p.)
8. Air release check valve
9. Vacuum line
10. Vacuum gauge
11. Dissolved oxygen controller
12. Solenoid valve – vacuum control
13. Water trap
14. Water flowmeter
15. Solenoid valve – water supply
16. Dissolved oxygen probe (temperature compensated)
17. Solenoid valve – bypass
18. Drain cap
19. Pump discharge check valve
20. Vacuum line

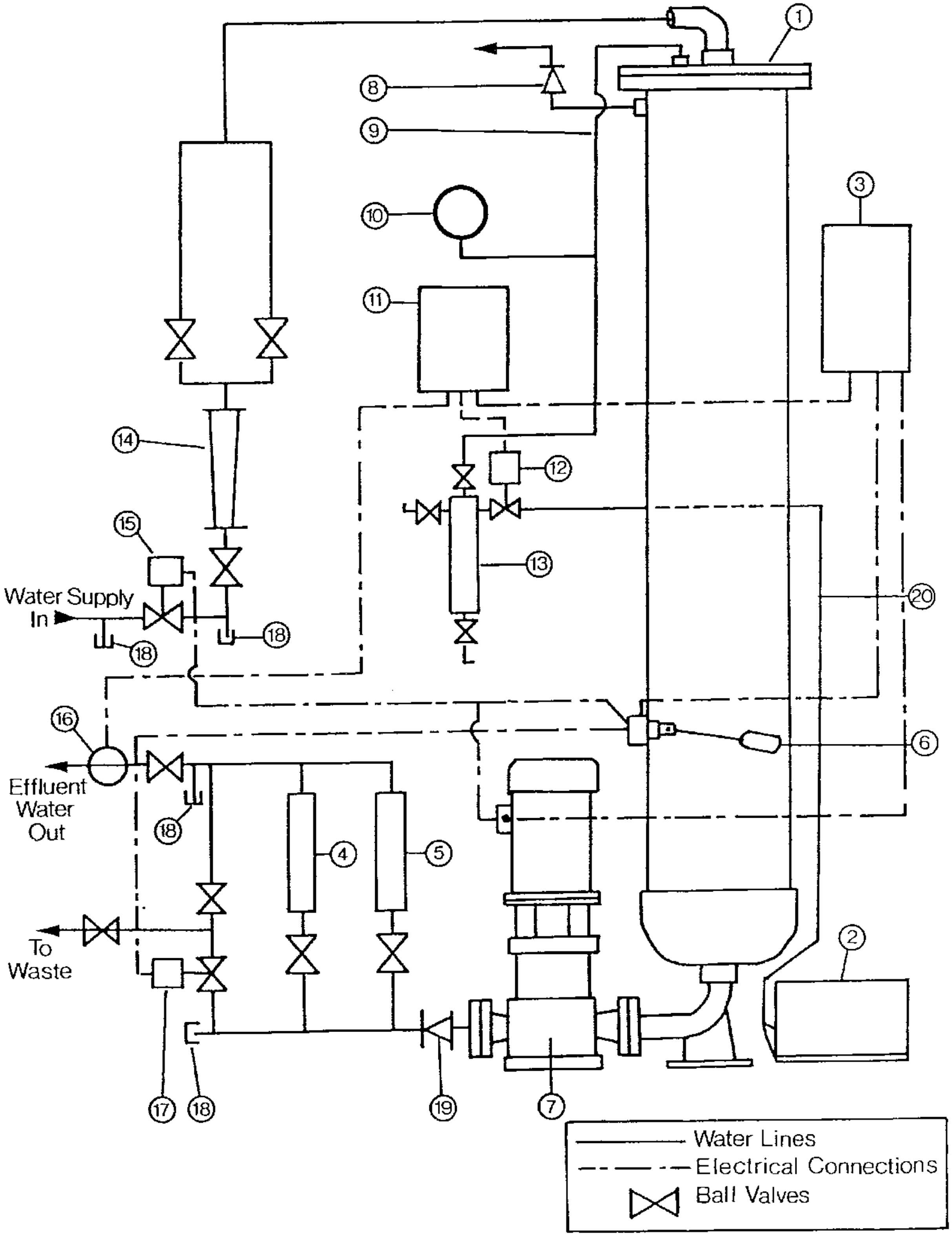

Figure 6. Schematic of the vacuum degasser apparatus

APPENDIX A - OPERATION

PREPARATION PROCEDURES

The following tasks should be performed before filling the WCS aquarium with water:

Flush water through gas equilibration columns and constant head reservoirs and check airtight seal (Figure 2).

Flush the water supply lines to clear out debris.

Fasten temperature data loggers at appropriate height on Velcro® strip inside the aquarium.

Close aquarium drain and pump drains (Figure 3, No. 34 and No. 6).

Open the 6" butterfly valves and air bleeds (Figure 3, No. 12, No. 13, No. 16 and No. 18).

Open the control, shunt and air bleed valves for the differential pressure indicator gauges (Figure 3, No. 17).

Adjust height of water level control standpipes (Figure 3, No. 21).

Open the bypass control valve (Figure 3, No. 8).

Install clean water filters if necessary (Figure 3, No. 10) (Figure 2, No. 8).

Inspect and calibrate all water quality meters and reinstall probes if removed.

Electrically activate temperature, conductivity and oxygen meters at instrument panel and set conductivity meters to the appropriate range.

Enable the data acquisition system with a new file name to identify the experiment and enter appropriate slopes and offsets for calibration screen of Genesis program.

Polish outside of Plexiglas® aquarium walls, if required, with Novus Plastic Polish.

Adjust the angle of occlusion on the photoperiod shutter to the appropriate seasonal setting and turn on the shutter motor and fan (Figure 3, No. 38).

Set the timer for the red filter illumination system (i.e. on 1 h before the start of "dusk" and off 1 h after the start of "dawn") (Figure 3, No. 40).

Set the timer for the metal halide lamp (i.e. off 0.5 h after the start of full dark and on 0.5 h before the start of "dawn") (Figure 3, No. 39).

Insert tape in video recorder and check date/time imprinter and all inter-equipment cable connections (Figure 1).

Turn on the dehumidifier and fans in front of the aquarium.

FILLING PROCEDURES

Fresh or Seawater Filling

The aquarium requires up to four hours to fill due to constraints on the water supply line pressure, which can vary widely with upstream use. To fill, open the fresh or seawater supply line valves until the water supply manifold flow meters (Figure 2, No. 4) for each zone of the aquarium reach the desired setting (usually 20 $L\cdot min^{-1}$). When filling with fresh water it is preferable to bypass the aeration tower (Figure 2, No. 27). If the flow rate is not balanced between water entering and exiting the aeration tower reservoir, it will drain, causing accumulated particulate matter on the bottom of the reservoir to enter the aquarium through the piping. If this occurs the aquarium must be drained and cleaned before refilling. Filling is complete when the water level is 2 cm above the highest acrylic wing (Figure 3, No. 29) at the left-hand side of the aquarium.

Stratified Water Column

Empirical testing of the WCS apparatus has shown that the most well defined vertical stratification of salinity is obtained when the tank is initially filled with fresh water. Seawater flow to the designated zones (e.g., middle and bottom) should then commence after the water pumps (Figure 3, No. 5) are in operation. Allow approximately 4 hours for the replacement of fresh with seawater in these zones.

Alternatively, if stratification is required in less than 4 hours, the aquarium can be filled and stratified before the pumps are turned on. In this situation it is essential to constantly monitor the filling process in order to prevent turbulence at the halocline as it forms. To fill, open the seawater supply line valves until the water supply manifold flow meters (Figure 2, No. 4) for bottom and middle zone reach the desired setting (usually 20 $L\cdot min^{-1}$). No seawater should be flowing into the top zone. Stop filling when the seawater is immediately below the top zone inlet (Figure 3, No. 25). Slowly add fresh water to the top zone so as not to create turbulence at the halocline. When the resulting fresh water layer is a few centimeters deep, lower the halocline by opening the main drain valve (Figure 3, No. 34) for a few minutes. To create a stable halocline, repeat these two steps until the top zone water level is 2 cm above the highest acrylic wing (Figure 3, No. 29) at the left-hand side of the aquarium and the halocline is approximately 3 cm below the middle acrylic wing.

START-UP PROCEDURES

Experimental Water Circulation System

1. It is essential that the aquarium is full of water before the main water pumps (Figure 3, No. 5) are started.

2. Close all air bleed valves (Figure 3, No. 13, No. 16 and No. 18).

3. Close the 6" butterfly valve (Figure 3, No. 19) and bypass control valve (Figure 3, No. 8).

4. Close the differential pressure indicator gauges "blue" control, "red" shunt and "bronze tap" air bleed valves for all zones of the aquarium (Figure 3, No. 17).

5. Activate the pump for the bottom zone by first switching the pump circuit breaker to the "on" position (located on the wall directly above the pumps) and then enable the pump with the black "start" button on the pump control box (located on the west wall of the WCS building, next to the circuit breaker panel).

6. Open the air bleed valves for the bottom zone water circulation loop (Figure 3, No. 13, No. 16 and No. 18).

7 To bleed air from the bottom differential pressure indicator gauge:
    - open "red" shunt valve
    - open "blue" control valves
    - open differential pressure indicator air bleed valves "bronze taps" for 10 seconds and then close
    - close "red" shunt valve
    - the gauge should read "0", if not gently tap the glass face of the gauge before repeating the procedure as the needle indicator sometimes sticks in a previous position

8. Water velocity is controlled by the 6" butterfly valves (Figure 3, No. 19) and measured by differential pressure indicator gauges (Figure 3, No. 17). Slowly open the 6" butterfly valve (Figure 3, No. 19) for the bottom zone until the desired differential pressure indicator gauge reading is attained. Typically, the gauge display should be set at "3" which corresponds to a water velocity of 3.2 $cm \cdot s^{-1}$ in the aquarium. Allow time for trapped air to exit the system. If the valve is opened too rapidly a geyser of water will spill from the vertical standpipes (Figure 3, No. 20). If this occurs, close the butterfly valve a couple of notches and wait a few minutes before recommencing the procedure, a notch at a time.

9. Close all air bleed valves for the bottom zone water circulation loop (Figure 3, No. 13, No. 16 and No. 18).

10. Repeat steps 5 to 9 for the middle and then the top zone of the aquarium, as logistically the pumps require sequential activation due to the intricate and labour intensive start-up protocol.

Water Level Control

1. Start water flow through level control siphon (Figure 3, No. 21). When the pumps are operating the level of the water surface should be approximately 1 cm above the top Plexiglas® wing at the upper left of the aquarium.

2. Adjust the height of the level control standpipes (Figure 3, No. 21). The standpipe permits a constant level of water to be maintained in the aquarium by providing an overflow for water displaced by the continual introduction of new water to the system. The standpipe allows for the adjustment of the total volume of water in each level and hence the adjustment of the positions of the interfaces between the levels. The height of the level control standpipes is particularly important when the aquarium is vertically stratified in terms of fresh water over seawater, warm water over cold water or both simultaneously. The height settings of the standpipes relate to one water velocity only and the elevations are set independently due to the water density differences in each level. If the water velocity in the aquarium is readjusted, the standpipe height will have to be readjusted as well. Adjustments should be made when the aquarium is full of fresh water, before seawater is introduced, to minimize disruption of the halocline. The halocline or thermocline should be approximately 3 cm below the Plexiglas® wing of the middle zone. If the halocline is less than 3 cm from the wing, turbulence is created and the halocline becomes less distinct. When an adjustment of the standpipe is made, allow 30 minutes for the new vertical stratification structure to reach equilibrium. The halocline or thermocline can be raised much more quickly than it can be lowered. It is therefore advisable to begin with the standpipes at or slightly below the desired setting. When "fine tuning" a standpipe height, very little adjustment is necessary for a response (i.e. 1 to 2 turns of the height adjustment mechanism can have a significant effect on an interface).

3. Readjust setting on water supply manifold flowmeters (Figure 2, No. 4) to balance the overflow at the level control standpipes (typically 20 $L\cdot min^{-1}$) and maintain the aquarium volume.

Refrigeration System

Operating Instructions - (The scope of this manual is supplementary to the manufacturer's instructions: Refer to chiller system manual for a more detailed description, wiring diagrams, safety precautions, maintenance and trouble shooting tips. Refer to Figure 4 for a schematic diagram of the refrigeration system):

1. Set compressor circuit breaker to "on" position (located on panel adjacent to compressor system in the annex) at least 24 h prior to starting the system in order to energize the compressor oil heater and thus prevent oil congelation. It is recommended that this circuit breaker be left "on" year-round.

2. Set the refrigeration system municipal water pump circuit breaker to the "on" position.

3. Check expansion tank (Figure 4, No 18) water level and set the flow of municipal water to the tank at 0.5 - 1 $L \cdot min^{-1}$ using valve No. 8 (Figure 4) and associated flow meter.

4. Check that all valves (water supply and drain lines) are in the appropriate water recirculation position (i.e. open or closed as required).

5. Ensure municipal water is flowing through condenser valve No. 14 (Figure 4) at compressor unit.

6. Check oil level in sight glass (Figure 4, No. 4) at the compressor base (Figure 4, No. 2). A minimum of $^1/_3$ to $^2/_3$ of the glass should be covered. When the system is operating the oil level cannot be accurately checked. The correct oil pressure will discharge oil from the relief valve against the sight glass. Therefore, oil spray at the sight-glass indicates sufficient oil pressure in the system.

7. Activate the refrigeration system municipal water centrifugal pump (Figure 4, No. 11) by pushing the black "start" button, which is located on the pump motor starter box. The refrigeration system including Freon™ compressor should start at this point.

8. With the system operating for at least 5 min, check the Freon™ sight-glass (located on the annex wall above the compressor, adjacent to the desiccant canister). A large flow of bubbles should not be apparent. The color indicator in the sight-glass should be blue after ½-1 h, to indicate appropriate moisture levels.

9. Set the 3 remote temperature signal controller units (i.e. one controller for each zone of the WCS aquarium) on WCS instrument panel (Figure 1) to desired setpoint temperature using the manufacturer's guidelines.

Boiler System

Operating Instructions - (The scope of this manual is supplementary to the manufacturer's instructions: Refer to boiler system manual for a more detailed description, wiring diagrams, safety precautions, maintenance and trouble shooting tips. Refer to Figure 5 for a schematic diagram of the boiler system):

1. Open experimental water supply into aeration tower reservoir (Figure 2, No. 24) through flow meter on the tower platform.

2. Confirm that the aeration tower reservoir has minimum water depth of 50 cm (preferably filled to the point that a trickle is displaced via the overflow drainspout which protrudes through the side of the reservoir) before starting the experimental water circulation loop pump.

3. Open municipal water supply valve at base of electric hydronic boiler (Figure 5) to establish circulation loop between the boilers and the titanium heat exchanger.

4. Confirm that 15 psi is displayed on the electric boiler pressure gauge which is located on the municipal water circulation loop.

5. Confirm that the 100 amp boiler system power supply circuit breaker switch is in the "on" position (located on separate grey panel from multi-circuit box on annex wall).

6. Set boiler control circuit breaker to "on" position (located on multi-circuit box on annex wall).

7. Confirm that the pump (located on top of the electric boiler) which circulates experimental water in a loop between the aeration tower reservoir and the titanium heat exchanger is operating.

8. Set remote temperature controller unit on WCS instrument panel (Figure 1) to desired setpoint temperature using the provided manufacturer's guidelines.

9. Establish experimental water flow to WCS aquarium (See Figure 2: A schematic diagram of the water delivery system to the WCS aquarium).

10. When the electric boiler is unable to maintain the desired setpoint on the remote temperature controller the gas-fired hydronic boiler boosts the temperature of the water in the municipal water circulation loop which flows through the titanium heat exchanger. The gas boiler water pump is automatically energized via the pump relay when the temperature controller calls for assistance in heating however the pilot light must be lit for the boiler to function. **Refer to the provided manufacturer's instruction and safety precaution booklets before attempting to manually light the pilot burner or supply propane to the unit.**

SHUT DOWN PROCEDURE

1. Stop logging water quality information on the Computerized Data Acquisition System, save and download the Genesis file to a back-up disk as a precaution. Follow procedures outlined in supplier's handbook.

2. Stop time-lapse video recording equipment and label videotape with relevant information for future analysis. Turn off camera and cover lens with protective cap.

3. Deactivate the pumps for the each zone of the WCS aquarium by first pressing the red "stop" button on the control box (located in the WCS building on the west wall) and then disengaging the pump circuit breakers (located on the wall directly above the pumps). The pumps may be shut down at any time without preparation. If however, the pumps are to be restarted while the aquarium remains filled the 6" butterfly valves (Figure 3, No. 19), bypass control valve (Figure 3, No. 8), and flowmeter valves (Figure 3, No. 2) should be closed and the air bleed valves opened (Figure 3, No. 16 and No. 18). See pump start-up procedures in Appendix A.

4. Turn off the refrigeration system by pushing the red "stop" button at the municipal water pump motor starter box (Figure 4, No. 11). Switch the municipal water pump circuit breaker to the "off" position. Leave system valves in open or nearly open position so valve plate does not corrode. Close the municipal water supply valves to the heat exchangers (Figure 4, No. 13) expansion tank (Figure 4, No. 8) and condenser (Figure 4, No. 14). Open valve (Figure 4, No. 10) to drain the expansion tank and valve (Figure 4, No. 23) to drain the chillers (Figure 4, No. 5) and heat exchangers (Figure 4, No. 1). Drain condenser (Figure 4, No. 3) if use is not expected in the near future. It is recommended that the compressor oil heater circuit breaker be left "on" year-round if possible. Turn the setpoint temperatures on the 3 remote temperature signal controller units to the lowest setting possible.

5. Turn off the boiler system by resetting the setpoint temperature on the remote temperature signal controller unit to the lowest setting possible. Sequentially switch the boiler control circuit breaker and then the 100 amp boiler power supply circuit breaker to the "off" position. Turn gas control knob to "off" position on gas-fired boiler and ensure that pilot light has been extinguished. Stop propane supply to regulator and gas-fired boiler by closing valve on cylinder. Turn off municipal water supply to circulation loop between gas and electric boilers and heat exchanger (Figure 5). Turn off replacement water flow to the aeration tower reservoir at the flowmeter on the tower platform. Open drain valves in both the municipal and experimental water circulation loop plumbing systems if operation is not expected in the near future (Figure 5).

6. Open the WCS aquarium drain (Figure 3, No. 34).

7. Open the air bleeds (Figure 3, No. 16 and No. 18).

8. Open the 6" butterfly valves (Figure 3, No. 19).

9. Close the differential pressure indicator gauges "blue" control valves (Figure 3, No. 17).

10. If the gas equilibration columns and constant head reservoir were filled with seawater, fresh water should be diverted to and flushed through them to limit algal and bacterial growth in the lines.

11. Close the water supply valves and drain the water supply system by opening the six valves of the water supply manifold (Figure 2, No. 4).

12. The inside of the WCS aquarium and especially the Nitex® screens should be rinsed with municipal water (do not use soap) through a hose equipped with an adjustable pressure nozzle attachment. Wash water remaining on the aquarium floor should be removed with a "wet/dry" shop vacuum or siphon connected to a long flexible hose.

13. Drain the level control standpipes (Figure 3, No. 21) and flush them with municipal water by hose.

14. Remove dissolved oxygen probes from the vertical standpipes (Figure 3, No. 20) and tensionometers from inside the aquarium then clean and inspect as instructed in provided manufacturer's manual.

15. Remove temperature data-loggers from Velcro® strip in the aquarium and download stored information to computer files following manufacturer's instruction manual.

16. De-energize the temperature and conductivity meters plus remote temperature controller units at circuit breaker box.

17. Turn off Light Pipe™, Luminaire, red filter illumination system, shutter mechanism and fan at circuit breaker box. If required, due to insufficient illumination in the WCS building, turn on the fluorescent ballast directly over aquarium which runs parallel to Light Pipe™ when performing tasks near the aquarium.

18. Open the main water pump drain valves (Figure 3, No. 6) which will drain water from the main water circulation loop plumbing.

19. Turn off the dehumidifier and fans in front of the aquarium.

COLD WEATHER PRECAUTIONS

Instructions for protecting the non-operational WCS apparatus in winter conditions:

1. Water lines that are above ground should be drained or have a continuous low flow of water through them.

2. The municipal water in the cooling system is fresh, and therefore susceptible to freezing in the winter. In order to prevent the destruction of the heat exchangers and their associated piping, it is essential that this system be drained in the winter if the aquarium is not being operated. In the refrigeration annex, remove the drain plugs from the condenser (Figure 4, No. 3) and open valve at compressor base (Figure 4, No. 14) in order to drain pipes. Leave the compressor oil heater circuit breaker in the "on" position.

3. Drain the municipal water circulation loop plumbing between the gas and electric boilers and titanium heat exchanger (Figure 5) after ensuring that the municipal water supply has been shut off. Drain the experimental water circulation loop plumbing between the aeration tower reservoir and the titanium heat exchanger by opening all drain valves. Drain the aeration tower and terminate water supply.

4. Leave the electric baseboard heaters in the WCS building and the refrigeration annex on continuously at low heat to prevent mold and mildew growth due to excess dampness.

5. Turn circuit breakers for equipment that is not in use to the "off" position

6. Move computer equipment and instruments that may be adversely effected by humidity to alternate location for the winter.

Tips for operating the WCS apparatus in winter conditions:

1. If operating the gas-fired hydronic boiler in the winter check the vent opening on the propane tank regulator after a freezing rain, sleet storm, or snow to ensure that ice has not formed in the vent.

2. To prevent accidental moisture damage to the videotape and recorder caused by condensation, be sure that the videotape is kept at room temperature for at least 1 hour before using. Use of a cold videotape in a warm recorder could result in the formation of moisture on the tape, which would hinder recording and image capture.

APPENDIX B - GENERAL MAINTENANCE AND METER CALIBRATION

WATER DELIVERY AND CIRCULATION

A red-brown iron oxide precipitate in the freshwater supply manifold and algal growth or invertebrate shells in the seawater supply manifold will form a coating on or clog components of the apparatus. This will impede flow and prevent accurate readings for the flow meters. To clean the flow meters, soak them for approximately two minutes in a solution of hydrogen chloride and water (1:10). For general cleaning purposes the flowmeters should be cleaned with a soft bottlebrush and a mild soap and water solution. Periodically, the biorings in the gas equilibration columns and aeration tower buckets and the mesh filters at the top of the columns will need to be removed and cleaned. When the biorings become clogged, water channels down through the buckets, making most of the dispersion medium ineffective. The seawater cartridge filters should be removed, rinsed with municipal water and replaced as necessary. If the filters are not cleaned, the flow rate of incoming seawater will decrease in proportion to the occlusion.

The 7.5 h.p. centrifugal recirculation pumps (Figure 3, No. 5) are of cast iron construction but the inside of the casing, pump impellers and all other exposed inside surfaces have been protected with a two-part plastic coating over a heavy-bodied primer. The pump lantern ring and packing gland have not been coated and will undoubtedly corrode. Although the pump packing glands are a dry seal type, some water will always seep from the stuffing box (containing the shaft seal packing, lantern ring etc.). If excessive dripping occurs, service is required. The seepage will contain a certain amount of rust, however this does not mean that the circulating water in the aquarium is contaminated. An inspection of the pumps and the temperature control system should be performed annually by certified professional service contractors. The pump shafts should be rotated every two months during periods when not in use.

LIGHTING AND VIDEOTAPE RECORDING

Plastic polish (No.2, Novus Inc., Minneapolis, MN) will clean, add antistatic and dust resistant properties to Plexiglas® and remove fine scratches, haziness and abrasions on the acrylic aquarium walls. Solvents such as acetone will score the surface and should not be used.

If the metal halide luminaire is switched off after it is warm, it must cool for approximately 15 min before being turned on again.

Avoid jarring or striking the camera as it contains a sensitive image pick-up tube, which can be damaged by improper handling. Always keep the camera in the horizontal position when transporting. Avoid turning the power on to the camera with the lens capped as this may shorten the life of the image pick-up tube. Whether the camera is in use or not, never face it toward the sun or an extremely bright object as this may damage the photoconductive layer on the image pick-up tube which acts as the photosensitive surface. The video system is designed for indoor use and therefore should not be used outside the ambient temperature range of $^{-}18$ °C to 50 °C and above humidities of 90%.

Avoid operating or storing the time-lapse video recorder or videotapes near strong magnetic fields, such as that produced by an electric motor, power transformer or large audio speakers. The magnetic field may accidentally erase the tape. Do not leave videotapes in the recorder when not in use. Operate the recorder in a horizontal position and do not place any heavy items on the top panel or block ventilation slots on the top and bottom of the unit. The recorder is designed exclusively for time lapse use and therefore, the recording system will not allow interchanging recorded programs with a standard VHS unit. Do not attempt to disassemble the recorder, camera or monitor, as there are no user serviceable parts inside. Refer any required servicing to qualified professional personnel.

WATER QUALITY MONITORING

Water Quality Manifold and Port Sampling System

Radiometer Calibration and Storage: The radiometer probe is housed in a glass cell through which water, thermoregulated by a water bath, is recirculated. Bath temperature should be as warm as feasible since warm temperatures reduce meter response time and create conditions less likely to cause retention of bubbles in the measuring chamber. A bubble may cause a "spike" in the oxygen partial pressure measurements and, therefore, should be removed by withdrawing the sample with a syringe.

Allow the oxygen partial pressure probe to polarize by setting the function switch to "PPO_2" at least 12 h before calibration. The "zero" and "air calibrate" settings on the meter should be checked at the start and end of each day. Since the air calibration setting is subject to drift, it should be checked each hour and adjusted if necessary. Calculations for drift correction should be performed as appropriate.

Adjust the air calibration setting by immersing a beaker of deionized, distilled water in the well of the water bath. Aerate with an aquarium pump until saturated and allow it to equilibrate to the bath (i.e. cell) temperature.

Calculate the air saturation setting with the following formula:

Air cal. = [BP-VP] x .2093

Where:
BP = barometric pressure (mm Hg)
VP = vapor pressure (mm Hg) of air at cell °C

Visually inspect for air bubbles under the radiometer membrane and replace if necessary. Slowly inject a sample of the air and temperature equilibrated water into the radiometer chamber and adjust the meter to the air calibration value generated from the formula. Check electronically for membrane damage by pressing "leak" and ensure that meter returns to a position within 20 mm Hg of the initial value. If it does not, the membrane is damaged and should be replaced. The membrane should be replaced monthly.

The zero setting of the meter is verified by injecting a solution of 100 mg sodium sulphite (which strips oxygen from the solution), dissolved in 5.0 ml distilled water, into the radiometer chamber. Adjust zero as necessary.

If each zone of the WCS aquarium is the same temperature, the water bath can be operated at the same temperature and no data corrections are necessary. However the WCS aquarium may be thermally stratified with the top zone at 20 °C and the middle and bottom zones at 12 °C. In this case the water bath should be set to an intermediate temperature (approximately 17 °C) and the measurements corrected for temperature using the formula below (J. Zavitz, Bach Simpson Ltd., London, Ont., personal communication):

$$R_t(\text{mm Hg}) = R_{meas}(\text{mm Hg}) \times \frac{\text{Sat Cell } (\text{mg}\cdot\text{L}^{-1})}{\text{Sat Sample } (\text{mg}\cdot\text{L}^{-1})}$$

Where:

R_t = oxygen partial pressure corrected for temperature

R_{meas} = oxygen partial pressure measured

Sat Cell = dissolved oxygen at saturation $\text{mg}\cdot\text{L}^{-1}$; cell °C, sample salinity

Sat Sample = dissolved oxygen at saturation $\text{mg}\cdot\text{L}^{-1}$; sample °C, sample salinity

If required, the oxygen partial pressure (R_t mm Hg) can be converted to oxygen concentration $\text{mg}\cdot\text{L}^{-1}$ by the following formula (J. Zavitz, Bach Simpson Ltd., personal communication):

$$\text{Dissolved Oxygen } (\text{mg}\cdot\text{L}^{-1}) = \frac{R_t(\text{mm Hg})}{\text{Air cal } (\text{mm Hg})} \times \text{Sat Sample } (\text{mg}\cdot\text{L}^{-1})$$

The concentration of oxygen at saturation (i.e. "Sat Cell") at a specified temperature and salinity are calculated using the formula of Benson and Krause (1984).

For long term storage of the radiometer electrode, remove the probe membrane and remove batteries from the meter. After long periods of radiometer storage, or if measurements are suspect, two independent calibration checks can be done.

Chemical verification of calibration

Fill a 300 ml "biochemical oxygen demand" bottle with deionized, distilled water, immerse in the water bath (Model FK10 70092, Haake Buchler Instruments Inc., Saddlebrook, NJ) and aerate with an aquarium air pump and air stone for 30 minutes. Withdraw several samples from the bottle with a glass syringe and measure oxygen partial pressure with the radiometer. Convert the measurements from mm Hg to $mg \cdot L^{-1}$ using the formula provided above. Perform a Winkler analysis on the bottle contents to obtain the concentration of oxygen in mg/L by chemical titration (Parsons et al. 1985).

Theoretical verification of calibration

Derive the theoretical concentration of oxygen in water at equilibrium with the atmosphere with the equation or table from Benson and Krause (1984). Aerate a beaker of deionized, distilled water for 30 min. Use the radiometer to measure the oxygen partial pressure in the beaker of water. Convert the oxygen partial pressure (mm Hg) to oxygen concentration ($mg \cdot L^{-1}$) using the formula provided above. Any discrepancies among the measurements derived from the above methods should be investigated.

Port Sampling: To begin oxygen partial pressure measurements on water from the aquarium, extract and discard 20 ml of water through the water-sampling manifold (Figure 3, No. 32). This is a precaution against oxygen partial pressure changes during residence time in the tubing (volume = 6.5m x 1.57 mm i.d. = 12.6 cm^3) and for use in salinity measurements. Use a 10 cm^3 syringe (Jelco 08869, Johnson and Johnson Medical Inc., Arlington, Texas) to extract 2 ml of aquarium water for salinity measurements. Salinity can be measured with an osmometer or a refractometer. Extract a sample for oxygen partial pressure measurement using a 2 ml glass syringe. Do not use a plastic syringe as oxygen can be transferred between the atmosphere and the sample (Scott et al. 1971). Inject the sample slowly into the radiometer measuring chamber. Allow the meter to stabilize (usually less than 2 minutes) before recording the measurement.

Temperatures near the thermocline (ports 2,3,4,5 and 6) are measured by a telethermometer (43TD, YSI Inc., Yellow Springs, OH) with probes inserted into the water sampling manifold (Figure 3, No. 32 and Figure 2). Sensors (Model RTP 120730120061, Action Instruments, San Diego, CA) at the left-hand side of the aquarium measure the temperature in each zone. An earlier modification of the WCS required removing water from the WCS via the manifold to a series of thermometers mounted in glass tubes (Piercey et al. 1992) on the right hand side of the aquarium. However, in spite of insulation and the installation of a pump to increase water flow through the tubes, the sample temperature increased up to 3 °C. Consequently, the in situ method of temperature measurement was employed.

Under vertically stratified salinity conditions, salinity determinations at each port are required to describe the stratification structure and location of the halocline. Extract samples for salinity measurement with a 10 ml syringe as described above. Place three drops on the sample plate of a temperature compensated salinity refractometer (Aquafauna, Argent Laboratories, Redmond, WA). Point the refractometer toward the light and read salinity from the scale on the lens. This method is quick and can be done during oxygen partial pressure measurement but is less accurate than the osmometer described below.

Salinity can also be measured with an osmometer (Model Osmette A, Precision Systems Inc., Natick, MA). These determinations are done after an experiment is completed using the 2 ml sample collected during oxygen partial pressure measurement. Osmolality is directly proportional to salinity and can be measured with small sample volumes (i.e. 0.2 ml). Measure osmolality in two replicates from each vial. If the difference between replicates exceeds 5 $mOsm \cdot L^{-1}$, analyze a third replicate. Calculate the mean of the two closest replicates. The relationship between osmolality ($mOsm \cdot L^{-1}$) and salinity (‰) was determined using a series of seawater dilutions on which osmolality and salinity (Model 8400, Autosalinometer, Guildline Instruments, Lake Mary, FL, ±0.003 ‰ accuracy, better than 0.0002 ‰ resolution) were measured. The following regression formula was generated:

$$\text{Salinity} = 0.034307\ (\text{Osmolality}) + 0.016492 \qquad (r^2 = 0.9997)$$

Dissolved Oxygen

The scope of this section is complimentary to the manufacturer's manual (Point Four Systems Inc., Port Moody, BC) which was supplied with the instrument. Refer to dissolved oxygen probe manual for a more detailed description of specifications, wiring diagrams and installation procedures, safety precautions, warranty information, maintenance and trouble shooting tips.

Dissolved oxygen sensors are inserted into the water mass flowing through standpipes that rise vertically from the 6" horizontal pipe at the left side of the aquarium (Figure 3, No. 20). The Oxyguard Stationary Probe is an active galvanic cell that generates an electrical signal proportional to the oxygen pressure it senses, whether in water, air or another medium. The probes' output is linear and therefore it was possible to connect directly to the Computerized Data Acquisition System, which displays dissolved oxygen on the computer monitor.

The probe consists of two parts, an upper part with cathode, anode and cable, and a lower part comprising a screw on membrane cap with fitted membrane. The cap is filled with electrolyte and simply screwed onto the top component. The probe is self-polarizing and requires no external power source. The probe's robust construction and simple design make maintenance and servicing straight forward. It utilizes a strong, easy-to-clean and easy-to-change membrane in a screw-on membrane cap. Cleaning the membrane is, apart from an occasional calibration, the only routine maintenance necessary. There is no need to send the probe back to the factory for servicing, as regular servicing is not required. The probe can be fully renovated in-situ, within five minutes, without the use of tools, at negligible cost. See manual supplied by Point Four Systems Inc. for further instruction. After renovation the probe should be hung up in air to stabilize for at least an hour before calibrating and should be recalibrated after 1 or 2 days.

A bacterial film can form on the membrane of an oxygen probe when in a biologically active system. This film acts as a barrier to the oxygen diffusing through the membrane. The probe must therefore be cleaned at regular intervals, the frequency depending on such factors as the biological loading of the system, the oxygen consumption of the probe and the accuracy required. Cleaning is accomplished by wiping the electrode surface with a cloth or soft paper, as the membrane is strong and not easily damaged.

A zero calibration can be done by immersing the probe in a solution of 10 g sodium sulphite (which strips oxygen from the solution) per 500 ml deionized water. Adjust zero as necessary.

Freezing can damage the probe cable. If the probe itself freezes the membrane will be damaged and will need replacing (See manual supplied by Point Four Systems Inc. for further instruction). Store the probe upright inside a plastic bag with a little water or in a bucket of water, keeping the cable end dry. If the probes are stored in air for long periods, loss of electrolyte through evaporation will occur via the small breather hole at the top of the probe. If the probe has completely dried out it will not be possible to calibrate the probe in air (the display will not read high enough after fully adjusting the calibration screw). Therefore, the probe will require more frequent servicing if routinely stored in air.

Conductivity

The scope of this section is complimentary to the manufacturer's manual (Uniloc, A Division of Rosemount Inc., Irvine, CA) which was supplied with the instrument. Refer to conductivity probe manual for a more detailed description of specifications, wiring diagrams and installation procedures, safety precautions, warranty information, maintenance and trouble shooting tips.

Conductivity is measured in each zone at the inlet pipe on the left-hand side of the aquarium by probes and meters. It is also measured at precise locations in the water column through a manifold. The Computerized Data Acquisition System displays conductivity and salinity on the computer monitor.

The conductivity meters are calibrated by correlating the meter reading of low and high conductivity samples with the readings of an autosalinometer (Model 8400, Guildine Instruments, Lake Mary, FL). Remove the probes from the Plexiglas® plate and immerse in a bucket containing seawater. If the probes have been stored dry they should be soaked either in municipal water for 12 hours or in a 1:100 soap/water solution for one minute and then flushed with clean water. Apply power to the unit and place the range selector switch in the proper position to obtain the desired full-scale conductivity range. Collect a sample of seawater and record the meter raw conductivity from the computer monitor and perform an independent analysis with an autosalinometer. Repeat with a sample of low salinity water. Adjust the meters to the autosalinometer values as described in section 4.3.8 of the Computerized Data Acquisition System manual. The temperature compensation circuit in the conductivity monitor and thermistor inside the conductivity sensor automatically correct the measurement to a reference temperature of 25 °C.

Check the calibration weekly while the WCS is in operation and when the salinity stratification between zones is stable. Collect a 250 ml water sample at the sampling port in the 6" pipe at the left side of the aquarium and record the conductivity as displayed on the monitor. Analyze the sample with the autosalinometer and compare with the meter conductivity. If there is a discrepancy, which is outside of the accuracy limits for the system, a correction must be applied to the data. Otherwise, if a conductivity meter malfunction is suspected, the source of the problem may be isolated (i.e. meter vs. probe) by means of a generic "resistor box" which is stored in the WCS building. The box contains 3 resistors, which are substituted for the conductivity probe and yield a mid-scale meter indication. A low resistance test is done by connecting the meter to the 169 and 19,000 ohm resistors simultaneously while a high resistance is simulated by connecting the meter to the 267 and 19,000 ohm resistors simultaneously. Connect the resistor box to the meter and set the range selector switch to the "check" position. Record the computer reading. This procedure should be done before experiments commence. Any change in the values indicates that there is a problem in the circuitry of the meter (i.e. not the probe). Recalibration and possibly service is required.

In order to obtain reliable and accurate readings when the system is in operation, the electrodes of the probe must be in fluid at all times and not in contact with air bubbles. Conductivity probes should be cleaned after approximately 50 days of use as fouling with a biological film may impair the current flow between the two electrodes at the sensor, preventing a stable reading from being obtained. Immerse the probe in a solution of 10 parts distilled water, 10 parts isopropyl alcohol and 1 part hydrochloric acid, for 5 minutes. Flush with clean water and recalibrate.

Temperature

The scope of this section is complimentary to the manufacturer's manual (Action Instruments Inc., San Diego, CA) which was supplied with the instrument. Refer to temperature probe manual for a more detailed description of specifications, wiring diagrams and installation procedures, safety precautions, warranty information, maintenance and trouble shooting tips.

The temperature probes for each zone of the WCS are inserted into a Plexiglas® plate at the left side of the aquarium. Temperature can also be measured at precise locations in the WCS through a manifold inside the aquarium which has been previously described (Figure 3, No. 32). The Computerized Data Acquisition System provides a conversion from raw to calibrated temperature values. The computer monitor displays the calibrated meter temperature values while the temperature meter output screen displays raw values.

The calibration involves mounting a rod stirrer (Type NSI-12, .07 h.p., Bodine Electric Co., Somerville, MA) on a retort stand and constantly stirring water at ambient temperature in an insulated container. Unfasten the connection (Swagelock Co., Solon, OH), which joins the temperature probe to the Plexiglas® plate and place the probe in the room temperature water. Measure the temperature of the ambient temperature water with a calibrated thermometer (± 0.1 °C, NBS certified) or with a digital platinum resistance thermometer (Model 9540, Guildline Instruments, Lake Mary, FL; accurate to ± 0.0017 °C). Record the temperature probe reading from the computer monitor. Adjust the computer monitor display of temperature values to match the digital platinum resistance thermometer (calibrated thermometer) reading as instructed in section 4.3.8 of the Computerized Data Acquisition System manual.

Check the calibration against a calibrated thermometer at the start of each experiment with the aquarium full of water and the 7.5 h.p. centrifugal recirculation pumps off (Figure 3, No. 5). Place a beaker in an insulated container and continuously fill from the sampling port at the left side of the aquarium. Measure temperature in the overflowing beaker with the thermometer and compare with reading temperature from the probes. If the readings are not within ± 0.24 °C (i.e. the thermometer accuracy of 0.1 °C added to the probe accuracy of 0.14 °C) a complete calibration is required.

Clean the temperature probes periodically by rinsing them with distilled water and wiping with a soft cloth. If a meter malfunction is suspected, the problem can be isolated (i.e. meter vs. probe) by the use of another generic "resistor box", similar to that used to test for conductivity meter problems. This box contains resistors of 100, 102, and 105 ohms that are connected to the meter individually to simulate three separate temperatures. This should be done before beginning a series of experiments and the output value of the meter and the computer recorded. A change or drift in the values over time indicates that the meter requires service.

Total Gas Pressure

The scope of this section is complimentary to the manufacturer's manual (Alpha Designs Ltd., Victoria, BC) which was supplied with the instrument. Refer to tensionometer manual for a more detailed description of specifications, wiring diagrams and installation instructions, safety precautions, warranty information, maintenance and trouble shooting tips.

A tensionometer probe is mounted in the center of each zone of the WCS and oriented vertically on the downstream side of the aquarium against the Nitex® screens (Figure 3, No. 31).

To check calibration and operation rotate the zero control fully clockwise and then counterclockwise noting the reading and the sign. Subtracting the counterclockwise reading from the clockwise reading derives the span. The span should be within 1-2 mm Hg of 250. It is suggested practice to return the tensionometer to the manufacturer yearly for inspection and calibration, as an accurate pressure source is required for calibration.

Adjust the zero control to 000. When the meter is at barometric equilibrium the reading should remain stable within ±1 mm Hg over a 5 min period. Once the tensionometer has been zeroed it is unnecessary to re-zero during the course of measurements unless the barometric pressure is changing. When re-zeroing, remove the probe from water and allow to reach barometric equilibrium. The power may be turned off to the meter any time during a measurement or throughout the course of a day without affecting the zero setting or the water reading.

When taking a total gas pressure measurement the probe should be agitated at least once per minute, if not continuously, in order to prevent bubble formation on the membrane. Bubbles on the membrane will slow the response time of the meter and cause erroneously low readings. The probe will have reached equilibrium with the sample water after approximately 10-15 min. High water temperature will shorten response time and lower temperature will lengthen it.

Although the functional life-span of a membrane (silicone tubing) that has received proper care is over 10 years, dirt, oil or algae accumulation on the membrane will slow the response time and is the principal maintenance problem. Soak the probe in a mild non-oily soap and rinse in a dilute solution of bleach. Flush thoroughly with clean water to remove all traces of cleaning solutions.

Mechanical damage to the membrane is possible and probably constitutes the second most likely cause for maintenance. Although the membrane is elastic and extremely rugged it is thin to facilitate diffusion. It can be damaged easily by sharp edges or abrasive materials and will disintegrate when in contact with acids.

Flush the probe with clean water before storing. Store the probe out of direct sunlight and protect it from excessive moisture. Remove the two 9 volt batteries from the meter when storing for long periods and replace batteries at least once a year.

If the probe is left in water for long periods, water vapor will eventually diffuse through the membrane wall where it can condense and create pockets of water. To remove entrained water, place the entire probe in a desiccating chamber for a few days.

APPENDIX C - EQUIPEMENT SUPPLIERS AND MANUFACTURERS

COMPONENT	DESCRIPTION	MANUFACTURER	LOCATION
REFRIGERATION SYSTEM	Supply of components	AC-Systems Engineering Ltd.	North Vancouver, BC
Refrigeration Unit	Model CCU10LR	Keeprite Products Ltd.	Brantford, ON
Compressor	Model 06DA537, 10 h.p., 208 volts, 60 cycles, FLA 36 amps, LRA 266 amps, refrigerant = R502	Carlyle Compressor Co.	Syracuse, NY
Water Cooled Condenser	Model CSTC 848	Dunham Bush Inc.	Hartford, CN
Freon™ Water Chillers	Capacity: 5 tons = 60,000 BTU/hr, Flow: 80 USgpm, Temperature Drop: 0.83°C, Pressure Drop: 5 psig	General Refrigeration Engineering	Vancouver, BC
Water Pump	C69, 2 h.p., centrifugal	KNM Begemann	Guelph, ON
3 Way Control Valves	Model V5013A1211, 1¼ "diameter	Honeywell Ltd.	North York, ON
Valve Actuators	Model ML7984	Honeywell Ltd.	North York, ON
Remote Temperature Controllers	Model T775E	Honeywell Ltd.	North York, ON
HEATING SYSTEM	Supply of components	Allied Engineering Co.	North Vancouver, BC
Electric Hydronic Boiler	Model E, Super Hot	Allied Engineering Co.	North Vancouver, BC
Gas Fired Hydronic Boiler	Model JV,Mini-Therm II	Teledyne Laars	Oakville, ON
Titanium Heat Exchanger	Superchanger	Tranter Inc.	Wichita Falls, TX
Municipal Water Pump	Model 0 00, Cartridge circulator	Taco Inc.	Cranston, RI
Experimental Water Pump	Model 5MD, 1/8 h.p., magnetic drive	Little Giant Pump Co.	Oklahoma City, OK
Remote Temperature Controller	Model T775E	Honeywell Ltd.	North York, ON
Automatic Changeover Regulators	Type R966	Fisher Controls International Inc.	McKinney, TX
WATER CIRCULATION			
Sea Water Filters	Wound Fiberglas high-rate pressure sand filters	Stark Aquatic Systems Inc.	Seattle, WA
Filters (30 and 50µm)	R30-BB and R50-BB	Amatek	Pittsburgh, PA
Filter Housings	No.10 and No.20 Blue Cold	Fabricated Plastics Ltd.	Toronto, ON
Bio-rings	Flexi-ring 2.5 cm	Koch Engineering Ltd.	Calgary, AB
Pressure gauge	0-60 psi	Marsh Bellofram Corp.	Newell, WV
Gas Flowmeter	Catalogue No. 36-541-31	Manostat	New York, NY
Water Flowmeters	CalQflo CF41000LN & CF40750LN	Blue and White Industries	Westminister, CA
Water Flowmeters	Catalogue No. FC-FI-A.¾	Filterchem Corp.	Alhambra, CA
Centrifugal Pump	Model 80-200MC, 7.5 h.p.	Ingersoll-Rand Company Ltd.	Gateshead, England
Induction Motor	3-phase, 7.5 h.p.	Toshiba International Corp.	Houston, TX
Differential Pressure Indicator	Model 227	ITT Barton Instruments	City of Industry, CA
Air Bleed Valve	Model PE-205 7446	Clay Adams	Parsippany, NJ
6" Butterfly Valve	Model WA-20-20	Chemtrol Division, Santa Barbara Control Systems	Santa Barbara, CA
Vacuum Degasser Apparatus	Prototype	Point Four Systems Inc.	Port Moody, BC
Effluent Water Pump	¾ h.p., single stage impeller pump	Grundfos Pumps Corp.	Clovis, CA
Water Current Meter	Propeller drive	Ott Messtechnik, GmbH & Co.	Germany
Portable Water Current Meter	Model 201D, electromagnetic	Marsh-McBirney Inc.	Gaithersburg, MD

COMPONENT	DESCRIPTION	MANUFACTURER	LOCATION
WATER QUALITY			
Dissolved Oxygen Monitoring System	Model 1W, Oxyguard, Single Channel	Point Four Systems Inc.	Port Moody, BC
Dissolved Oxygen Display Unit	PT4 Multi-channel Oxygen Monitor	Point Four Systems Inc.	Port Moody, BC
Dissolved Oxygen Probe	Oxyguard Probe	Point Four Systems Inc.	Port Moody, BC
Portable Oxygen Meter	Handyguard Mk III	Point Four Systems Inc.	Port Moody, BC
Portable Oxygen Meter	Model 7932	Leeds & Northrup	Covington, GA
Dissolved Oxygen Meter	Model 57	YSI Inc.	Yellow Springs, OH
Dissolved Oxygen Display Unit	Model PHM71b Acid-Base Analyzer	Radiometer	Copenhagen, Denmark
PO_2 Electrode	Model E5046	Radiometer	Copenhagen, Denmark
Thermostatted Cell	Model D616	Radiometer	Copenhagen, Denmark
Polyethylene Tubing	PE-205 Intramedic	Clay Adams	Parsippany, NJ
Varistaltic Pump	Junior Model, Cat No. 72-300-000	Manostat	New York, NY
Hypodermic Needle	16G 1½ 5198	Becton, Dickinson and Co.	Rutherford, NJ
Plastic Syringe, 1ml	No. 2A 370 14	Becton, Dickinson and Co.	Rutherford, NJ
10 cm^3 Syringe	Jelco 08869	Johnson and Johnson Medical Inc.	Arlington, TX
Rod Stirrer	Model NSI-12, 0.7 hp	Bodine Electric Company	Somerville, MA
Water Bath and Heat Controller	Model FK10 70092 and Model F4921	Haake Buchler Instruments Inc.	Saddlebrook, NJ
Conductivity Display Unit	Model 750C-0304, Conductivity Analyzer/ Transmitter	Rosemount Analytical Inc. / Uniloc Div.	Irvine, CA
Conductivity Sensor	Sensor 112-09	Rosemount Analytical Inc. / Uniloc Div.	Irvine, CA
Portable Conductivity Meter	Model 30	YSI Inc.	Yellow Springs, OH
Salinometer	Model 8400, Autosal	Guideline Instruments	Lake Mary, FL
Refractometer	Aquafauna, Temperature Compensated	Argent Laboratories	Redmond, WA
Salinity Meter	Model 33	YSI Inc.	Yellow Springs, OH
Osmometer	Model Osmette A	Precision Systems Inc.	Nattick, MA
Temperature Display Unit	VIP501 RTD Input Digital Indicator	Action Instruments Inc.	San Diego, CA
Temperature Sensor	Model RPT 120730120061	Action Instruments Inc.	San Diego, CA
Temperature Data Loggers	Waterproof Stowaway Tidbit	Onset Computer Corp.	Pocasset, MA
Digital Thermometer	Model 9540	Guideline Instruments	Lake Mary, FL
Telethermometer	Meter Model 43TD / Switcher Model 4002	YSI Inc.	Yellow Springs, OH
Telethermometer Probe	Model 401	YSI Inc.	Yellow Springs, OH
Total Gas Pressure	Tensionometer 300C	Alpha Designs Ltd.	Victoria, BC
COMPUTERIZED DATA ACQUISITION SYSTEM	supply of components	Bentek Systems Inc.	Vancouver, BC
Data Acquisition Software	Genesis Control Series .	Iconics Inc.	Foxborough, MA
VIDEOTAPE RECORDING & DIGITAL IMAGE ANALYSIS			
Video Processor	PIP 640B Digitizer Board	Matrox Electronic Systems Ltd.	Dorval, QUE
Videotape Analysis Software	SNAP Digital Image Analysis	Sci Tech Consultants Inc.	Vancouver, BC
High Resolution Camera	Model WV 1850	Panasonic Industrial Co.	Secaucus, NJ
Camera Lens	Model 8125, 12.5 mm APC Auto Iris	Computar, Chugai International Corp.	New York, NY
Time-lapse Video Recorder	Model AG6750 SVHS	Panasonic Industrial Co.	Secaucus, NJ
Video Monitor	Model WV5470	Panasonic Industrial Co.	Secaucus, NJ
Remote Controller	Model AG-A670	Panasonic Industrial Co.	Secaucus, NJ
Time/Date Generator	Model NV-F85	Panasonic Industrial Co.	Secaucus, NJ

COMPONENT	DESCRIPTION	MANUFACTURER	LOCATION
LIGHTING			
Light Pipe	Light Pipe	TIR Systems Ltd.	Vancouver, BC
Luminaire	HQI-T400 W/DH Powerstar	Osram	Germany
Motor	Model BK1356, 100 -60	Synchron	USA
Red Light	ENH 250 W	Phillips Lighting Co.	Somerset, NJ
Radiation/Light Meter	LI 188B Integrating Quantum Photometer	LI-Cor Inc.	Lincoln, NE
Spherical Quantum Sensor	Underwater LI-193SB	LI-Cor Inc.	Lincoln, NE
Dichroic Far Red Filter	RG 695	Schott-Fostec, LLC.	Auburn, NY
MISCELLANEOUS			
MACtac	pressure sensitive, self-adhesive film	Bemis Co. Inc	Stow, OH
Wet Surface Epoxy Coating	Intergard EXA 008	International Marine Coatings Ltd.	North Vancouver, BC
Plexiglas® Polish	Plastic Polish No.2	Novus Inc.	Minneapolis, MN