

SWIM BLADDER STRESS SYNDROME IN
ARCTIC CHARR (*Salvelinus alpinus*)

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ABSTRACT

I investigated the condition known as Swim Bladder Stress Syndrome (SBSS) in a Nauyuk Lake, Northwest Territories strain of Arctic charr (*Salvelinus alpinus*). Induction of the condition was attempted through an extended period of acute sessions of applied handling stress. Water quality was controlled throughout the entire 5 month project. Blood analyses were performed (packed cell volume, plasma cortisol and glucose) to confirm that the fish had been physiologically stressed. In addition, bacteriology, virology, histopathology and a tissue homogenate injection trial were performed in an attempt to ascertain the exact etiology of this condition.

The charr were subjected to two stress treatments (stressed and non-stressed), both treatments occurring at each of two density levels (75 kg/m³ and 150 kg/m³). The incidence of SBSS in the treatment groups was not statistically significant (Log-likelihood ratio; $P > 0.05$), although seven out of a total of eight (87.5%) cases were fish from high density groups. Cortisol levels were higher in the low density groups as opposed to the high density groups for all three samplings, significantly so at the third sampling.

Bacteriology, virology, histopathology and the tissue homogenate injection trial all failed to demonstrate any source of the condition.

It is questionable whether Swim Bladder Stress Syndrome in Arctic charr is in fact due to stress as previously indicated in the scientific literature.

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INTRODUCTION

Swim Bladder Stress Syndrome (SBSS) is a condition which has been found to occur in Arctic charr (*Salvelinus alpinus*), as well as some other cultured species. Briefly, the condition is characterized internally by an over-inflation of the swim bladder, sometimes with an accompanying distortion in its shape. Behaviourally, the fish loses its ability to maintain normal positive rheotaxis in the water column. A typical sequence of events, in the most classic case, may include the following: the fish tends to float with the dorsal fin out of the water; the fish will swim on its side; a vertical posture may be assumed, usually with the anterior end up; ultimately the fish will float in an inverted posture, with a greatly distended abdomen, and become unable to respond even to touch stimulus. While it may take several weeks, the final outcome of this condition is death by exhaustion and starvation.

SBSS has been experienced by fish reared at Polar Sea Fisheries Ltd. since the facility's existence in late 1987. This condition has been observed at a variety of temperatures, stocking densities and fish sizes. While overall mortalities at the facility have been extremely low (approximately 5%/year), SBSS is basically the only cause of significant mortality at this facility. The literature suggests that the condition is stress related (Clary and Clary, 1978; Carmichael and Tomasso, 1984; Johnson and Katavic, 1984).

Swim Bladder Stress Syndrome (SBSS) was first reported in the literature by Clary and Clary (1978). While that was a somewhat speculative work, based on casual observation, it served to identify a condition in rainbow trout (*Oncorhynchus mykiss*) which was not dealt with previously as a specific syndrome. Stages of development of the condition were identified and enumerated, and inadequate dissolved oxygen was identified as the specific cause, with an acknowledgement of stress in general, causing exhaustion of the adrenal tissue

(chromaffin cells), which would otherwise allow for the reabsorption of the excess gas from the swim bladder. While specific data were not generated, the descriptions were consistent with the condition in Arctic Charr at Polar Sea Fisheries Ltd. (PSF).

Carmichael and Tomasso (1984) identified SBSS in largemouth bass (*Micropterus salmoides*) in Texas. The authors attributed the condition to stress due to handling and short-term transportation. They also described specific stages of the condition, being somewhat different but distinctly similar to those described by Clary and Clary (1978). As proof that stress was indeed being induced, they were able to demonstrate elevated blood glucose and corticosteroid levels in affected fish, as well as decreased plasma chloride. They suggested physical (pressure, temperature, supersaturation) and physiological/anatomical (gas gland, pneumatic duct) discrepancies as possible causes. They were unable to determine a specific cause of the syndrome.

At about the same time, Johnson and Katavic (1984) described SBSS as an incidental finding in their work with sea bass (*Dicentrarchus Labrax*) larvae. They suggested a considerable number of environmental stressors and water quality parameters as possible causes of this condition, and seemed to demonstrate that increased water temperature and light may be significant contributing factors in the condition. Bagarinao and Kungvankij (1986) also described the condition in sea bass (*Lates calcarifer*) larvae. They attributed the condition to environmental stressors, but were unable to stipulate a specific etiology.

Finally, Kolbeinshavn and Wallace (1985) induced SBSS in Arctic charr (*Salvelinus alpinus*) through decreasing water depths and low stocking densities, although the density (10 kg/m³) and water depth (12 cm) that produced the highest number of cases were both not representative of realistic culture pa-

rameters. One would also expect other stress factors to come into play at the water levels described. This same experiment indicated increased levels of dopamine (a catecholamine precursor) in the stressed fish. However, a previous study had already determined that dopamine was not affected by stress (Mazeaud and Mazeaud, 1981).

An appropriate definition of stress has been provided by Brett (1958). He refers to those adaptive responses to the physical, chemical and biological environment that extend beyond the normal range such that the organism's chances of survival are significantly reduced. The overall objective of this project was to demonstrate the specific stress-related nature of SBSS by attempting to induce a significant number of cases in Arctic charr through the application of handling induced stress in fish at two stocked densities. Water quality parameters, and to a large extent other environmental conditions, were controlled so that the possibility of other stressors was kept to a minimum. To confirm that stress was being induced in the charr, three blood parameters were evaluated: packed cell volume (pcv), cortisol concentration and glucose concentration. Some of the measurable effects of stress on fish have been known for some time. Primary effects (plasma hormone concentrations) and secondary effects (biochemical/physiological responses, i.e., blood glucose) of stress have been documented and reviewed, and account for the analysis of cortisol and glucose in order to determine a state of stress (Wedemeyer, 1972; Mazeaud et al., 1977; Mazeaud and Mazeaud, 1981). In addition, possible transmissibility of the condition was investigated through routine virology, bacteriology, and a tissue homogenate injection.

LITERATURE REVIEW

The swim bladder in the various fishes serves one or more of four primary functions. These functions include hydrostatic, respiratory, sensory and acoustical (Jones and Marshall, 1953). The function of concern in charr is that of a hydrostatic organ. This function of the swim bladder allows the fish to maintain neutral buoyancy at virtually any depth, depending on the efficiency of the swim bladder to produce gas against a pressure gradient. This function is crucial in most free-swimming fishes as it allows them to remain motionless in water with a minimum of energy expenditure, and greatly reduces the energy required to swim horizontally (Steen, 1970). The swim bladder represents approximately 5% of total fish volume in marine fishes and approximately 7% in fresh water fishes (Steen, 1970; Hughes, 1963).

There are two basic types of swim bladders. They are classified as physoclistous and physostomous. The physoclistous bladder is a hollow, gas-filled, sac-like organ with no connection outside of the peritoneal cavity. In contrast, the physostomous bladder possesses a muscular tube, the pneumatic duct, which provides a passageway between the lumen of the bladder and the esophagus (Steen, 1970; Fänge, 1976; Fahlén, 1971). This pneumatic duct is thought to allow for a rapid release of gas from the bladder in the event of a sudden increase in bladder pressure (i.e., a sudden upward migration), and is cited by some individuals as being the primary means for the introduction of gases into the physostome swim bladder (Fänge, 1976; Jones and Marshall, 1953), although this belief has been discounted by others (Alexander, 1966; Saunders, 1953).

The swim bladder is derived embryologically from the foregut (Jones and Marshall, 1953). It is supplied by the coeliaco-mesenteric artery and drained by the hepatic-portal system (Jasinski, 1963; Fänge, 1976; Steen, 1970; Fahlén,

1968). Innervation is supplied by the vagus nerve, and supplemented by some adrenergic fibers. The parasympathetic system (vagus) seems to be generally responsible for the filling of the bladder, while the sympathetic system (adrenergic fibers) seems to be generally responsible for the diffusion out or release of gas from the bladder (Fänge, 1953; Jones and Marshall, 1953).

The swim bladders of the salmonids are classified as physostomous. The physoclistous fish, while lacking a pneumatic duct, are in possession of highly specialized structures known as the rete mirabile and the gas gland. These structures are credited with gas production in physoclists, and are usually not present in physostomes, although the eel and the whitefish are notable exceptions (Fahlén, 1959; Steen, 1963). A brief explanation of the function of the physoclistous swim bladder follows, as it is considerably more complex than the physostomous swim bladder, and it is hoped that an understanding of the function of the physoclistous bladder will aid in the explanation of the operation of the physostomous bladder.

The physoclistous swim bladder can be of two basic types, the paraphysoclist and the euphysoclist. The former has an indistinct separation between the secretory portion and resorbent portion (the oval), while the latter does form a sharp separation of the two sections, sometimes to the extent of containing a muscular sphincter, which can completely isolate one area from the other (secretory and resorbent). The respective areas of the euphysoclist bladder are capable of contraction and expansion in some fishes, which greatly enhances the appropriate function, as required (Steen, 1970; Fänge, 1973). Gases must be introduced against pressures of up to several atmospheres (Scholander and van Dam, 1953; Steen, 1963), depending on the depth of the fish at the time of filling of the bladder. The pressure in the bladder, especially the partial pressure of certain gases, may be several hundred times greater than the partial

pressure of that same gas in the surrounding water (Steen, 1963). This would indicate the need for some type of active gas transfer mechanism in order to force gases into the swim bladder against potentially substantial pressures (Wittenberg et al., 1964; Ball et al., 1955; Kuhn et al., 1963).

The swim bladder wall consists of four basic layers (specific nomenclature varies among histologists): the internal epithelium, the muscularis, the submuscularis or submucosa (connective tissue), and the external serosa (Fahlén, 1971; Yasutake and Wales, 1983). The rete mirabile is an intricate network of up to thousands of arterial and venous capillaries, running parallel and counter-currently to each other, which are in intimate proximity to each other, supplying and draining the swim bladder wall. The arterial vessels fuse to form a single vessel, after exiting the rete and prior to entry into the gas gland, and the exiting venous vessel from the gas gland divides upon re-entry into the rete. The rete is located just outside the epithelial layer of the bladder and is supplied by the normal swim bladder vasculature. Number and size of these structures are variable, usually depending on the level of efficiency of gas production required. The gas gland consists of a group of specialized swim bladder epithelial cells, through which the arterial (afferent) flow from the rete mirabile passes and becomes the venous (efferent) flow back into the rete mirabile. Numerous excellent reviews and descriptions of the anatomy and physiology of these structures exist (Alexander, 1966; Steen, 1970; Steen, 1963; Fänge, 1973; Fänge, 1953; Jones and Marshall, 1953; Scholander, 1954; Wittenberg et al., 1964; Kuhn et al., 1963).

Gas production is generally considered to be accomplished by the following mechanism. Blood flow into the rete carries normal atmospheric gases dissolved in the blood, namely oxygen, nitrogen, carbon dioxide, argon, neon and helium. The latter three are considered to be in very small volumes, and gen-

erally of no consequence. However, some authors feel that carbon dioxide may form a significant portion of the secreted gas, as well as aiding in the pH reduction of the blood at the gas gland (Steen, 1963; Wittenberg et al., 1964; Scholander, 1956). It may be a product of aerobic glycolysis and directly proportional to the amount of lactic acid produced (Copeland, 1952; Wittenberg et al., 1964). Molecular oxygen is carried by the hemoglobin fraction of the red blood cells. As this blood passes through the rete mirabile and into the gas gland, a primary substance produced by the gas gland cells is released. This substance is thought to be lactic acid which has been produced by metabolic glycolysis, but in the presence of oxygen (Deck, 1970; D'aoust, 1970; Ball et al., 1955; Steen, 1963; Kuhn et al., 1963; Copeland, 1952; Fänge, 1953; Fänge, 1976; Steen, 1962). The produced lactic acid is responsible for the onset of two significant metabolic functions, one being a "salting out" process, and the other being a rapid decrease in pH.

Salting out refers to the decreased solubility of any gas in solution due to the addition of any solute or molecular ions to the liquid (Battino and Clever, 1966). The process is due simply to the hydration of these ions and the subsequent decrease in available water, thereby producing, in effect, a case of supersaturation of any saturated or near saturated gases present. The level of dissolved gases decreases while the gas tension, or available gases, increases (Kuhn et al., 1963; Steen, 1970). Lactic acid, through its dissociation to lactate ion, adds additional solute to the blood, producing a salting out effect.

The rapid decrease in pH is responsible for the release of oxygen which is attached to the hemoglobin fraction of the red blood cells. The mechanisms for this event are known as the Root and Bohr effects. The Root effect allows for a decreased capacity of hemoglobin for oxygen in the presence of decreasing pH, while the Bohr effect allows for the decreasing affinity of hemoglobin for

oxygen under the same acidic conditions (Scholander, 1954; Scholander and van Dam, 1954; Kuhn et al., 1963; Berg and Steen, 1968; Steen, 1970). The resulting effect is a substantial release of oxygen from the oxyhemoglobin of the red blood cells into the fluid portion of the blood, causing an increased oxygen tension.

The combination of the two primary gas-releasing mechanisms, "salting out" and Bohr and Root, and the increase in gas tension of the blood, is termed the single concentrating effect, as the localized elevation in gas tension has been generated by these two processes, due almost exclusively to the release of a primary substance, lactic acid. Both of these processes were initiated by the gas gland at the vertex of the vessel exiting the rete (afferent), just prior to its return to the rete (efferent).

The final metabolic activity, termed the countercurrent multiplication effect, directly involves the rete mirabile. As gas tension, primarily oxygen, is increased immediately "downstream" from the gas gland, a diffusion gradient is established in the rete mirabile between the afferent (arterial) and efferent (venous) components of this structure. The venous side of the rete, nearest the gas gland, will now exhibit a substantially increased gas tension as compared to the arterial side. At some point the gradient will equalize, with a venous side decrease in gas tension accompanied by a concurrent increase in gas tension in the arterial side. The arterial blood again exits the rete and enters the gas gland area, where more lactic acid is produced and more gas is caused to be added to the already increased tension from the arterial side. The gradient equalization process continues until such time as the gas tension, at the entrance to the gas gland, is greater than the gas pressure inside the swim bladder. At that point, the gas from the blood will diffuse into the bladder. This process is described in detail by a number of authors (Scholander, 1954; Kuhn et

al., 1963; Steen, 1963; Alexander, 1966; Fänge, 1966; Steen, 1970; Fänge, 1973). The method of actual gas transfer into the swim bladder has been under some debate, but it is believed to simply diffuse into the bladder (Wittenberg, 1958; Wittenberg and Wittenberg, 1961; Alexander, 1966) or to collect in a series of intercellular secretory ducts which are continuous with the lumen of the swim bladder (Fänge, 1953). A mathematical model has been designed which confirms the extreme efficiency of the rete mirabile countercurrent mechanism design (Rodin and Jacques, 1989). The efficiency of this entire system is dependent on the rete length, surface area available for diffusion and rate of blood flow (Alexander, 1966). Regarding the surface area in the rete which is available for diffusion, calculations made (Steen, 1963) from data supplied (Scholander, 1954) from work on the long nosed eel indicated a total diffusion area to rete volume ratio of $1200 \text{ cm}^2 : \text{cm}^3$. Maintenance of high pressures in the swim bladder is aided by the presence of various gas-impermeable substances in the wall of the secretory portion of the bladder, such as overlapping guanine crystals (Lapennas and Schmidt-Nielsen, 1977; Fänge, 1958), specialized collagen fibers (Fänge, 1973) and highly organized lipids which “freeze” and “melt” at specific temperatures (Wittenberg et al., 1980). At the exact area of the gas gland, it would be unlikely that a crystal layer or other diffusion barrier would be present. An attempt has been made to demonstrate that the gas gland itself may act as a one-way valve against gas diffusion from the swim bladder (Wittenberg and Wittenberg, 1961). In addition, and as mentioned previously, the secretory and resorbent portions of the bladder can expand and contract in many species, significantly aiding whichever process is in operation (Fänge, 1953).

One notable experiment has cast some doubt on the generally accepted gas secretion process just described, as it pertains to deep-sea fishes. It was

demonstrated that, under certain pressure conditions and at a pH as low as six, in certain species of deep-sea fishes, less than 10% of the oxygen bound by blood hemoglobin was released. The authors concluded that the Root Effect was not the primary reaction responsible for the release of oxygen into the blood and subsequently into the swim bladder (Scholander and van Dam, 1954).

Fishes which utilize a physostomous type of swim bladder do not, with a few exceptions, have rete mirabilia or gas glands. Arctic charr were specifically examined and determined not to possess any discernible rete structure (Sundnes et al., 1958). It is the lack of these structures that raises serious questions as to how the physostomes are able to add gases to their swim bladders against considerable pressure gradients. As previously mentioned, a few individuals have indicated that this process is accomplished by gulping air at the surface. A thorough examination of the "surface gulping technique" casts serious doubt on the feasibility of this technique as a primary means of introducing gases into the swim bladder. It would appear to be impossible to introduce enough air at the surface which would allow for neutral buoyancy at several atmospheres depth (Alexander, 1966). It has also been pointed out that, under certain conditions, it may not be possible for fish to obtain access to the surface, as in the cases of ice covering during winter or a prohibitively warm epilimnion during the summer (Saunders, 1953). As further evidence for the existence of some alternative method to surface gulping, rainbow and brown trout swim bladders were completely deflated by artificial means, and the fish were then denied access to the surface via a wire screen. All individuals refilled their swim bladders within approximately 11 days (Wittenberg, 1958). The need for some alternative active secretory mechanism seems mandatory in physostomes lacking a rete/gas gland structure. Numerous authors have indicated that the exact method of gas

secretion in these physostomes is unknown, or at best, questionable (Saunders, 1953; Fahlén, 1968; Scholander and van Dam, 1953; Scholander et al., 1956; Sundnes et al., 1958; Sundnes et al., 1969; Fahlén, 1971).

A notable condition which has been found to exist in some physostomes is an extremely high (up to 99%) nitrogen content (Saunders, 1953; Scholander et al., 1956; Tait, 1956) in the swim bladder. This fact alone causes one to seriously consider a method of gas secretion other than the one generally accepted (previously described), even in those physostomes that possess rete mirabile/gas gland structures.

There are three primary theories regarding an alternative method of gas secretion (other than via rete mirabilia and gas glands) by physostomes which are subscribed to by various authors. I will address them in random order.

THEORY #1: Surface Gulping Theory - this theory has been previously mentioned and discounted as being a primary mechanism for the introduction of gases to the swim bladder.

THEORY #2: Rete Precursor Theory - this theory suggests that, even though no rete/gas gland structure exists, the vasculature of the swim bladder in certain physostomes is extensive enough and arranged in such a way (parallel and adjacent arterial and venous capillaries in close proximity) that some diffusion may take place between arterial and venous vessels, generating a small gas tension gradient with ultimate addition of gases to the swim bladder, although a slow and inefficient process (Fahlén, 1968). This theory was substantially disputed as a blanket solution, however, in a very common physostome, rainbow trout, due to the absence of a vascular structure which might allow any consequential amount of gaseous exchange to take place (Jasinski, 1963).

THEORY #3: Active Cellular Secretion Theory - this theory suggests that the epithelium lining the lumen of the swim bladder may be capable of secreting

specific gases directly into the swim bladder against a substantial pressure gradient. This concept has been proposed, or at least accepted, by various individuals (Scholander and van Dam, 1954; Sundnes et al., 1958). One particularly intensive investigation identified three distinct cell types present in the swim bladder epithelium of four species of the genus *Salmo* (Fahlén, 1971). These cells were designated chief cells, ciliated cells and ovoid cells. The chief cells contained vacuoles which, in many cases, opened directly to the lumen of the swim bladder. The vacuoles were found to contain, in part, a mucopolysaccharide. It was speculated that the mucopolysaccharide may have been transformed into gaseous products which were ultimately routed into the swim bladder. Similar anatomical structures were observed by the same author in the physostome grayling (Fahlén, 1968).

As previously referred to, the most substantial difference noted regarding gas content in the two swim bladder types (physoclistous and physostomous) was that of the extremely high nitrogen content in some physostomes. As the actual mechanism for gas secretion in physostomes is uncertain, it has been suggested that nitrogen is secreted directly into the swim bladder by the same method, whatever that may be, as oxygen. The high nitrogen content is ultimately achieved by preferential reabsorption of oxygen and carbon dioxide from the swim bladder, allowing for a gradual build-up of nitrogen over time (Copeland, 1952; Scholander and van Dam, 1953; Kuhn et al., 1963; Sundnes, 1963; Sundnes et al., 1969; Abernethy, 1972). This explanation, however, assumes some common mechanism for oxygen and nitrogen secretion, and this has not been specifically established. This theory of nitrogen build-up, due to a greater diffusion rate and therefore greater reabsorption of oxygen from the swim bladder, seems to be supported by the consistent finding that the argon/nitrogen ratio in fish has consistently been found to be approximately the

same as that of the respective surrounding water and air. If nitrogen was selectively secreted by some chemical means, one would expect argon to be left behind and therefore substantially decrease the argon/nitrogen ratio in the swim bladder (Scholander, 1954; Tait, 1956; Abernethy, 1972). However, this lowered ratio may also be explained by the greater diffusion coefficient of argon as opposed to nitrogen (Wittenberg, 1958; Abernethy, 1972; Scholander and van Dam, 1953).

A final theory pertaining to the secretion of inert gases into the swim bladder suggests that small bubbles of nitrogen diffuse into larger bubbles of oxygen which then enter the bladder. This would allow nitrogen to enter the swim bladder, presumably independently of the existing partial pressure of nitrogen already present in the bladder (Wittenberg, 1958). This may aid in explaining the extremely high nitrogen component found in many physostome investigations.

The literature investigation into swim bladder function found nothing which conclusively determined the method of gas secretion into the swim bladder of physostome fish lacking rete mirabile/gas gland structures.

The preceding review was done in an attempt to assist in determining the cause of SBSS. It seemed reasonable to investigate the overall structure and function of the swim bladder in hopes that such knowledge would aid in determining deficiencies which would cause or aggravate an over-inflation of this organ, and also provide a better understanding of the results obtained in this study.

METHODS AND MATERIALS

EXPERIMENTAL DESIGN

The effect of handling stress on Arctic charr was examined at two stocking densities. There were a total of eight tanks, four of which were low density (75 kg/m³) and four of which were high density (150 kg/m³). Within each density level, there were two stress levels, one tank containing a stressed group and one tank containing a non-stressed group. The tank system was designed so that tanks were stacked vertically, two high. One set of two upper tanks and one set of two lower tanks held the non-stressed groups. Conversely, the opposing sets of tanks held the stressed groups.

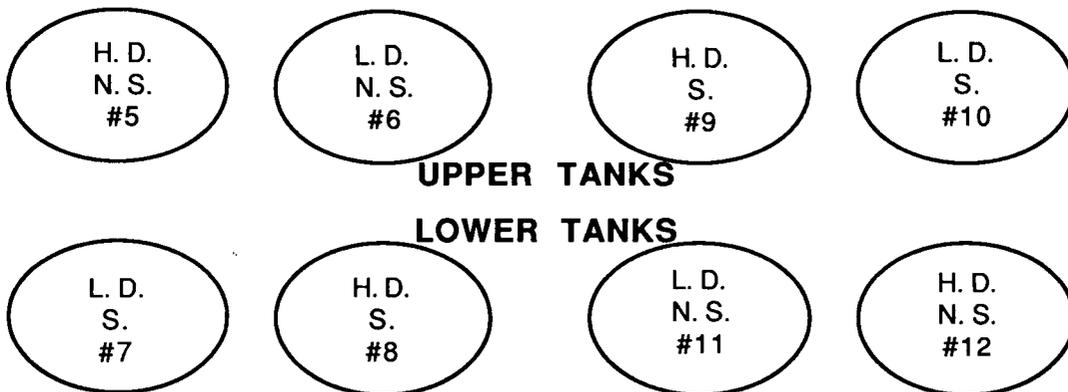


FIGURE 1. Distribution of Treatments Among Upper and Lower Tanks. H. D. = high density. L. D. = low density. S. = stressed. N. S. = non-stressed.

Each of the eight fiberglass tanks measured 1.1 m in diameter and 0.4 m in depth, and had a capacity of approximately 335 l. The outflow control configuration in each tank consisted of a 5.1 cm diameter center standpipe with a 10.2 cm diameter outer standpipe which directed the flow upward from the bottom of the tank via notches at the base.

There were two water lines available to each tank, both originating from the same ground water source. One line delivered cold water directly from the

source, and one was from an inside header tank, the water from which had been passed through a heat exchanger. Each tank was equipped with a small aeration column (25.4 cm dia. x 38.1 cm ht.) containing 5.1 cm diameter Tri-Paks®. Small holes were bored in the sides of the aeration columns to facilitate gas exchange. Water flow was initiated on January 24, 1990, and the entire system of eight tanks was allowed to operate for 5 days prior to the introduction of fish.

The target stocking densities were 150 kg/m³ (208 fish) in the high density groups, and 75 kg/m³ (104 fish) in the low density groups. These densities were chosen to reflect densities at which Arctic charr have been successfully maintained, at this facility and elsewhere (Wallace et al., 1988).

On January 31, 1990, the group of fish which had been designated for the experiment were sorted into two size groups, those that weighed less than 200 g and those that weighed more than 200 g. This was done using an Ewos® fixed space sorting device. Samples consisting of 30 fish were taken from each group, and the average of each group weight was determined. All of these fish were of Nauyuk Lake, Northwest Territories, stock, and were a mixture of anadromous and non-anadromous types. They arrived at the facility in January of 1988 as fingerlings. These fish were originally obtained from the Freshwater Institute, Department of Fisheries and Oceans (DFO) Canada, near Winnipeg, Manitoba.

The appropriate biomass of fish was obtained, from these two groups, to make up the density levels as previously determined. The total biomass of each experimental tank was made up of approximately 68% from the larger individual size group, and the remaining biomass, approximately 32%, was completed with individuals from the smaller individual size group. This was done to maintain a consistent variation in size among all eight of the experimental tanks.

The charr were allowed to acclimate for several days. Feeding began on February 8, 1990, at a rate determined from the experience of this facility and a Moore-Clark® feeding table for salmonids. The water temperature at this time was 3 °C. Over the next few days, the water flows in each tank were initially established so that a minimum of 1 liter per minute (L/min) per 5 kg of fish was being delivered in the high density tanks, which was 10 L/min. The lower density tanks were maintained at 10 L/min as well, or just slightly less. As adequate oxygen was being supplied to all tanks at this rate of flow, the primary concern became one of producing enough current to keep the tank bottoms free from organic debris.

On February 14, 1990, the water temperature was elevated from an ambient 3 °C to 5 °C. It was the intention that the water temperature remain at approximately 5 °C for the duration of the project.

On February 20, 1990, a curtain of black, 4 mil polyethylene plastic was hung from the ceiling to approximately 1 m from the floor, surrounding the experimental tanks and at a distance about 1.5 m from the tanks. This was done to reduce and equalize any random hatchery-related stress which might have impacted on the experimental fish. Whenever additional lighting was needed in this enclosed area, a lamp with a red bulb was used, as it has been the experience of this facility that the use of a red light invoked a substantially reduced reaction by the charr when applied in the vicinity of the tanks.

Any individuals showing signs consistent with those of Swim Bladder Stress Syndrome were removed from the experimental tanks prior to the initiation of the project.

WEEKLY STRESS PROTOCOL/WATER QUALITY

On March 13, 1990, the weekly stress protocol began (Table 1). Water quality parameters were determined prior to the stress protocol that morning, with the exception of ammonia, which was measured on the afternoon before due to the length of time involved in performing the test.

The fish in the stressed tanks underwent 5 min each of harassment with a dip net, during which period small groups of fish (3 - 5) were held out of the water for approximately 30 s. While the stress treatment was being applied to the fish in tanks #9 and #10, which were upper tanks, the two tanks directly below them, #11 and #12, were covered. The time at which the 5 min stress period ended was noted, and water quality samples were taken from each of the four stress treatment tanks at 1 h, 2.5 h, 5 h and 8 h after the noted time. The parameters measured were temperature, pH, dissolved oxygen and total nitrogen. The total nitrogen value was subsequently converted to un-ionized ammonia (described below). As there was only one tensionometer available for the project, the pressure sensing probe was placed in one of the tanks prior to the stress period, total gas pressure (TGP) was obtained, and the probe was left in that tank for the duration of the 8 h sampling period. The TGP was calculated at the same intervals as the other water quality parameters. The tank being evaluated for TGP was varied weekly, so that all four stressed tanks would be sampled several times during the course of the experiment, and at the same post-stress time intervals as those of the other parameters.

Beginning with the first stress protocol procedure on March 13, 1990, a water quality sampling routine was established and was adhered to as closely as possible over the duration of the project.

TABLE 1. Weekly and Daily Water Quality Sampling Procedures

TUESDAY: Stressed tanks only sampled. All water quality parameters (except ammonia and TGP) were sampled in all stress treatment tanks prior to the initiation of the stress protocol. The ammonia was sampled the afternoon before (Monday) due to the time required for the salicylate-based ammonia determination test. Sampling was done at 1 h, 2.5 h, 5 h and 8 h intervals from the end of each stress period for each of the four tanks. TGP was determined in one of the stressed tanks prior to the stress protocol, and was left in the tank during the entire day. TGP readings from that tank were taken at the time intervals listed above. In that way, all stressed tanks were completely sampled for TGP several times throughout the project.

WEDNESDAY, FRIDAY AND SUNDAY: Dissolved oxygen and temperature were measured in all treatment tanks.

THURSDAY AND MONDAY: pH and ammonia were measured in all treatment tanks.

DAILY: TGP was determined in an individual treatment tank each week day.

TABLE 2. Complete Sampling Protocol - SBSS Cases

PHYSICAL PARAMETERS: Length (cm) and weight (g) were obtained. The external aspect of each individual was examined and abnormalities noted. Condition and abnormalities of the swim bladder and all other viscera were noted. This information was included with the tissues sent for histopathology.

AEROBIC BACTERIOLOGY: Each individual was opened aseptically and inoculating loop samples were taken from the mid-kidney and the inner swim bladder epithelium. These inocula were smeared onto TSA agar plates. One sample of each was incubated at room temperature and one sample of each was incubated at approximately 4 °C. All samples were held for two weeks.

ANAEROBIC BACTERIOLOGY: Each individual was opened aseptically and inoculating loop samples were taken from the kidney and the inner lining of the swim bladder epithelium. These inocula were immersed into fluid thioglycollate medium. One sample of each was incubated at room temperature and one sample of each was incubated at approximately 4 °C. All samples were held for two weeks.

VIROLOGY: Each individual was opened aseptically and small tissue samples of the kidney and swim bladder were taken. These samples were placed into a sterile plastic bag and frozen at -25 °C, to be analyzed for the presence of viral pathogens at the Pacific Biological Station, DFO, Nanaimo, B. C.

HISTOPATHOLOGY: Tissue samples of most tissues were taken and fixed according to the procedure described under METHODS and MATERIALS/HISTOPATHOLOGY.

On May 1, 1990, it was decided that the levels of un-ionized ammonia measured over the past few weeks, following the 5 min stress period, were generally

well below any levels which had been consistently associated with causing any stress and/or pathological changes in fish. Therefore, the monitoring of ammonia levels during the weekly stress protocol was reduced from weekly to approximately every third week, and the routine monitoring of ammonia in all tanks was reduced from twice weekly to weekly. At the same time, a decision was made to decrease the routine pH sampling frequency from twice a week to once a week, as the pH determinations in the various tanks up to that point had been extremely consistent, and remained so throughout the duration of the project. The weekly, post-stress sampling of pH was continued as originally proposed.

Average water quality parameters recorded following each weekly stress episode are shown in Figures 2 and 3.

Water quality sampling began on February 19, 1990. Temperature and dissolved oxygen were sampled every other day in order to obtain normal, routine levels of these parameters. Samples were also taken twice weekly for pH and ammonia evaluations. The un-ionized ammonia levels in various tanks were determined using the salicylate method via a Hach® DR/2000 spectrophotometer. This method is concerned with the initial formation of monochloramines, which in turn react with salicylate to form 5-aminosalicylate. This compound is then oxidized, in the presence of nitroferricyanide, to indosalicylate, which gives a blue colour. The blue colour is initially shrouded by a yellow colour produced by excess nitroferricyanide. As the ammonia reacts to produce more monochloramine, the blue intensifies and, in combination with the yellow, produces a green coloured solution, the intensity of the green being directly proportional to the production of the ammonia-based monochloramine (Chemical Procedures Explained, 1989). Calculations were then performed to determine the amount of un-ionized ammonia present, based on pH and temperature (Emerson et al., 1975).

Un-ionized ammonia was of primary concern as it is the most toxic of the ammonia based by-products from fish metabolism (Alabaster and Lloyd, 1980). The readout on the spectrophotometer was in mg/l of total nitrogen. This value was then multiplied by a constant which represented the amount of aqueous ammonia as compared to the total nitrogen, that constant being 1.214, a value calculated by dividing the molecular weight of ammonia by the molecular weight of nitrogen. This product was then multiplied by a pre-determined value, which gave the amount of un-ionized ammonia in aqueous solution. This pre-determined value was dependent on the pH and temperature of the sample being tested (Emerson et al., 1975). This value was usually 1.2% (.012), as it corresponded with a temperature of 5°C and a pH of 8. However, as the water warmed up in June, an appropriate value was used to correspond to the increasing water temperature. Considering that this experiment was utilizing a flow-through system, ammonia concentrations approaching unacceptable levels were not anticipated. This particular parameter was not checked frequently in the non-stressed groups. However, these levels were checked more frequently in the stressed groups, at specific intervals after the stress regime had been initiated, as previously indicated.

The dissolved oxygen levels and temperatures were determined using a YSI® Dissolved Oxygen meter, model 54A. Periodic inspections were made of the probe during the project and the membrane was cleaned or replaced as required. The meter itself was periodically checked for accuracy with the DR/2000 spectrophotometer, using the Hach® Dissolved Oxygen Reagent, High Range AccuVac test. The pH was determined with a Fisher® Accumet pH meter, model 956. This instrument was calibrated weekly using Fisher® Buffer Solution pH 7.00. All water quality parameters (except TGP) were measured from water samples collected from the outflowing water between the inner standpipe and

outer standpipe. In the case of D. O. and temperature, the YSI® probe was inserted into this area. For pH and ammonia determinations, water samples were collected from these areas via a plastic aquarium hand pump siphon, and taken to the lab for evaluation.

TGP was determined using a Novatech® 300C tensionometer, and was measured with the sensing probe lying on the bottom of the tank. Atmospheric pressure was initially obtained using this instrument. It was then zeroed and placed into the appropriate tank. After approximately 45 min, the change in pressure could be obtained via the digital readout on the instrument. This change was subtracted from the original atmospheric pressure value, and the difference divided by the original atmospheric pressure value to obtain the % TGP. TGP was also calculated using a mathematical formula which had been entered on a spreadsheet (Cook and Canton, 1988). The spreadsheet computed % TGP as well as % oxygen and % nitrogen. When determining the TGP in the stressed tanks, it was necessary to place the probe into the tank the night before so that the reading could stabilize. Otherwise, it would be necessary to wait up to an hour on the day of the stress protocol so that the pre-stress TGP could be determined. The placing of the probe into the tank also disturbed the fish and, had it been done on the day of the sampling, may have altered the pre-stress dissolved oxygen readings, and possibly the subsequent un-ionized ammonia determinations. At each post-stress water sampling, the computer unit was attached to the probe and the pressure change was read without disturbing the fish. The tensionometer used in the project was sent to Novatech® in Victoria, B. C., immediately prior to the onset of water sampling, for a complete inspection and calibration.

BLOOD SAMPLING

Blood samples were collected on three occasions, once at the start, once during the experiment and once at the end of the experiment. This was done so that the concentrations of cortisol and glucose could be established to assure that the stressed fish were experiencing stress relative to the the non-stressed fish.

An evaluation trial was conducted to determine the pH of the anesthetic solution which would be used during the blood sampling. Two tests of tricaine methanesulfonate (MS 222) solutions were made, each at a concentration of 100 mg/L, and the pH was checked. In both cases, the pH went from 8.0 in the water only, to 7.3 in the 100 mg/L solution of MS 222. This was not anticipated to be a concern. In addition, on March 6, charr of the same strain and year class as those involved in the project were placed in a 100 mg/L MS 222 solution and individuals were withdrawn for blood sampling after 5 min, 8 min, 12 min and 15 min. All of the fish in this test survived the anesthetic and the sampling. The possibility of the anesthetic itself causing an increase in plasma cortisol or glucose levels, under the conditions of use in this project, was not considered to be a concern (Wedemeyer, 1970).

Initial Blood Sampling - Non-Stressed Fish

On March 8, 1990, blood samples were collected from fish in the four non-stressed tanks. Six individuals from each of the non-stressed tanks were anesthetized and 1.0 to 1.5 ml blood was collected from each fish (procedure described below). The anesthetic used was MS 222 at a dosage of 100 mg/L. The anesthetic solution was stabilized at a temperature of 5 °C (± 0.5 °C) so as to correspond to the tank water temperature. After carefully approaching the tanks in order to avoid startling the fish, six fish were netted from each tank at

the same time. The effect that removal of the sampled fish may have had on the others in the tank was not anticipated to be a problem (Barton et al., 1980). Adjacent tanks were covered during the netting so as not to disturb the fish in those tanks. After placing the fish in the anesthetic solution, which was supplemented with pure oxygen, the entire procedure was timed. The first fish was taken from the anesthetic solution after 5 min, at which time the majority were found to have reached stage II, plane 2 of anesthesia (Ross and Ross, 1984). The first fish was retrieved from the anesthesia and the extraction of blood began. This was considered time 0, and the times at which blood was drawn from the other five were recorded relative to time 0.

Each fish was laid on its back in a v-trough. Using a 2.54 cm, 23 gauge needle and a 3 cm³ sterile disposable syringe, blood was withdrawn from the caudal vein. The point of insertion was approximately 1 cm posterior to the anal fin on the ventral mid-line of the fish. Between 1 and 1.5 ml of blood was taken from each fish. A smear was made from each sample of the whole blood. The top was then removed from a 3 ml Vacutainer[®] tube containing lithium heparin as an anticoagulant, and the blood was gently injected into this tube, after having first removed the needle from the syringe. The Vacutainer[®] tube, having been chilled to approximately 4 °C. and held on top of a cloth-covered container of ice, was inverted several times and replaced on the ice container.

During blood collection, the fish was weighed, the fork length measured, and any abnormalities noted. Finally, a permanent Visible Implant Tag[®] (Northwest Marine Technology, Ltd.), containing an identification code which consisted of a letter and a number, was installed subcutaneously in the transparent superficial tissue just posterior to the left eye. After the total procedure was completed on each fish, it was placed in a recovery tank at 5 °C which was supplemented with pure oxygen. After the fish appeared to have recovered from the anesthetic

(approximately 20 min), all six individuals were returned to the tank from which they had been taken. The same anesthetic solution was used for the first two groups of six fish. A fresh solution was prepared for the remaining two groups. Recovery tank water was also replaced after the recovery of two sample groups.

After the fish were sampled, the blood samples were taken to the Polar Sea Fisheries lab and processed. Initially, blood from each sample was drawn into a microhematocrit capillary tube. These were immediately centrifuged to determine the pcv of each fish sampled. This was done with a Clay Adams® Readacrit centrifuge. The samples were spun for 5 min, after which the pcv (%) was read directly from the centrifuge rotor, using a reading device which was installed there for that purpose. While the hematocrit tubes were being centrifuged, the blood from each Vacutainer® sample was poured into a chilled centrifuge tube and spun down in order to separate the cells from the plasma. This was done in a Clay Adams® Analytical centrifuge at a speed of 3500 rpm for 10 min, a time which was sufficient for complete pelleting of blood cells (Robyt and White, 1987). The plasma supernatant was then collected from each tube with a sterile glass pipet. This plasma sample was placed in a 1.5 ml Eppendorf® plastic serum sample tube. After each group of six fish was completed, all serum samples were placed in a freezer at -25 °C. All 24 samples were handled in this manner. The samples were then, within a few days, transported to the University of British Columbia, where analyses for cortisol and glucose were performed.

Plasma cortisol levels were determined using the Coat-A-Count® Cortisol kit (Diagnostic Products Corporation). This is a radioimmunoassay procedure in which ¹²⁵I-labeled cortisol is added to the sample tube along with the plasma sample. The radiolabeled cortisol competes with plasma cortisol for the specific antibodies which have been bound to the sides of the tube. Based on a cali-

bration curve prepared by the use of standards, the amount of cortisol present in the plasma sample can be determined through the use of a gamma counter.

Plasma glucose levels were determined using a Sigma® Glucose Determination kit (#635). Blood plasma is reacted with ortho-toluidine in glacial acetic acid. The absorbance of the resulting colour change is read at 635 nm. A standard curve of O.D. vs. glucose had been previously prepared using known concentrations of glucose, and was compared to the test results.

Initial Blood Sampling - Stressed Fish

On March 9, 1990, blood samples were collected from the stressed fish in a nearly identical manner as for the non-stressed fish on March 8, and closely following the procedure used for determining post-stress cortisol levels in coho salmon (Barton et al., 1985). The only deviation from the above procedure was that the fish in each of the four stressed treatments were stressed by a standardized protocol 1 h prior to blood collection. The stress protocol consisted of a 5 min period during which the fish of the tanks designated as stressed were moderately harassed with a dip net, including the netting of several small groups of fish which were held out of the water for approximately 30 s at a time. The time at which the 5 min stress period ended was noted, and six fish were netted from the treatment tank and placed into the anesthetic solution 1 h after the noted time.

Second Blood Sampling - Non-Stressed Fish

On May 15, 1990, the second blood sampling of the non-stressed groups was accomplished. The technique used was identical to that described in the section on Initial Blood Sampling - Non-Stressed Fish.

Second Blood Sampling - Stressed Fish

On May 17, 1990, the second blood sampling of the stressed groups was accomplished. The technique used was identical to that employed in the Initial Blood Sampling - Stressed Fish section described previously. It should be noted that one of the six samples from tank #9 (high density, non-stressed) was not processed to completion due to the sample leaking out during centrifugation. For the purpose of statistical analyses, this value was formulated from the average of the other five values within that same group.

Third Blood Sampling - Non-Stressed Fish

On July 23, 1990, the third blood sampling of the non-stressed groups was accomplished. The technique used was identical to that employed in the Initial Blood Sampling (see Initial Blood Sampling - Non-Stressed Fish section).

Third Blood Sampling - Stressed Fish

On July 24, 1990, the third blood sampling of the stressed groups was accomplished. The technique used was identical to that employed in the Initial Blood Sampling (see Initial Blood Sampling - Stressed Fish section).

HISTOPATHOLOGY

Preparation for the histopathological aspect of the project was made prior to the actual initiation of the stress protocol and subsequent related activities. Several normal appearing charr of the same strain and age class as the experimental fish were killed, tissue samples were taken, and sent to Dr. Rob Armstrong (Provincial Fisheries Veterinarian, Animal Health Branch, Ministry of Agriculture and Fisheries, Abbotsford, B. C.). Tissues taken included gill, eye, brain, pseudobranch, heart, anterior kidney, posterior kidney, liver, spleen,

posterior intestine, pyloric caecae, esophagus with approximately 1 cm of pneumatic duct attached, swim bladder, gonad, muscle with skin, and stomach. The gills, intact eye, muscle with skin, and pseudobranch were fixed in Bouin's fluid (Kiernan, 1981) for 24-48 h (eye - 12 h) and then placed in 70% methanol for an additional 24 h for clearing of the picric acid (eye - two changes of methanol). All other tissues were fixed in 10% buffered formalin, and the formalin was changed in 24 h. The brain and eye were left intact. The swim bladder was opened by a longitudinal incision and then spiraled around a pencil shaft prior to fixation. In the esophagus/pneumatic duct sample, the esophagus was incised down one side and opened flat, and the pneumatic duct was infused with formalin. After appropriate fixation times, all tissue samples were drained of their respective fixatives and placed in Whirl-pak[®] plastic bags with a paper towel which had been moistened with the appropriate fixative.

Individuals that showed signs of SBSS were collected at a certain stage of the syndrome (inability to respond to touch stimulus), tissues were collected and fixed as described for normal tissues, and extensive histopathological analyses were performed by Dr. Armstrong. Any abnormalities were noted and evaluated, and specifically, any pathological signs were to be documented. Particular attention was paid to the presence, if any, of specific structures that might be involved in secreting gases into the swim bladder against substantial pressure gradients, such as a rete mirabile/gas gland structure.

BACTERIOLOGY

Aerobic and anaerobic bacterial cultures from tissue of SBSS fish were attempted at the Polar Sea Fisheries laboratory. Shortly after the initiation of the project, 1 l of fluid thioglycollate medium, USP (BBL[®]) was prepared and 5 ml were added to each of several 13mm X 100 mm Kimax[®] glass culture tubes.

Also, approximately 1 l of trypticase soy agar (BBL®), or TSA, was prepared. Both solutions were autoclaved at 121 °C for 15 min in a Barnstead® Laboratory Autoclave, model #C2250. The TSA was poured into sterile petri dishes in aliquots of approximately 20 ml each, and allowed to cool and firm.

All SBSS individuals, prior to tissue sampling for histopathology, were aseptically sampled (kidney and swim bladder lining) and cultured for aerobic bacterial growth on Trypticase Soy Agar. Anaerobic bacterial growth was encouraged using fluid thioglycollate medium. These inoculated media were incubated at room temperature and at approximately 4 °C.

Samples of any bacterial growth were sent to the Pacific Biological Station, DFO, Nanaimo, B. C. for identification. The techniques used for identification included standard laboratory techniques (Gram's stain, colony appearance, motility determination, size of organisms, etc.) and the API 20E® System (Analytab Products), a self-contained laboratory analysis kit which has been demonstrated to be a satisfactory diagnostic tool (Kent, 1982).

VIROLOGY

The first five SBSS cases, prior to tissue sampling for histopathology, were aseptically sampled (kidney, spleen, swim bladder) and tissues were frozen and subsequently sent to the Pacific Biological Station, Department of Fisheries and Oceans (DFO) in Nanaimo, B. C., for virology screening. This was done by applying the sample tissue homogenate to a Rainbow trout Gonad 2 (RTG2) monolayer and incubating for 14 days at 15 °C.

HOMOGENATE INJECTION TRIAL

A tissue homogenate injection was performed using kidney and swim bladder tissue prepared as described below. This homogenate, which had been prepared from an individual obviously affected with SBSS, was injected into apparently normal individuals. This was done to investigate whether a transmissible disease agent from those tissues could be involved.

On August 7, 1990, a fish showing signs of SBSS was taken from experimental tank #8 (high density/stressed). This individual was aseptically opened and portions of the swim bladder and anterior kidney were removed, using sterile technique. Using sterile technique throughout the procedure, the tissue samples were weighed (0.808 g) and a volume of sterile Cortland's saline adequate to produce a 5% homogenate (15.35 ml) was added. The tissue/saline mixture was poured into a sterile mortar and was ground thoroughly until a reasonably homogeneous tissue/saline suspension was obtained. This suspension was held at 4 °C for approximately 10 min, while eight charr of the same strain and age class were anesthetized. The fish were each injected intraperitoneally with 0.5 cm³ of the tissue suspension. The injected fish were all placed in an experimental tank for recovery.

As part of the homogenate test, eight individuals of the same strain and age class were inoculated in exactly the same manner with sterile Cortland's saline only. Those individuals were placed in a separate but identical experimental tank to serve as controls.

A stress routine, identical to the stress protocol applied to the experimental fish in the Weekly Stress Protocol aspect of the project, was initiated in both tanks approximately 5 weeks after injection of the homogenate. This was done in an attempt to determine if the transmissibility of the condition was stress related. The application of the stress routine began on September 18, 1990, and

continued on a weekly basis through to October 23, 1990. The fish were held and observed until October 30, 1990.

STATISTICAL ANALYSES

Statistical analyses involving ANOVA were performed using Statpak® (Northwest Analytical, Inc.). Packed Cell Volumes, glucose concentration and cortisol concentration results were evaluated using ANOVA in a 2 X 2 factorial, random design (Appendix I).

Due to technical difficulties during the cortisol tests, six of the samples were not evaluated. In those cases, an average from the other five samples in that same group (n = 5) was obtained and substituted for the missing value.

A Log-likelihood ratio test was performed to determine the significance of the distribution of SBSS cases among the treatment groups (Appendix I).

ANOVA values of $P \leq 0.05$ were interpreted as significant.

RESULTS AND DISCUSSION

MORTALITIES - SBSS AND OTHER

There was a total of 14 fish removed from the tanks during the project; eight of those were SBSS fish and six were "routine mortalities". The distribution of all mortalities (SBSS cases and non-SBSS mortalities) are presented in Table 3.

TABLE 3. Distribution of SBSS Cases and Non-SBSS Mortalities in Arctic Charr. Within respective treatment groups, numbers indicate the SBSS cases or Non-SBSS mortalities from that treatment. There was no significant difference in the ratio of SBSS cases among treatments (Log-likelihood ratio; $P > 0.05$) or in the ratio of non-SBSS mortalities among treatments (Log-likelihood ratio; $P > 0.05$).

| TREATMENTS | SBSS CASES | OTHER MORTALITIES |
|---------------------------|------------|-------------------|
| High Density Stressed | 3 | 1 |
| Low Density Stressed | 0 | 0 |
| High Density Non-Stressed | 4 | 2 |
| Low Density Non-Stressed | 1 | 3 |
| Total | 8 | 6 |

Seven of the eight cases of SBSS were from high density groups, with only one case occurring in the low density groups (Table 3). There was no obvious explanation for this occurrence. When analysed by the Log-likelihood ratio test, the null hypothesis regarding the occurrence of SBSS among the treatments was accepted ($P > 0.05$), i.e., that there were no significant differences in the occurrence of SBSS among the four treatments. One important study (Wallace et al., 1988) indicated increased stress in Arctic charr in lower densities when compared to densities at or above 100 kg/m³. Wallace speculated that higher densities stimulate the development of schooling behaviour while inhibiting the development of stressful aggressive behaviour. Wallace's study also indicated that lower densities in charr may stimulate aggressive behaviour and more stress. When considering Wallace's study, the prevalent occurrence of SBSS in the high density environments, while not statistically significant, is contrary to

the general consensus that this condition is due to stress (Clary and Clary, 1978; Johnson and Katavic, 1984; Bagarinao and Kungvankij, 1986).

WATER QUALITY

Routine Water Quality Sampling

The average values of the routine water quality parameter samplings are shown in Table 4.

TABLE 4. Routine Average Water Quality Values. Values are mean values calculated from values taken over the term of the project during non-stressed periods (\pm S. D.). NH₃ refers to un-ionized ammonia.

| GROUPS | D.O. (mg/L) | pH | TGP (%) | TEMP (°C) | NH ₃ (mg/L) |
|--------------|----------------|----------------|-----------------|----------------|------------------------|
| High Density | 7.7 \pm 0.74 | 7.8 \pm 0.04 | 96.7 \pm 0.82 | 5.5 \pm 0.72 | 0.0015 \pm 0.0007 |
| Low Density | 8.5 \pm 0.68 | 7.9 \pm 0.04 | 98.2 \pm 0.83 | 5.5 \pm 0.71 | 0.001 \pm 0.0004 |
| All | 8.1 \pm 0.71 | 7.9 \pm 0.04 | 97.5 \pm 0.83 | 5.5 \pm 0.71 | 0.0012 \pm 0.0006 |

The high density tanks had dissolved oxygen, pH and TGP values that were slightly lower, and un-ionized ammonia levels which were slightly higher, than those in the low density tanks. The temperature was very consistent in all of the tanks, showing a difference between the extreme low and high average values (over all tanks) of only 0.15 °C. The routine water quality samples from all tanks exhibited values which were well within accepted ranges for the culture of salmonids (Piper et al., 1982; FRED, 1983).

Stress Protocol Water Quality Sampling

Summaries of the average values from stressed treatments are displayed in Figures 2 and 3.

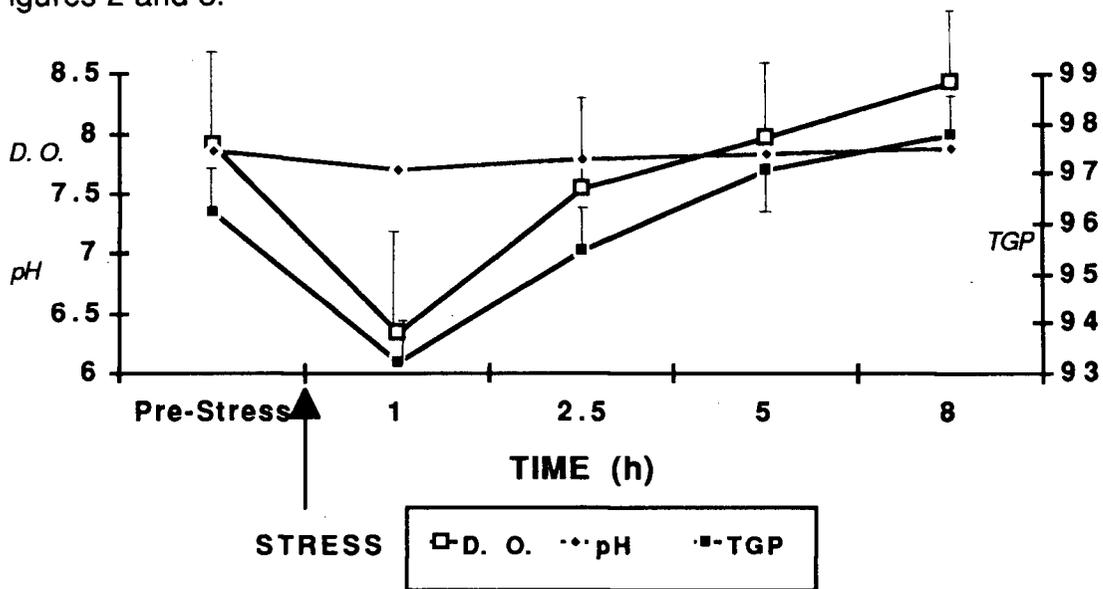


FIGURE 2. Mean Water Quality Parameters From Stressed Groups. D. O. = dissolved oxygen (mg/L). TGP = total gas pressure (%). Pre-stress category indicates values taken within 1 h prior to stress application. Hour categories indicate values taken at specific times after completion of stress application. Vertical lines indicate ± 1 S. D.

DISSOLVED OXYGEN

There was an initial drop in D. O. (average 1.58 mg/L) at the 1 h sampling, followed by a gradual increase through the remainder of the day. The increased consumption of oxygen in association with stress has been documented (Barton and Schreck, 1987). In some cases, by the 8 h sampling, the D. O. reached levels higher than those determined at the pre-stress samplings. The lower values obtained in the pre-stress category were thought to be associated with the morning "start-up" activities at the hatchery (turning on the lights, people moving about, additional noises). It was shortly after these series of activities that the stress protocol and subsequent water parameter measurements began. It is likely that, due to increased metabolism resulting from the start-up

activities, somewhat lower than normal oxygen levels (Barton and Schreck, 1987) were being experienced at the time of the experimental stress application, but were compensated for over the course of the day, allowing for recovery levels at the 8 h sampling which were slightly higher than those recorded at the pre-stress sampling. With two exceptions, the dissolved oxygen levels, even at 1 h post-stress, were above those values indicated to be of concern for salmonid culture (Piper, 1982; FRED, 1983). On two occasions, and for only brief periods during the early stages of the project, two tanks experienced dissolved oxygen levels down to approximately 4 mg/L. These isolated incidences were due to improperly functioning valves in two of the experimental tanks. Once recognized as a mechanical problem, the situation was corrected and those notably low levels were not encountered again. It should be noted that the low levels just described were determined through routine dissolved oxygen measurement, and had not produced any observable behavioural changes in the fish.

pH

The very small fluctuation in the pH of the water was consistent with the initial decrease and subsequent increase in dissolved oxygen. Concurrent with the decreased dissolved oxygen, one would expect an increased carbon dioxide level, hydration of which would have produced carbonic acid, thereby lowering the pH slightly (Boyd, 1979). Average fluctuation in pH across all tanks was approximately 0.15 pH unit. The water at Polar Sea Fisheries is quite hard (approximately 300 mg/L) and well buffered against pH changes. These small changes were not considered to be of concern.

TOTAL GAS PRESSURE

The TGP values consistently demonstrated the same pattern as the dissolved oxygen, showing an initial drop followed by a gradual increase back to pre-

stress levels, or even higher. This initial decrease in % TGP could be correlated with the corresponding initial decrease in % dissolved oxygen by multiplying the decrease in % oxygen saturation by 0.21 (% atmospheric oxygen) and comparing that value with the decrease in TGP. The % nitrogen remained basically unchanged throughout all water samplings. Over-inflated swim bladders have been observed in rainbow trout during conditions of supersaturation by Shrimpton et al. (1990). Their work also determined that TGP levels in excess of 100% are required for downloading of gas into the swim bladder. In addition, fish held in water at or below saturation slowly lost gas from the swimbladder. At no time during the experiment did the water in any of the experimental tanks achieve supersaturation. None of the typical signs of Gas Bubble Disease, also associated with supersaturated water (Pauley and Nakatani, 1967) were observed in the histopathological aspect of the project .

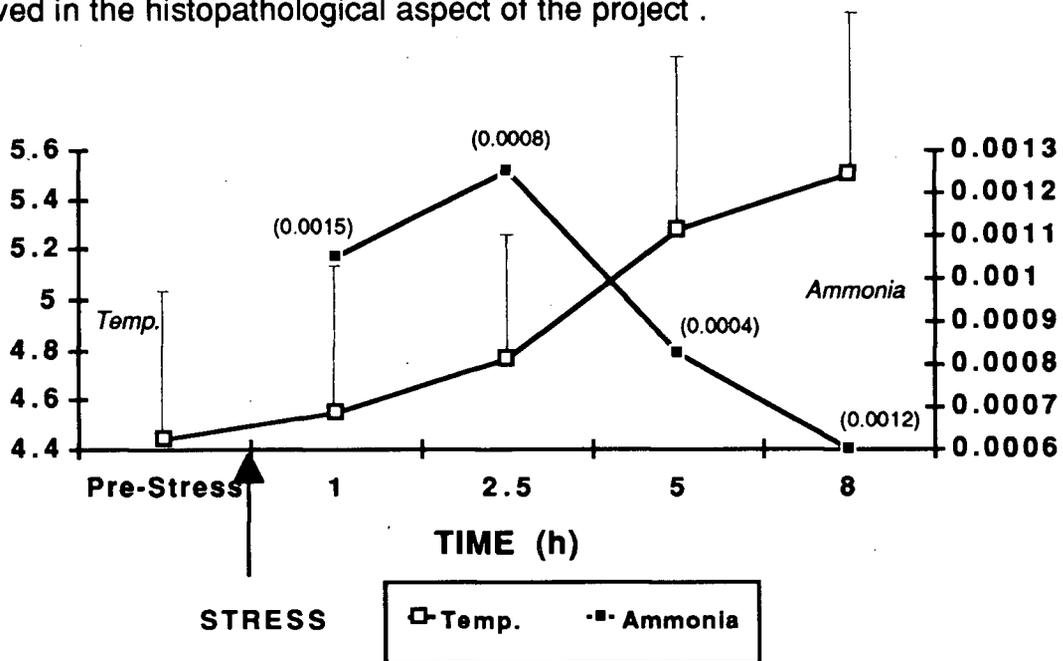


FIGURE 3. Mean Water Quality Parameters From Stressed Groups. Temp. = temperature (°C). Ammonia = un-ionized ammonia (mg/L). Pre-stress category indicates values taken within 1 h prior to stress application. Hour categories indicate values taken at specific times after completion of stress application. Pre-stress values for un-ionized ammonia were not measured. Vertical lines indicate ± 1 S. D. (Temp.). Brackets indicate ± 1 S. D. (Ammonia).

TEMPERATURE

The temperature pattern in all tanks was consistent. Even the most dramatic change was rarely more than a 1.5 °C increase throughout an entire day. These changes occurred in all of the tanks, and were due to increasing summer temperatures outside warming the main-line water flow into the hatchery.

UN-IONIZED AMMONIA

As explained in the Methods and Materials section, pre-stress ammonia values were not obtained (Fig. 3). It was intended that these "pre-stress" values be obtained the afternoon prior to the stress day. However, it was found that these afternoon ammonia levels were subject to fluctuation and did not necessarily indicate realistic pre-stress ammonia levels. The routine water quality values (Table 4) indicated an overall average un-ionized ammonia level of 0.0012 mg/L. The maximum average value of un-ionized ammonia in the stressed groups, following stress application, was 0.0013 mg/L at the 2.5 h sampling (Fig. 3). The average routine ammonia values did not exceed the maximum levels reached in the stressed groups at 2.5 h after stress application (0.0013 mg/L). At no time did any of the un-ionized ammonia values reach levels which have been determined to be detrimental to fish (Meade, 1985; Daoust, P.-Y. and Ferguson, H. W., 1984; Thurston, R. V. and Russo, R. C., 1983).

BLOOD SAMPLING

Packed Cell Volumes

Blood samples were collected from the stressed and non-stressed fish at the initiation, mid-point and termination of the project. Summaries of the pcv values are presented in Figures 4 - 7.

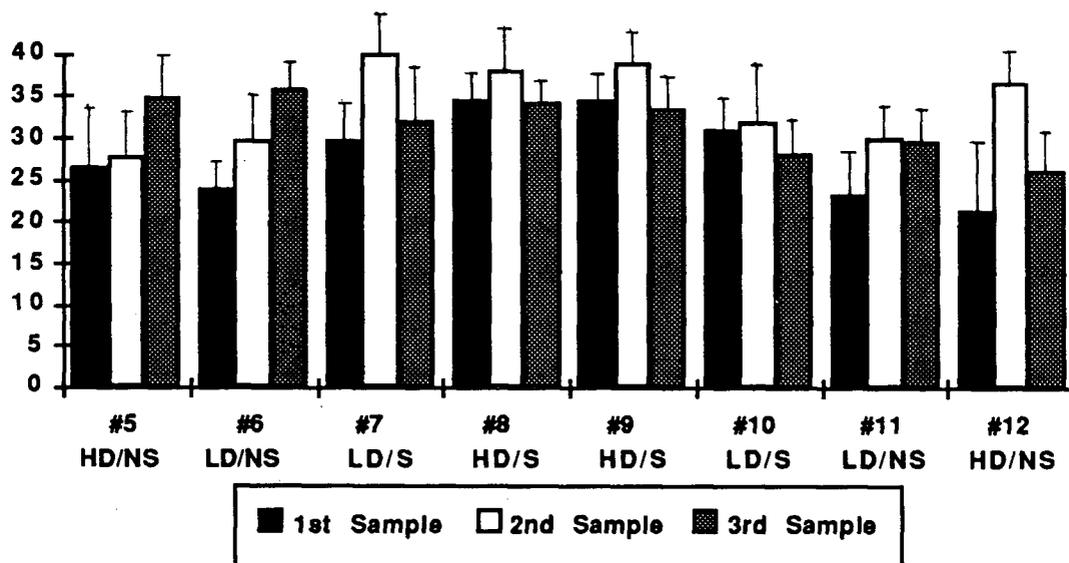


FIGURE 4. Mean Packed Cell Volumes (%) from 1st, 2nd and 3rd Blood Samplings of Arctic charr from All Tanks. Each treatment value is the mean of sample values (n=6). HD=high density. LD=low density. S=stressed. NS=non-stressed. Vertical lines indicate ± 1 S. D.

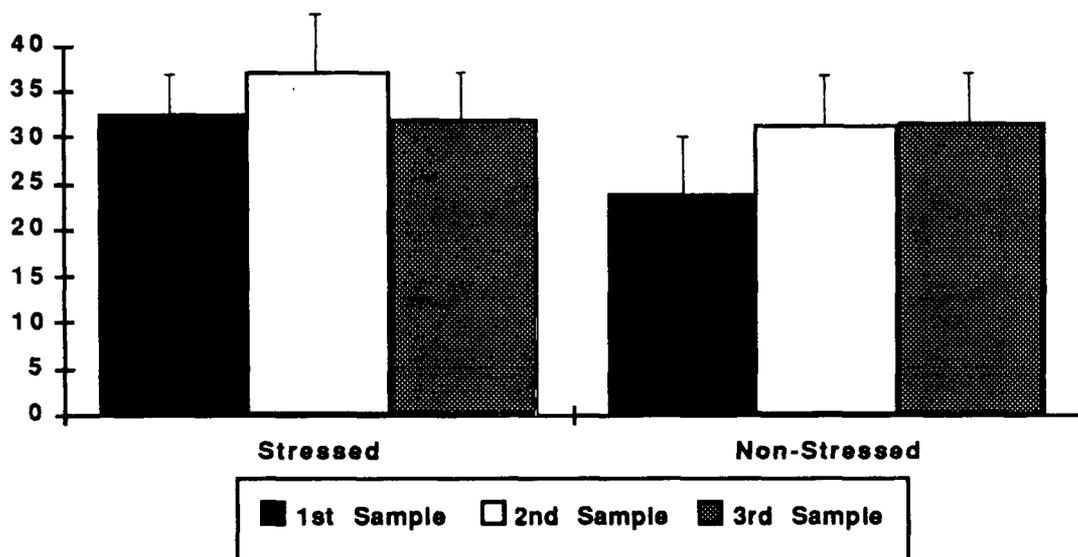


FIGURE 5. Mean Packed Cell Volumes (%) from 1st, 2nd and 3rd Blood Samplings of Arctic charr, Stressed vs. Non-Stressed Treatments. Each treatment value is the mean of four treatment groups; each group value is the mean of sample values (n=6). Vertical lines indicate ± 1 S. D.

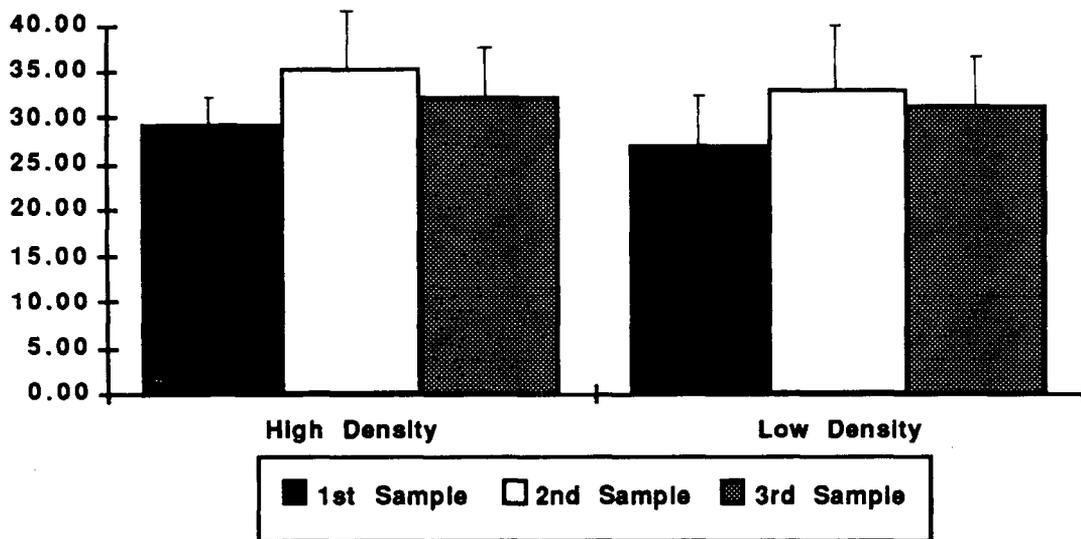


FIGURE 6. Mean Packed Cell Volumes (%) from 1st, 2nd and 3rd Blood Samplings of Arctic charr, High Density vs. Low Density Treatments. Each treatment value represents the mean of four treatment groups; each group value is the mean of sample values (n=6). Vertical lines indicate ± 1 S. D.

TABLE 5. Significance of Packed Cell Volumes From 1st, 2nd & 3rd Blood Samplings of Arctic Charr Among Treatment Groups - ANOVA (n=6). $P \leq 0.05$ was considered significant.

| SAMPLE | TREATMENT | SIGNIFICANCE |
|------------------|------------------|-----------------|
| 1st Sample - PCV | Stress | ≤ 0.001 |
| | Density | Not Significant |
| | Stress X Density | Not Significant |
| 2nd Sample - PCV | Stress | ≤ 0.001 |
| | Density | Not Significant |
| | Stress X Density | Not Significant |
| 3rd Sample - PCV | Stress | Not Significant |
| | Density | Not Significant |
| | Stress X Density | ≤ 0.05 |

The respective average packed cell volumes (pcv's) from stressed and non-stressed fish, regardless of density, each followed a fairly consistent pattern within each of those treatments, but showed a different pattern between those two treatments (Fig. 4).

The average pcv value from the stressed fish showed significantly elevated levels over the non-stressed fish, in the first and second samplings. In the third sampling, pcv levels in each stressed group dropped to approximately the level of the first pcv values of those same groups (Fig. 5). The pcv values from the third sampling of the non-stressed fish tended to continue to rise, or at least remain about the same, producing a level which corresponded closely to the third pcv average level of the stressed groups (Fig. 5). Specifically, the final average pcv value of the stressed groups, which had decreased, was almost identical to the corresponding value in the non-stressed groups, which had increased or remained the same. ANOVA indicated a significant stress/density interaction ($P \leq 0.05$) in the third sampling, although this difference is not readily apparent when the values are compared by density (Fig. 6) or stress (Fig. 5), respectively. It does become apparent when all treatments are compared together (Fig. 7). Stress levels, as measured by pcv, show opposite trends when compared within the density levels. This configuration readily explains the lack of a significant difference in cortisol values at the third sampling between stressed and

non-stressed treatments, and also between high and low density treatments, as the means of the extreme values are almost identical within each treatment (mean value of stressed treatments = 31.8 %; mean value of density treatments = 31.6 %). Keeping in mind that this interaction occurred during the third blood sampling, it may indicate acclimation (Schreck, 1981; Pickering and Stewart, 1984) to the stress treatment over the course of the project. It is possible that, due to a preference for higher stocking densities by Arctic charr (Wallace et al., 1988), the experimental fish in the stressed treatment groups had been experiencing more stress in the low density groups throughout the first part of the project and, due to the combination of the stress treatment and the low density stress, had achieved a state of compensation by the time of the third blood sampling. This could explain the lower pcv value in the low density/stressed groups at the third sampling. It would also follow that, in the non-stressed, low density treatment groups, a relatively low level, gradual stress was building over the course of the project due to the low density environment, and this was demonstrated in the elevated pcv values in the final blood sampling from the low density groups. These stress levels were not enough to “activate” a compensatory response, however. This phenomenon also appears to have occurred based on the cortisol values, and will be discussed in more detail below, in the Cortisol section of the thesis.

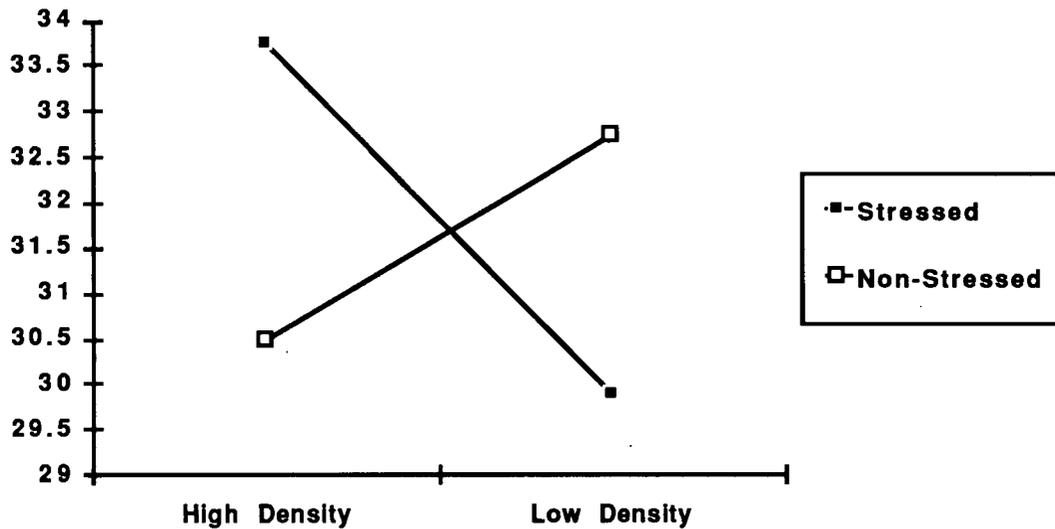


FIGURE 7. Mean Packed Cell Volumes (%) - Interaction Between Stress and Density Treatments at 3rd Blood Sampling of Arctic Charr. Each treatment value is the mean of pooled sample values (n=12).

The significant increases (1st sample - $P \leq 0.001$; 2nd sample - $P \leq 0.001$) in pcv found in the stressed groups in the first and second sampling was in accord with current information pertaining to the effect of physiological stress (adrenalin response) on the osmotic balance of the red blood cells, that being an increase in the size of the erythrocytes due to the movement of sodium ions and potassium ions into the cells, with a subsequent influx of water (Nikinmaa, 1982; Ling and Wells, 1985). One must also consider the fact that, in addition to adrenalin-induced erythrocyte swelling, catecholamines (adrenalin and noradrenalin) have been credited with responsibility for the dilation of the pneumatic duct of the physostome swim bladder and subsequent release of the gases within it (Fänge, 1953; Jones and Marshall, 1953), a situation which would be antagonistic to an over-inflated swim bladder.

The further increased average pcv of the stressed fish obtained from the second sampling demonstrated that we had continued to stress those individuals and had produced even greater pcv values. In the second sampling, non-

stressed average values increased also, reaching a level approximately one percentage unit below the first sampling value obtained from the stressed groups, but still indicated a level approximately six percentage units below the stressed tanks of the same sampling. These second sampling results point to two occurrences: (1) the stressed groups were continuing to be stressed to about the same degree relative to the non-stressed groups and (2) the stress levels were rising in all groups. The latter may have been due to the conditions under which the experimental fish were maintained, i.e., small tanks, relatively shallow water, restricted movements, frequent water quality samplings. The latter portion of the experiment occurred during a period in which mature, wild charr migrate to the sea for the summer. Even though the experimental fish were only 2 yr old charr, this may have had some effect on the increased overall packed cell volumes, as cortisol levels have been shown to increase during the period of smoltification of coho salmon (Barton et al., 1985). Attempted migratory behaviour is believed to have been previously observed in one year old Arctic Charr at Polar Sea Fisheries.

Third sampling results of the stressed groups showed an obvious decrease in the average pcv value, down to a level which was slightly below the value obtained from the initial sampling of the same groups. The non-stressed pcv value from the third sampling remained basically the same as the non-stressed pcv value from the second sampling, and was within 0.2 percentage unit of the corresponding value from the stressed groups. The behavioural reaction of the fish in the stressed tanks to each stress encounter remained about the same throughout the project. The fish would react violently to the net as it was moved about the tank, some of them attempting to jump out of the tank. After the 5 min stress application, the fish would settle to the bottom of the tanks and remain there for 1 h to 2.5 h before resuming their normal positions in the water column.

The decreased oxygen and increased ammonia values in the water indicated that the fish were experiencing a state of increased metabolism, a condition which accompanies stress (Mazeaud and Mazeaud, 1981; Barton, 1988). The fact that the average pcv value of the stressed groups dropped to essentially the same value as the non-stressed groups in the third sample may indicate that some sort of adaptation or compensation to the applied stress may have been occurring (Schreck, 1981), or that pcv values are not a totally reliable indication of physiological stress induced by handling.

The patterns of average pcv value fluctuations as just described could generally be observed in the fish in the individual tanks of the respective treatments. A fairly obvious exception was tank #12, containing a non-stressed group. It showed a pcv pattern similar to those demonstrated by the stressed groups, that being a substantial increase in pcv in the second sampling, followed by a marked decrease in the third. There may be a plausible explanation for this occurrence. Tank #12 was located on the lower level and at the extreme right of the experimental tank configuration (see Fig. 1, Methods and Materials). Entry into the experimental area through the plastic curtain was located at exactly this point and very close to the tank. This entry was used almost exclusively whenever anyone entered the experimental area. As a result of this activity, it is quite possible that the fish in this tank were subjected to additional stress, perhaps even to the same extent as the stressed tanks. There was usually a noticeable reaction by the fish in this tank whenever the experimental area was entered. The apparent compensation to stress, which was thought to be observed in the stressed tanks, may also have been demonstrated in this tank. Tank #11, containing another non-stressed group which was right next to tank #12 but removed from the entrance, showed a pcv pattern very similar to those which were demonstrated for the other non-stressed treatments.

CORTISOL

The elevation of plasma cortisol in fish as an indicator of stress from handling has been documented (Strange et al., 1977). Summaries of the cortisol values found in the present experiment are shown in Figures 8 - 12.

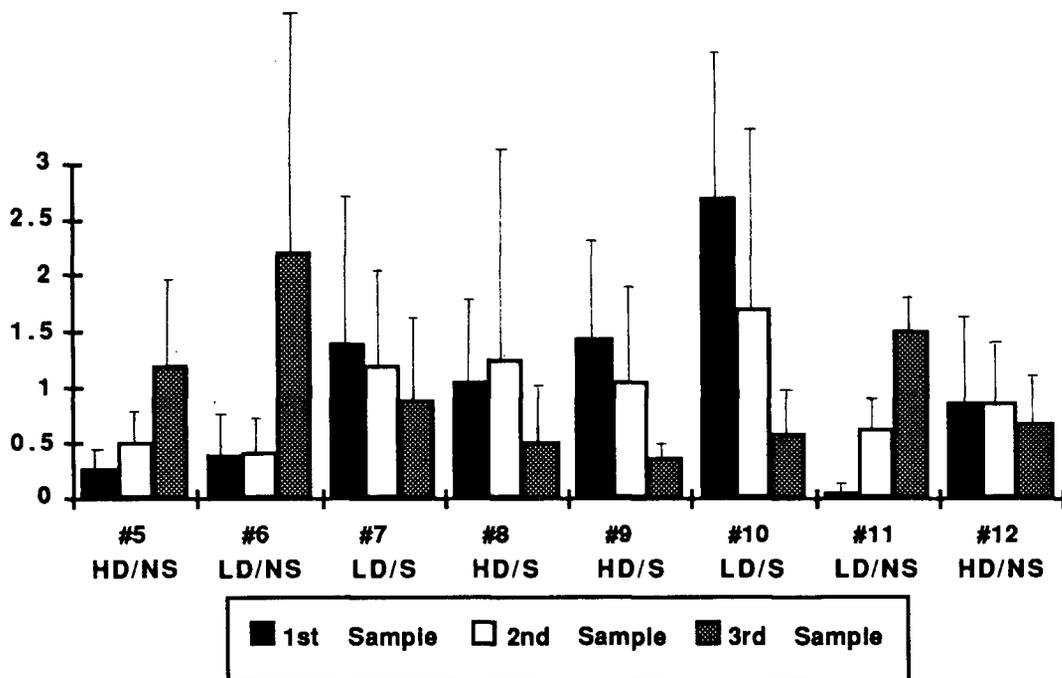


FIGURE 8. Mean Cortisol Values ($\mu\text{g/dl}$) from 1st, 2nd and 3rd Blood Samplings of Arctic Charr from All Tanks. Each treatment value is the mean of sample values ($n=6$). HD=high density. LD=low density. S=stressed. NS=non-stressed. Vertical lines indicate ± 1 S. D.

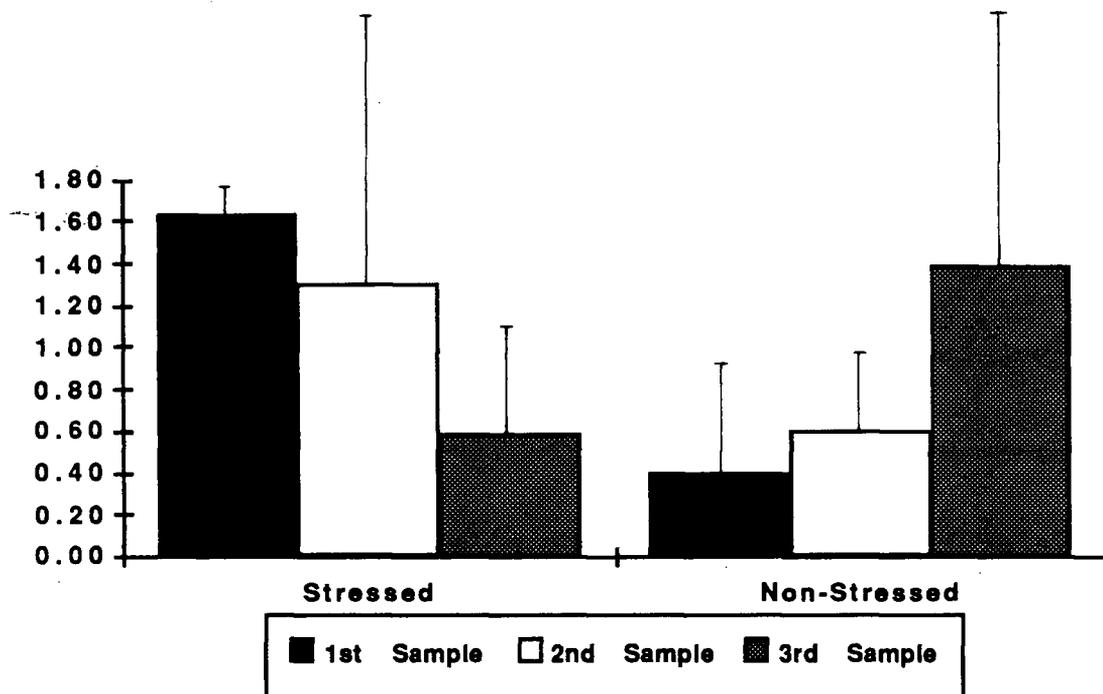


FIGURE 9. Mean Cortisol Values ($\mu\text{g/dl}$) from 1st, 2nd and 3rd Blood Samplings of Arctic Charr, Stressed vs. Non-Stressed Treatments. Each treatment value is the mean of four treatment groups; each group value is the mean of sample values ($n=6$). Vertical lines indicate ± 1 S. D.

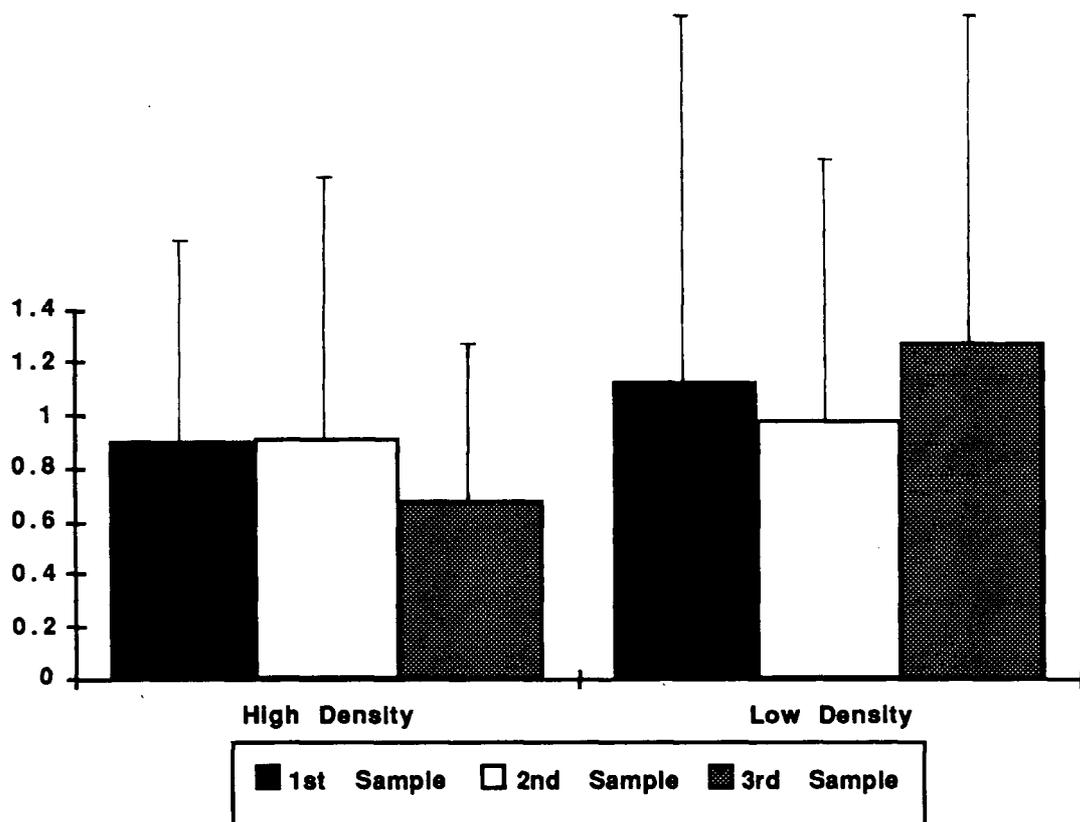


FIGURE 10. Mean Cortisol Values ($\mu\text{g/dl}$) from 1st, 2nd and 3rd Blood Samplings of Arctic charr, High Density vs. Low Density Treatments. Each treatment value is the mean of four treatment groups; each group value is the mean of sample values ($n=6$). Vertical lines indicate ± 1 S. D.

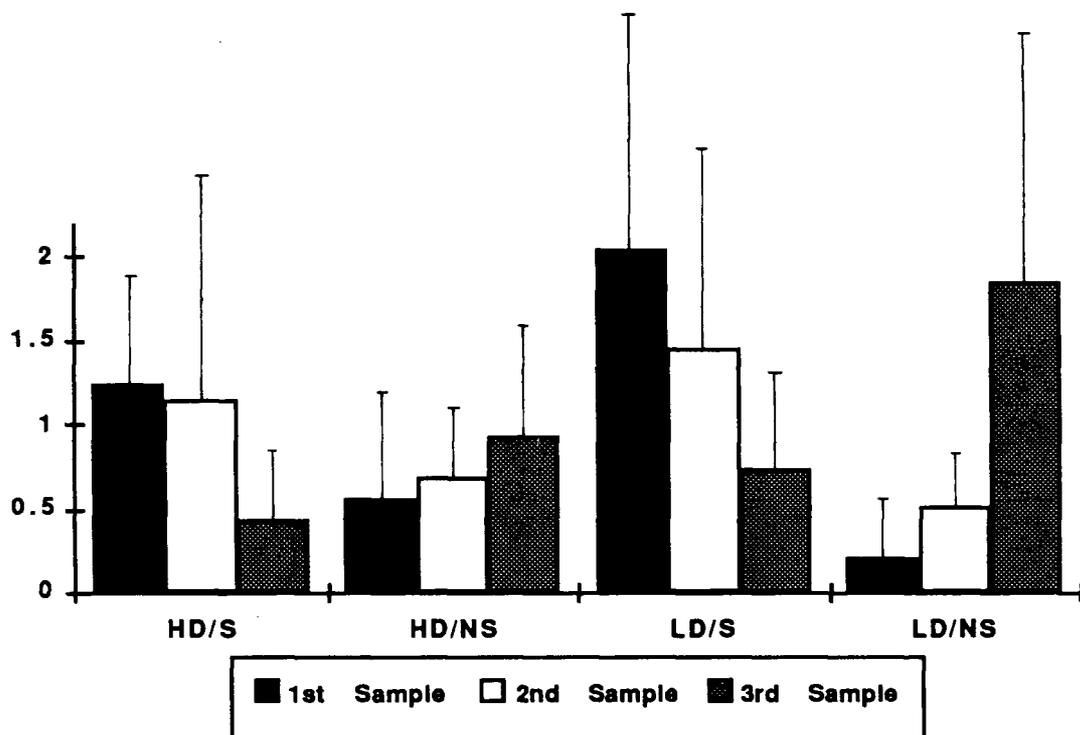


FIGURE 11. Mean Cortisol Values ($\mu\text{g/dl}$) from 1st, 2nd and 3rd Blood Samplings of Arctic charr, All Treatments. Each treatment value is the mean of two treatment groups; each group value is the mean of sample values ($n=6$). HD=High Density. LD=Low Density. S=Stressed. NS=Non-Stressed. Vertical lines indicate ± 1 S. D.

TABLE 6. Significance of Cortisol Values From 1st, 2nd & 3rd Blood Samplings of Arctic Charr Among Treatment Groups - ANOVA (n=6). $P \leq 0.05$ was considered significant.

| SAMPLE | TREATMENT | SIGNIFICANCE |
|-----------------------|------------------|-----------------|
| 1st Sample - CORTISOL | Stress | ≤ 0.001 |
| | Density | Not Significant |
| | Stress X Density | ≤ 0.05 |
| 2nd Sample - CORTISOL | Stress | ≤ 0.025 |
| | Density | Not Significant |
| | Stress X Density | Not Significant |
| 3rd Sample - CORTISOL | Stress | ≤ 0.005 |
| | Density | ≤ 0.025 |
| | Stress X Density | Not Significant |

The average values from the stressed groups showed a steady decrease over all three samplings (Fig. 9). The first two samplings were significantly higher in the stressed groups when compared to the respective values of the non-stressed groups. The gradual decline of cortisol values in the stressed treatments appears to represent an acclimation to the weekly stress protocol which was applied to the stressed groups. As the fish became accustomed to this routine, acute stress application, the primary response was decreased. The concept of acclimation or compensation to certain types of stress has been documented (Schreck, 1981; Pickering and Stewart, 1984). While average cortisol levels were shown to be significantly higher in the stressed groups as compared to the non-stressed groups for samplings #1 and #2, for the third sampling, average cortisol levels were significantly higher in the non-stressed groups as opposed to the stressed groups. In the non-stressed groups, the cortisol levels underwent an increase over the three samplings, each average sample value being higher than the one before.

When evaluated from a density standpoint, the increased cortisol levels in the third sampling can be attributed primarily to the low density tanks (Fig. 10). It is probable that the reaction to the applied stress was the most conspicuous and may have overshadowed any obvious density implications during the first two samplings, although a significant stress/density interaction was also indicated in

the first sampling, indicating even then that the low density environment may have been influencing the levels of cortisol production between the two stress treatments (Fig. 12).

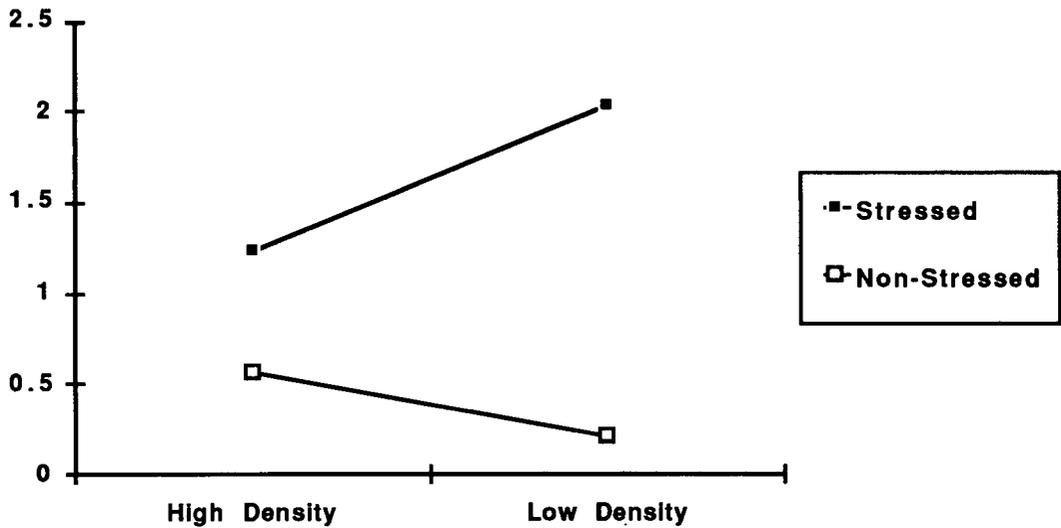


FIGURE 12. Cortisol ($\mu\text{g}/\text{dl}$) Mean Values - Interaction Between Stress and Density Treatments at 1st Blood Sampling of Arctic Charr. Each treatment value is the mean of pooled sample values ($n=12$).

Upon reaching a stage of acclimation or compensation in the stressed groups by the time of the third sampling, the effects of stress induced by a lower density became apparent. This hypothesis gains some merit when all categories are compared (Fig. 11). While the stressed groups and the non-stressed groups showed the same pattern respectively (overall decrease in stressed groups and overall increase in non-stressed groups), it could be seen that the cortisol values of the low density/stressed groups were consistently higher than those of the high density/stressed groups. The same relationship did not occur, however, between the low density/non-stressed groups and the high density/non-stressed groups except at the third sampling. It has been documented (Wallace

et al., 1988; Baker and Ayles, 1990) that Arctic Charr prefer higher stocking densities than other salmonids. It should be kept in mind that 87.5% of all cases of SBSS induced during this project were from charr at the high density levels. Density appears to have had some effect in the overall cortisol values.

I was only able to demonstrate significantly higher stress levels (cortisol) in the stressed groups, as compared to those in the non-stressed groups, for samplings one and two. This was not demonstrated for sampling three. This apparent adaptation to the stress protocol was not anticipated. It should be noted, however, that because of the nature of the stressor or the fish's ability to compensate, the lack of a significant physiological response does not necessarily mean that the fish were not stressed (Schreck, 1981).

It should also be noted that during the period between the second and third blood sampling, the period in which the cortisol levels of the non-stressed groups became significantly higher than those in the stressed groups, there were three cases of SBSS observed. Two of these cases occurred in high density, stressed groups. As the average cortisol levels in both the stressed groups and the high density groups became significantly lower than their respective counterparts during this period (based on the third sampling results - Table 6), these occurrences seem contradictory to a stress related condition.

The objective of the cortisol measurements was to test whether the fish were indeed stressed by the stress protocol. The fact that the stressed groups generated relatively more blood cortisol seems to confirm that condition, at least for the first two samplings.

GLUCOSE

Summaries of the glucose values are presented in Figures 13 - 14.

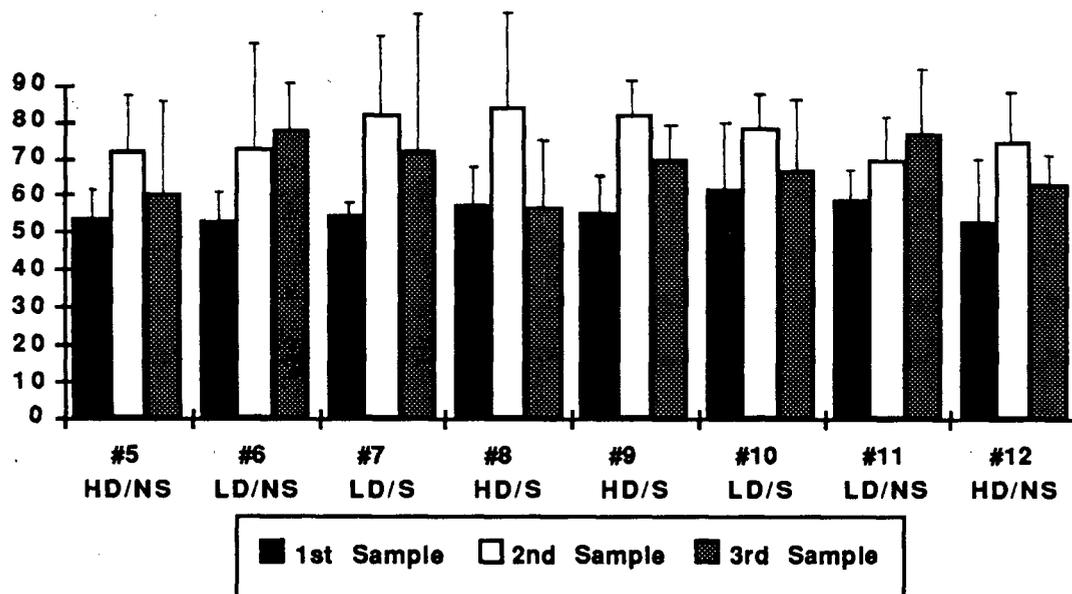


FIGURE 13. Mean Glucose Values (mg/L) from 1st, 2nd and 3rd Blood Samplings of Arctic charr from All Tanks. Each treatment value is the mean of sample values (n=6). HD=high density. LD=low density. S=stressed. NS=non-stressed. Vertical lines indicate ± 1 S. D.

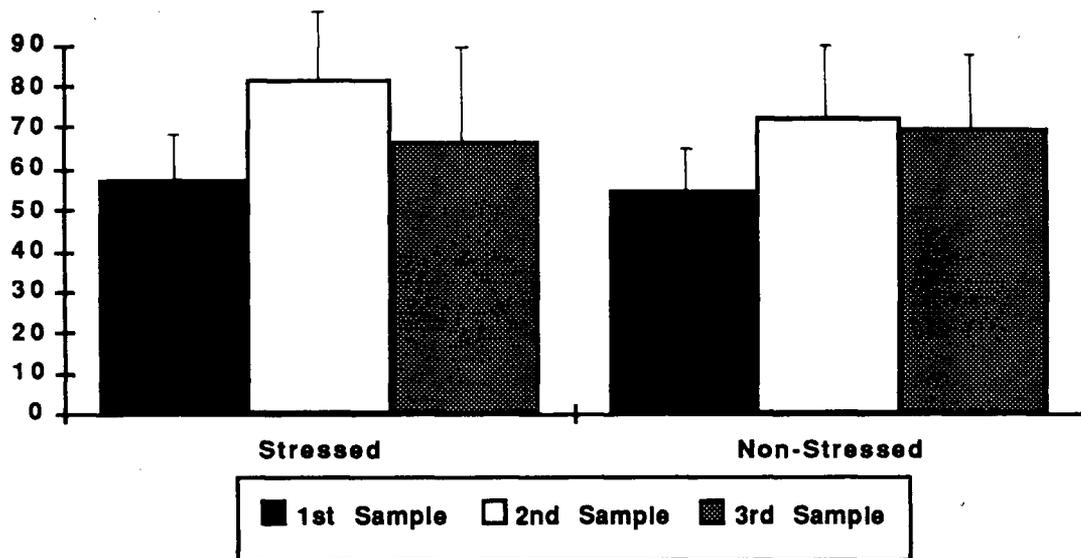


FIGURE 14. Mean Glucose Values (mg/L) from 1st, 2nd and 3rd Blood Samplings of Arctic charr, Stressed vs. Non-Stressed Treatments. Each treatment value is the mean of four treatment groups; each group value is the mean of sample values (n=6). Vertical lines indicate ± 1 S. D.

TABLE 7. Significance of Glucose Values From 1st, 2nd & 3rd Blood Samplings of Arctic Charr Among Treatment Groups - ANOVA (n=6). $P \leq 0.05$ was considered significant.

| SAMPLE | TREATMENT | SIGNIFICANCE |
|----------------------|------------------|-----------------|
| 1st Sample - GLUCOSE | Stress | Not Significant |
| | Density | Not Significant |
| | Stress X Density | Not Significant |
| 2nd Sample - GLUCOSE | Stress | Not Significant |
| | Density | Not Significant |
| | Stress X Density | Not Significant |
| 3rd Sample - GLUCOSE | Stress | Not Significant |
| | Density | Not Significant |
| | Stress X Density | Not Significant |

None of the differences in the glucose concentrations were found to be statistically significant among the test categories within the respective blood samplings (Table 7). Mazeaud et al. (1977) indicated that secondary stress responses (plasma glucose elevation) are generally slower to occur than primary responses (plasma corticosteroid elevation), and this may account for the non-significant changes in glucose as opposed to cortisol, as all blood samples were collected at 1 h after the stress application. These results are somewhat in keeping with those of Carmichael and Tomasso (1984), who identified SBSS in largemouth bass (*Micropterus salmoides*), and demonstrated that glucose levels showed no trend and were, in fact, lower in fish after transport than before, except in those that actually exhibited SBSS.

In a study involving brown trout, plasma cortisol levels increased almost immediately after handling stress and peaked at 2 h post stress, whereas plasma glucose levels remained the same for the first 2 h after handling stress and peaked at 4 h post stress (Pickering et al., 1982).

TRANSMISSIBILITY

Histopathology

Of the eight cases of SBSS which were observed, tissues were collected and fixed for histopathological examination in only seven. One of the fish was in an

advanced moribund state, and it appeared that some of the tissues had begun autolysis.

In five of the six cases submitted, mild, diffuse, acute congestion with mild scattered acute epithelial cell degeneration and mild multifocal acute telangiectasis (dilated capillary) were observed in the gill lamellae.

In two of the submissions, mild, diffuse scalloping of the swim bladder epithelial lining (mucosa) was observed.

In five of the submissions, the swim bladder wall demonstrated mild to moderate inflammation of the bladder wall epithelial cells (non-suppurative sub-acute perivascular pneumocystitis) with lymphocytes dominating the cellular infiltrate.

In three of the submissions, there was mild, scattered degeneration of smooth muscle fibers (myodegeneration) in the smooth muscle aspect of the swim bladder wall (pneumocyst muscularis).

It was specifically requested that close attention be paid to determining the existence of rete mirabilia and gas gland structures, or the equivalent. None of these structures were found.

Dr. Armstrong, who performed the pathological analyses, concluded the following:

1) The gill lesions were consistent with those caused by agonal death or terminal trauma, probably induced by a blow to the head which was administered to induce unconsciousness.

2) The scalloping of the epithelium was consistent with what would be expected from the contraction of a distended swim bladder wall during fixation.

3) The apparent inflammatory responses (perivascular lymphocytic clustering) could not be fully explained. They may have been normal occurrences. A similar condition was found in two of the normal sets of tissues.

4) The occasional degenerating myofibres in the swim bladder walls were consistent with what may have been produced from the pressure produced from a distended swim bladder.

Dr. Armstrong indicated that he "would be unable to distinguish consistently between normal and SBSS fish, based on findings from the swim bladder tissue submitted during this project". The histopathological examination of the specimens submitted failed to indicate any specific and consistent pathology associated with SBSS.

Bacteriology

Bacterial growth was observed in two of the eight cultured samples. One was from a fish from a low density, non-stressed treatment and the other was a fish from a high density, stressed treatment. In both cases, the growth was obtained from within the swim bladder and grew only at room temperature and only in the anaerobic medium. There was no bacterial growth from the kidney smears from either individual.

To determine if the organisms were true anaerobes, an inoculum from each sample was transferred to TSA plates. Overnight growth occurred from one sample, and slower but still enhanced growth occurred from the other, both at room temperature. It appeared that the anaerobic medium had acted as an enhancement medium. The subsequent growth on the TSA allowed the two samples to be recognized as different colony types.

Laboratory investigations showed that the bacteria from one was a *Pseudomonas* while the bacteria from the other sample was a mixture of two types. While these two bacteria could not be specifically identified, they were concluded to be "sewer bacteria", these being bacteria of a genus which are generally associated with contamination and/or secondary or opportunistic infections.

The bacteria from both fish were determined not to be primary pathogens, and generally are encountered only when the fish have already been placed in a state of decreased immunological competence, quite likely from some type of stress. Considering these results and the fact that only two of eight cases demonstrated any bacterial growth at all, the bacteria which were identified were not considered to be causative agents of SBSS.

A swim bladder disease has been identified in fingerling lake trout with the causative agent identified as *Pseudomonas fluorescens* (Wolf, 1937). However, this condition was manifested as a deflated swim bladder, with affected individuals sinking to the bottom of the tank and remaining there until death.

Virology

Tissue samples from all five individuals submitted for virology screening were found to be negative for any viral activity. It is highly unlikely that a virus was responsible for SBSS cases which occurred during this study.

Homogenate Injection

After being held in the experimental tanks for over 2 months, neither the injected nor the control fish exhibited any signs of SBSS.

The lack of any evidence of SBSS, or any other abnormal condition or behaviour, resulting from the tissue homogenate injection would tend to indicate that a transmissible agent is not involved in the inducement of SBSS.

SUMMARY AND CONCLUSIONS

In an attempt to understand the important factors in the inducement of SBSS, Arctic charr were subjected to four experimental treatments (stressed/non-stressed X high density/low density) in an attempt to induce a significant number of SBSS cases. Only eight cases were observed overall. There was no significant effect of the experimental treatments on the incidence of SBSS

among the treatments (Log-likelihood ratio - $P > 0.05$), although seven out of eight (87.5%) of the SBSS cases occurred in the high density groups.

PCV values and plasma glucose and cortisol levels were determined on three occasions during the project. I was able to demonstrate that the fish in the stressed groups had been stressed throughout most of the study (approximately 3 months), as compared to the non-stressed groups. It should be noted that, in the three previous studies which dealt specifically with SBSS (Clary and Clary, 1978; Carmichael and Tomasso, 1984; Kolbeinshavn and Wallace, 1985), the condition was initiated during a period of time from 5 h to 2 months, depending on the study, after the onset of the stress situation.

Some degree of compensation to the applied stress was apparent in the stressed groups, as indicated by the pcv and cortisol results. There is the possibility that more cases of SBSS might have been induced had the stress levels (as indicated by blood parameters) been maintained for a longer period of time.

There was an indication of a low density related stress by the cortisol levels from the third blood sampling, which seems in direct contrast to the fact that 87.5% (not statistically significant) of all SBSS cases occurred in high density groups. This is an area which warrants more study, especially considering the unusual density parameters which pertain to Arctic Charr.

The condition might be induced by a different type, frequency or duration of stress. In a study by Barton et al. (1986) involving juvenile chinook salmon, it was determined that a series of multiple, acute handling stresses produced a cumulative stress response as measured by blood cortisol levels. Each subsequent stress application produced a significant increase in blood cortisol over the previous one. However, after the brief stress assaults, and within a 30 h period, the cortisol levels began returning to levels which were only slightly elevated over initial stress levels. The cortisol levels were continuing to drop, and

it was assumed that the levels would continue to fall over the next day or two, possibly to pre-stress levels. Cortisol concentrations were not measured past a 30 h period. When considering the situation of chronic stress, that being a rise of cortisol to a plateau and remaining there (Strange et al., 1977; Barton et al., 1980; Schreck, 1981), this may be more likely to produce a condition such as SBSS than would a series of brief, acute stresses which occur over a greater period of time. Mazeaud et al. (1977) determined that variations in stress responses were due to different types of stress, and that "this variation in response permits the evaluation of handling techniques on the basis of the induced hormonal response".

The attempt to control the water quality of the experimental tanks, especially during the stress periods, was successful. The emphasis was on producing handling stress only, while minimizing additional stress or alternative stress responses related to diminished water quality (Pickering and Pottinger, 1987).

Histopathological analysis of tissues from SBSS fish failed to reveal any pathology which could be considered diagnostic of the condition.

Bacterial and viral screening did not identify any organisms which were considered primary pathogens.

A tissue homogenate injection test performed on charr of the same strain and age class did not induce the condition.

The fact that numerous cases of SBSS were not observed neither substantiates nor rules out stress as an integral part of the etiology of this syndrome. Regarding the stress to which these fish were subjected, it forces one to consider that another, or at least an additional, factor may be involved in the onset of this condition. There is the possibility that a genetic predisposition may exist. A nutritional explanation seems unlikely, as all fish were on the same diet and feeding program.

This study was unable to substantiate other studies which have indicated that SBSS is a stress-related condition.

The physiological control over the physostome swim bladder has not been adequately described to allow knowledgeable speculation regarding the effect of stress on the swim bladder function. This is a handicap to designing appropriate studies which may shed more light on this condition.

APPENDICES

APPENDIX I. STATISTICAL ANALYSES.

A. ANOVA

| SAMPLING | DESIGNATION | SUM OF SQUARES | DEGREES OF FREEDOM | MEAN SQUARE | F-TEST RATIO |
|-------------|-----------------------|----------------|--------------------|-------------|--------------|
| PCV #1 | Treatment A (Stress) | 884.0859 | 1 | 884.0859 | 34.2277 |
| | Treatment B (Density) | 56.33594 | 1 | 56.33594 | 2.181066 |
| | A X B | 36.7461 | 1 | 36.7461 | 1.422638 |
| | Error | 1136.5 | 44 | 25.82955 | |
| PCV #2 | Treatment A (Stress) | 438.0235 | 1 | 438.0235 | 12.78546 |
| | Treatment B (Density) | 72.52344 | 1 | 72.52344 | 2.116886 |
| | A X B | 0.015625 | 1 | 0.015625 | 0.00045607 |
| | Error | 1507.418 | 44 | 34.2595 | |
| PCV #3 | Treatment A (Stress) | 0.5234375 | 1 | 0.5234375 | 0.02016009 |
| | Treatment B (Density) | 7.523438 | 1 | 7.523438 | 0.2897637 |
| | A X B | 111.0156 | 1 | 111.0156 | 4.275745 |
| | Error | 1142.418 | 44 | 25.96405 | |
| GLUCOSE #1 | Treatment A (Stress) | 72.51563 | 1 | 72.51563 | 0.6400016 |
| | Treatment B (Density) | 44.85938 | 1 | 44.85938 | 0.3959156 |
| | A X B | 2.515625 | 1 | 2.515625 | 0.02220217 |
| | Error | 4985.438 | 44 | 113.3054 | |
| GLUCOSE #2 | Treatment A (Stress) | 1038.938 | 1 | 1038.938 | 3.479683 |
| | Treatment B (Density) | 65.6875 | 1 | 65.6875 | 0.2200052 |
| | A X B | 0.375 | 1 | 0.375 | 0.00125597 |
| | Error | 13137.19 | 44 | 298.5725 | |
| GLUCOSE #3 | Treatment A (Stress) | 135.9688 | 1 | 135.9688 | 0.3519149 |
| | Treatment B (Density) | 1432.25 | 1 | 1432.25 | 3.706956 |
| | A X B | 244.8438 | 1 | 244.8438 | 0.6337057 |
| | Error | 17000.2 | 44 | 386.3683 | |
| CORTISOL #1 | Treatment A (Stress) | 18.57916 | 1 | 18.57916 | 25.037 |
| | Treatment B (Density) | 0.5841217 | 1 | 0.5841217 | 0.7871535 |
| | A X B | 3.910759 | 1 | 3.910759 | 5.270079 |
| | Error | 32.65101 | 44 | 0.7420684 | |
| CORTISOL #2 | Treatment A (Stress) | 5.925576 | 1 | 5.925576 | 6.426368 |
| | Treatment B (Density) | 0.04445648 | 1 | 0.04445648 | 0.04821367 |
| | A X B | 0.6790238 | 1 | 0.6790238 | 0.7364106 |
| | Error | 40.57118 | 44 | 0.9220722 | |
| CORTISOL #3 | Treatment A (Stress) | 8.224533 | 1 | 8.224533 | 10.59763 |
| | Treatment B (Density) | 4.241768 | 1 | 4.241768 | 5.465683 |
| | A X B | 1.281506 | 1 | 1.281506 | 1.65127 |
| | Error | 34.14721 | 44 | 0.7760729 | |

B. Log-likelihood ratio - From Table 3. SBSS Cases.

| | HIGH DENSITY STRESSED | HIGH DENSITY NON- STRESSED | LOW DENSITY STRESSED | LOW DENSITY NON- STRESSED |
|-----------------------|--------------------------|----------------------------------|-------------------------|---------------------------------|
| OBSERVED FREQUENCY | 3 | 4 | 0 | 1 |
| EXPECTED FREQUENCY | 2.7 | 2.7 | 1.3 | 1.3 |

$$G = 4.60517 \left[\sum f_i \log_{10} f_i - \sum f_i \log_{10} F_i \right]$$

G = 3.25 V (degrees of freedom) = K - 1 = 3 P > 0.05

Log-likelihood ratio - From Table 4. Non-SBSS Mortalities.

| | HIGH DENSITY STRESSED | HIGH DENSITY NON- STRESSED | LOW DENSITY STRESSED | LOW DENSITY NON- STRESSED |
|-----------------------|--------------------------|----------------------------------|-------------------------|---------------------------------|
| OBSERVED FREQUENCY | 1 | 2 | 0 | 3 |
| EXPECTED FREQUENCY | 2 | 2 | 1 | 1 |

$$G = 4.60517 \left[\sum f_i \log_{10} f_i - \sum f_i \log_{10} F_i \right]$$

G = 2.6 V (degrees of freedom) = K - 1 = 3 P > 0.05

APPENDIX II. TIME LINE OF MAJOR EVENTS

- Fish placed in tanks- January 31, 1990.
- Feeding began - February 8.
- Water temperature elevated to 5 °C - February 14.
- Initial blood sampling - Non-stressed - March 8.
- Initial blood sampling - Stressed - March 9.
- Weekly stress treatments - March 13.
- Weekly stress treatments - March 20.
- Weekly stress treatments - March 27.
- Weekly stress treatments - April 3.
- Weekly stress treatments - April 10.
- Weekly stress treatments - April 17.
- Weekly stress treatments - April 24.
- Weekly stress treatments - May 1.
- Weekly stress treatments - May 8.
- Second blood sampling - Non-stressed - May 15.
- Weekly stress treatments - Second blood sampling - Stressed - May 17.
- Weekly stress treatments - May 22.
- Weekly stress treatments - May 29.
- Weekly stress treatments - June 5.
- Weekly stress treatments - June 12.
- Weekly stress treatments - June 19.
- Weekly stress treatments - June 26.
- Weekly stress treatments - July 3.
- Weekly stress treatments - July 10.
- Weekly stress treatments - July 17.
- Third blood sampling - Non-stressed - July 23.
- Final weekly stress treatments - Third blood sampling - Stressed - July 24.
- Homogenate injection trial - August 7.
- Weekly stress treatments began in injected and control fish - September 18.
- Weekly stress treatments in injected and control fish - September 25.
- Weekly stress treatments in injected and control fish - October 2.
- Weekly stress treatments in injected and control fish - October 9.
- Weekly stress treatments in injected and control fish - October 16.
- Weekly stress treatments in injected and control fish - October 23.
- Injected fish held for observation until October 30, 1990.

REFERENCES

- Abernethy, J. D. 1972. The Mechanism of Secretion of Inert Gases into the Fish Swimbladder. *Aust. J. exp. Biol. med. Sci.* 50:365-374.
- Alabaster, J. S. and Lloyd, R. Ammonia. In: Water Quality Criteria for Freshwater Fish. Food and Agriculture Organization of the United Nations. Toronto: Butterworths, 1980. 85-102.
- Alexander, R. McN. 1966. Physical Aspects of Swimbladder Function. *Biol. Rev.* 41:141-176.
- Bagarinao, Teodora and Kungvankij, Pini. 1986. An Incidence of Swimbladder Stress Syndrome in Hatchery-Reared Sea Bass (*Lates Calcarifer*) Larvae. *Aquaculture*. 51:181-188.
- Baker, R. F. and Ayles, G. B. 1990. The Effects of Varying Density and Loading Level on the Growth of Arctic Charr (*Salvelinus alpinus* L.) and Rainbow Trout (*Oncorhynchus mykiss*). *World Aquaculture*. 21(1):58-62.
- Ball, E. G., Strittmatter, C. F. and Cooper, O. 1955. Metabolic Studies on the Gas Gland of the Swimbladder. *Biol. Bull.* 108:1-17.
- Barton, B. A., Peter, R. E. and Paulencu, C. R. 1980. Plasma Cortisol Levels of Fingerling Rainbow Trout (*Salmo gairdneri*) at Rest, and Subjected to Handling, Confinement, Transport, and Stocking. *Can. J. Fish. Aquat. Sci.* 37:805-811.
- Barton, B. A., Schreck, C. B., Ewing, R. D., Hemmingsen, A. R. and Patino, R. 1985. Changes in Plasma Cortisol During Stress and Smoltification in Coho Salmon, *Oncorhynchus Kisutch*. *General and Comparative Endocrinology*. 59:468-471.

- Barton, B. A., Schreck, C. B. and Sigismondi, L. A. 1986. Multiple Acute Disturbances Evoke Cumulative Physiological Stress Responses in Juvenile Chinook Salmon. *Trans. Am. Fish. Soc.* 115:245-251.
- Barton, B. A. and Schreck, C. B. 1987. Metabolic Cost of Acute Physical Stress in Juvenile Steelhead (*Salmo gairdneri*). *Trans. Am. Fish. Soc.* 116:257-263.
- Barton, B. A. 1988. Endocrine and Metabolic Responses of Fish to Stress. *Proceedings from the International Association of Aquatic Animal Medicine.* 19:41-55.
- Battino, R. and Clever, H. L. 1966. The Solubility of Gases in Liquids. *Chem. Rev.* 66:395-463.
- Berg, T. and Steen, J. B. 1968. The Mechanism of Oxygen Concentration in the Swim-Bladder of the Eel. *J. Physiol.* 195:631-638.
- Boyd, C. E. Water Quality in Warmwater Fish Ponds. Auburn University Agricultural Experiment Station. 1979. 13-14.
- Brett, J. R. 1958. Implications and Assessment of Environmental Stress. pp. 69-83 in The Investigation of Fish-Power Problems. Ed. P. A. Larkin. H. R. MacMillan Lectures in Fisheries, University of British Columbia, Vancouver, B. C.
- Bry, C. 1982. Daily Variations in Plasma Cortisol Levels of Individual Female Rainbow Trout *Salmo gairdneri*: Evidence for a Post-Feeding Peak in Well-Adapted Fish. *Gen. and Comp. Endocrin.* 48:462-468.
- Carmichael, Gary J. and Tomasso, J. R., 1984. Swim Bladder Stress Syndrome in Largemouth Bass. *The Texas Journal of Science.* 35(4):315-321.
- Chemical Procedures Explained - APPENDIX A. In: Water Analysis Handbook. Ed. Gregg L. Walters. Hach Company, Loveland, Colorado. 1968. 638.

- Clary, J. R. and Clary, S. D. 1978. Swim Bladder Stress Syndrome. Salmonid. March/April:8-9.
- Cook, M. F. and Canton, S. P. 1988. Calculation of Percent Gas Saturation in Water by Use of a Spreadsheet. Progressive Fish Culturist. 50:248-250.
- Copeland, E. 1952. The Histophysiology of the Teleostean Physoclist Swimbladder. J. Cellular. comp. Physiol. 40:317-336.
- D'aoust, B. G. 1970. The Role of Lactic Acid in Gas Secretion in the Teleost Swimbladder. Comp. Biochem. Physiol. 32:637-668.
- Daoust, P.-Y. and Ferguson, H. W., 1984. The Pathology of Chronic Ammonia Toxicity in Rainbow Trout, *Salmo Gairdneri* Richardson. J. Fish Dis. 7:199-205.
- Deck, J. E. 1970. Lactic Acid Production by the Swimbladder Gas Gland *In Vitro* as Influenced by Glucagon and Epinephrine. Comp. Biochem. Physiol. 34:317-324.
- Emerson, K., Russo, Rosemarie C., Lund, R. E. and Thurston, R. V. 1975. Aqueous Ammonia Equilibrium Calculations: Effect of pH and Temperature. J. Fish. Res. Bd. Can. 32:2379-2383.
- Fahlén, G. 1959. Rete Mirabile in the Gas Bladder of *Coregonus Lavaretus*. Nature (Engl.). 184(4691):1001-1002.
- Fahlén, G. 1968. The Gas Bladder as a Hydrostatic Organ in *Thymallus Thymallus* L., *Osmerus Eperlanus* L. and *Mallotus Villosus* Mull. FiskDir. Skr. Ser. HavUnders. 14:199-228.
- Fahlén, G. 1971. The Functional Morphology of the Gas Bladder of the Genus *Salmo*. Acta. anat. 78:161-184.
- Fänge, R. 1953. The Mechanism of Gas Transport in the Euphysoclist Swimbladder. Acta physiol. scand. 30(suppl. 110):102 pp.

- Fänge, R. 1958. The Structure and Function of the Gas Bladder in *Argentina Silus*. Quart. J. Microscop. Sci. 99:95-102.
- Fänge, R. 1966. Physiology of the Swimbladder. Physiol. Rev. 46:299-322.
- Fänge, R. Gas Exchange in the Swimbladder. In: Respiration of Amphibious Vertebrates. Ed. G. M. Hughes. London: Academic Press, 1976. 189-211.
- Fänge, R. The Physiology of the Swimbladder. In: Comparative Physiology. Ed. L. Bolis, K. Schmidt-Nielsen and S. H. P. Maddrell. North-Holland Publishing Company, 1973. 135-159.
- FRED Staff. 1983. Fish Culture Manual. Alaskan Department of Fish and Game, Division of Fisheries Rehabilitation, Enhancement and Development. Juneau, Alaska. 16.
- Hughes, G. M. Air Breathing in Fishes. In: Comparative Physiology of Vertebrate Respiration. Toronto: Heinemann, 1963. 29-39.
- Jasinski, A. 1963. The Vascularization of the Air Bladder in Fishes. Part I. Air Bladder of the Bleak (*Coregonus Albula* L.) and Rainbow Trout (*Salmo Irindeus* Gibb.), and the Ductus Pneumaticus of the Eel (*Anguilla Anguilla* L.). Acta Biologica Cracoviensia. Series: Zoologia. VI:2-31.
- Johnson, D. W. and Katavic, I. 1984. Mortality, Growth and Swim Bladder Stress Syndrome of Sea Bass (*Dicentrarchus Labrax*) Larvae Under Varied Environmental Conditions. Aquaculture. 38:67-78.
- Jones, F. R. H. and Marshall, N. B. 1953. The Structure and Functions of the Teleost Swimbladder. Biol. Rev. 28:16-83.
- Kent, M. L. 1982. Characteristics and Identification of *Pasteurella* and *Vibrio* Species Pathogenic to Fishes Using API - 20E (Analytab Products) Multi-tube Test Strips. Can. J. Fish. Aquat. Sci. 39:1725-1729.

- Kiernan, J. A. Histological and Histochemical Methods: Theory and Practice. Pergamon Press, 1981.
- Kolbeinshavn, Arne and Wallace, Jeff C. 1985. Observations on Swim Bladder Stress Syndrome in Arctic Charr (*Salvelinus Alpinus*), Induced by Inadequate Water Depth. *Aquaculture*. 46:259-261.
- Kuhn, W., Ramel, A., Kuhn, H. J. and Marti, E. 1963. The Filling Mechanism of the Swimbladder. *Experientia*. 19:497-511.
- Lapennas, G. N. and Schmidt-Nielsen, K. 1977. Swimbladder Permeability to Oxygen. *J. exp. Biol.* 67:175-196.
- Ling, N. and Wells, R. M. G. 1985. Plasma Catecholamines and Erythrocyte Swelling Following Capture Stress in a Marine Teleost Fish. *Comp. Biochem. Physiol.* 82(1):231-234.
- Mazeaud, M. M. and Mazeaud, F. Adrenergic Responses to Stress in Fish. In: Stress In Fish. ED. A. D. Pickering. New York: Academic Press, 1981. 49-75.
- Mazeaud, M. M., Mazeaud, F. and Donaldson, E. M. 1977. Primary and Secondary Effects of Stress in Fish: Some New Data with a General Review. *Trans. Am. Fish. Soc.* 106:201-212.
- Meade, J. W., 1985. Allowable Ammonia for Fish Culture. *The Progressive Fish Culturist*. 47(3):135-145.
- Nikinmaa, M. 1982. Effects of Adrenaline on Red Cell Volume and Concentration Gradient of Protons Across the Red Cell Membrane in the Rainbow Trout, *Salmo gairdneri*. *Molec. Physiol.* 2:287-297.
- Pauley, G. B. and Nakatani, R. E. 1967. Histopathology of "Gas-Bubble" Disease in Salmon Fingerlings. *J. Fish. Res. Bd. Can.* 24(4):867-871.

- Pickering, A. D. and Pottinger, T. G. 1987. Poor Water Quality Suppresses the Cortisol Response of Salmonid Fish to Handling and Confinement. *J. Fish. Biol.* 30:363-374.
- Pickering, A. D. and Stewart, A. 1984. Acclimation of the Interrenal Tissue of the Brown Trout, *Salmo trutta* L., to Chronic Crowding Stress. *J. Fish Biol.* 24:731-740.
- Pickering, A. D., Pottinger, T. G. and Christie, P. 1982. Recovery of the Brown Trout, *Salmo trutta* L., from Acute Handling Stress: A Time Course Study. *J. Fish Biol.* 20:229-244.
- Piper, R. G., McElwain, I. B., Orme, L. E., McCraren, J. P., Fowler, L. G. and Leonard, J. R. 1982. Fish Hatchery Management. United States Department of the Interior, Fish and Wildlife Service, Washington, D. C. 14-15.
- Robyt, F. J. and White, B. J. Biochemical Techniques - Theory and Practice. Monterey: Brooks/Cole Publishing Company, 1987. 254-258.
- Rodin, E. Y. and Jacques, S. 1989. Countercurrent Oxygen Exchange in the Swim Bladders of Deep-Sea Fish: A Mathematical Model. *Mathl. Comput. Modelling.* 12(3):389-393.
- Ross, L. G. and Ross, B. Anesthetic and Sedative Techniques for Fish. Institute of Aquaculture, University of Stirling, Stirling, Scotland. 1984. 7.
- Saunders, R. L. 1953. The Swimbladder Gas Content of Some Freshwater Fish with Particular Reference to the Physostomes. *Can. J. Zool.* 31:547-560.
- Scholander, P. F. 1954. Secretion of Gases Against High Pressures in the Swimbladder of Deep Sea Fishes. II. The Rete Mirabile. *Biol. Bull.* 107(2):260-277.
- Scholander, P. F. 1956. Observations on the Gas Gland on Living Fish. *J. Cell. Comp. Physiol.* 48:523-528.

- Scholander, P. F. and van Dam, L. 1953. Composition of the Swimbladder Gas in Deep Sea Fishes. *Biol. Bull. mar. biol. Lab., Woods Hole.* 104:75-86.
- Scholander, P. F. and van Dam, L. 1954. Secretion of Gases Against High Pressures in the Swimbladder of Deep Sea Fishes. I. Oxygen Dissociation in the Blood. *Biol. Bull. mar. biol. lab., Woods Hole.* 107:247-259.
- Scholander, P. F., van Dam, L. and Enns, T. 1956. Nitrogen Secretion in the Swimbladder of Whitefish. *Science.* 123:59-60.
- Schreck, C. B. Stress and Compensation in Teleostean Fishes: Response to Social and Physical Factors. In: Stress In Fish. Ed. A. D. Pickering. New York: Academic Press, 1981. 295-321.
- Shrimpton, J. M., Randall, D. J. and Fidler, L. E. 1990. Factors Affecting Swim Bladder Volume in Rainbow Trout (*Oncorhynchus mykiss*) Held in Gas Supersaturated Water. *Can. J. Zool.* 68:962-968.
- Sigma Environmental Consultants Ltd. 1983. Summary of Water Quality Criteria for Salmonid Hatcheries. 163 pp. Report to Department of Fisheries and Oceans, Vancouver, B. C.
- Smart, G. 1976. The Effect of Ammonia Exposure on Gill Structure of the Rainbow Trout (*Salmo gairdneri*) . *J. Fish Biol.* 8:471-475.
- Steen, J. B. 1962. Action of the Rete Mirabile in the Secretion of Gases by Blood-Acidification in the Swimbladder of the Eel. *Nature, London.* 96:906-907.
- Steen, J. B. 1963. The Physiology of the Swimbladder in the Eel, *Anguilla Vulgaris*. II. The Reabsorption of Gases. *Acta physiol. scand.* 58:138-149.
- Steen, J. B. The Swim Bladder as a Hydrostatic Organ. In: Fish Physiology. Ed. W. S. Hoar and D. J. Randall. Volume 4. New York: Academic Press, 1970. 413-443.

- Strange, R. J., Schreck, C. B. and Golden, J. T. 1977. Corticoid Stress Response to Handling and Temperature in Salmonids. *Trans. Am. Fish. Soc.* 106:213-2
- Sundnes, G. 1963. Studies on the High Nitrogen Content in the Physostome Swimbladder. *Rept. Norweg. Fishery Invest.* 13:1-8.
- Sundnes, G., Bratland, P. and Strand, E. 1969. The Gas Content of the Coregonid Swimbladder. *FrisDir. Skr. Ser. HavUnders.* 15:274-278.
- Sundnes, G., Enns, T. and Scholander, P. F. 1958. Gas Secretion in Fishes Lacking a Rete Mirabile. *J. Exp. Bio.* 35(3): 671-676.
- Tait, J. S. 1956. Nitrogen and Argon in Salmonid Swimbladders. *Can. J. Zool.* 34:58-68.
- Thurston, R. V. and Russo, R. C., 1983. Acute Toxicity of Ammonia to Rainbow Trout. *Transactions of the American Fisheries Society.* 112:696-704.
- Wallace, Jeff C., Kolbeinshavn, Arne G. and Reinsnes, Trond G. 1988. The Effects of Stocking Density on Early Growth in Arctic Charr, *Salvelinus alpinus* (L.). *Aquaculture.* 73:101-110.
- Weber, R. E. Intraspecific Adaptation of Hemoglobin Function in Fish to Oxygen Availability. In: Exogenous and Endogenous Influences on Metabolic and Neural Control. Ed. A. D. F. Addink and N. Spronk. Vol. 1. Oxford: Pergamon Press, 1982. 87-102.
- Weber, R. E., Wood, S. C. and Lomholt, J. P. 1976. Temperature Acclimation and Oxygen Binding Properties of Blood and Multiple Haemoglobins of Rainbow Trout. *J. Exp. Biol.* 65:333-345.
- Wedemeyer, G. 1970. Stress of Anesthesia with MS 222 and Benzocaine in Rainbow Trout (*Salmo gairdneri*). *J. Fish. Res. Bd. Can.* 27:909-914.

- Wedemeyer, G. 1972. Some Physiological Consequences of Handling Stress in the Juvenile Coho Salmon (*Oncorhynchus kisutch*) and Steelhead Trout (*Salmo gairdneri*). J. Fish. Res. Bd. Can. 29:1780-1783.
- Wittenberg, J. B. 1958. The Secretion of Inert Gases into the Swim-Bladder of Fish. J. Gen. Physiol. 41:783-804.
- Wittenberg, J. B. and Wittenberg, B. A. 1961. The Secretion of Oxygen into the Swim-Bladder of Fish. II. The Simultaneous Transport of Carbon Monoxide and Oxygen. J. Gen. Physiol. 44:527-542.
- Wittenberg, J. B., Copeland, D. E., Haedrich, R. L. and Child, J. S. 1980. The Swimbladder of Deep-Sea Fish: The Swimbladder Wall is a Lipid-Rich Barrier to Oxygen Diffusion. J. Mar. Biol. Ass. U.K. 60:263-276.
- Wittenberg, J. B., Schwend, M. J. and Wittenberg, B. A. 1964. The Secretion of Oxygen into the Swim-Bladder of Fish. III. The Role of Carbon Dioxide. J. Gen. Physiol. 48:337-355.
- Wolf, L. E. 1937. An Air Bladder Disease in Lake Trout Fingerlings. Trans. Am. Fish. Soc. 66:359-363.
- Yamamoto, K. 1988. Contraction of Spleen in Exercised Freshwater Teleosts. Comp. Biochem. Physiol. A. 89:65-66.
- Yasutake, W. T. and Wales, J. H. Microscopic Anatomy of Salmonids: An Atlas. Resource Publication/U. S. Department of the Interior, Fish and Wildlife Service; No. 150. 1983.