BIOCHEMICAL AND BEHAVIOURAL INDICATORS
OF SENSORY PERCEPTION IN ATLANTIC SALMON (Salmo salar)

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Faculty of Veterinary Medicine
University of Prince Edward Island

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Charlottetown, P.E.I.
March, 1996

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ABSTRACT

The salmonid industry is rapidly expanding worldwide. Every year thousands of tonnes of Atlantic salmon are produced generating millions of dollars to the aquaculture industry. As one of the most commonly farmed salmonids in Canada, Atlantic salmon are often used in research to enhance the knowledge of the biology of this species. Either in a commercial or research environment, fish encounter a variety of potential stressors including handling, netting, transport, confinement, social stress and probably others that are not currently recognized. Stress can affect the well-being of the fish. The response to stress can be divided into three main categories - primary, secondary, and tertiary, each of which involves different levels of organization. Considerable progress has been made in understanding the response of teleost fish to stressors but the relationship between biochemical and behavioural responses to stress has not been dealt with in much detail. The objectives of this current study were: a) to quantify the biochemical and behavioural responses of Atlantic salmon to different external stimuli, and b) to determine if different classes of stimuli elicit reproducibly distinct responses.

The study was divided into two replicate experiments each employing 18 adult Atlantic salmon (0.9 - 1.5 kg). Each group of 18 was held in artificial seawater and randomly placed in groups of three per section within two fiberglass circular tanks, each of which was subdivided into three sections. Chronic indwelling cannulae were surgically implanted into the dorsal aorta of two fish from each section. The cannula of one fish (experimental) extended to the surface of the water for purposes of repeated blood sampling. The second cannulated fish received a shorter closed cannula and was employed as a replacement fish. The third remaining fish within the section was designated as the control.

Circulating levels of plasma cortisol, catecholamines (norepinephrine and epinephrine) and plasma glucose were measured and analysed during four different time periods: Baseline 1 (before surgery), Post-surgery, Baseline 2 (after surgery), and Experimental. Concurrently, Atlantic salmon movement and interactive behaviours were video-recorded and quantified. Comparisons were then made between behavioural and biochemical data during Baseline 1, Baseline 2, and Experimental periods. The experimental period involved exposing each experimental fish to a non-mechanical (light, formalin) or mechanical (pinch, probe) stimulus according to a random schedule over a five week period.

Evidence of the stressful effects of surgery was observed in the biochemical data. Plasma cortisol and glucose concentrations were elevated significantly following catheterization of the dorsal aorta. Concentrations returned to baseline within 24 hours. Plasma catecholamines were not elevated significantly. There appeared to be a slight increase in epinephrine immediately following surgery, but these data were
characterised by large variability among fish. Baseline concentrations for each biochemical parameter before and after surgery were comparable to baseline data in the literature. Baseline behavioural observations indicated a significant reduction in movement and interactive behaviour after surgery when compared to pre-surgery baseline data. This reduction in behaviour may have implied further acclimation of the fish to their confined environment. Alternatively, the fish may have been displaying phlegmatic behaviour in response to the stress of confinement.

The behavioural and biochemical changes during the experimental period varied according to the stimulus. Plasma cortisol was elevated significantly after light, formalin, and pinch stimulation but did not change in response to the probe stimulus. There was a second peak in plasma cortisol that occurred six hours after introduction of each of the four stimuli and corresponded to the time the lights were turned off during the regular photoperiod. Plasma catecholamine and glucose were not affected significantly by any of the stimuli and remained within baseline concentrations throughout the experimental period. Overall, each external stimulus produced a significant change in behaviour among the treatment groups but different behaviours were seen in association with different stimuli. Changes in movement behaviour were observed in response to light, pinch, and probe stimulus, and changes in displacement interaction were displayed following introduction of formalin.

The results from the biochemical analysis and corresponding behavioural observations indicated that Atlantic salmon responded differently to different external stimuli. Consequently, Atlantic salmon appear to possess a rudimentary form of sensory discrimination. However, the biochemical and behavioural changes observed following each stimulus in the current study were subtle, possibly because of the short duration and relative lack of severity of the stimuli employed. Further research on the relationship between external stimuli and the response of salmonids to stress is clearly required.
DEDICATION

I dedicate this thesis to my parents, Freda and John, and to my husband Marc for all their love, patience and support.
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<th>Description</th>
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<tr>
<td>ACTH</td>
<td>adrenocorticotropic hormone</td>
</tr>
<tr>
<td>ANS</td>
<td>autonomic nervous system</td>
</tr>
<tr>
<td>ASD</td>
<td>asynchronous displacement</td>
</tr>
<tr>
<td>C</td>
<td>control</td>
</tr>
<tr>
<td>°C</td>
<td>degrees celsius</td>
</tr>
<tr>
<td>CM</td>
<td>continuous movement</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CRH</td>
<td>corticotropin releasing hormone</td>
</tr>
<tr>
<td>DHBA</td>
<td>3, 4 dihydroxybenzylamine</td>
</tr>
<tr>
<td>E</td>
<td>epinephrine</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetra - acetic acid</td>
</tr>
<tr>
<td>GAS</td>
<td>general adaptation syndrome</td>
</tr>
<tr>
<td>GC</td>
<td>glucocorticoids</td>
</tr>
<tr>
<td>HCl</td>
<td>hydrochloric acid</td>
</tr>
<tr>
<td>HPA</td>
<td>hypothalamic - pituitary - adrenal</td>
</tr>
<tr>
<td>HPI</td>
<td>hypothalamic - pituitary - interrenal</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>LC</td>
<td>long cannulated fish</td>
</tr>
<tr>
<td>NE</td>
<td>norepinephrine</td>
</tr>
<tr>
<td>PO</td>
<td>pattern of occurrence</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
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</table>
PVC  polyvinyl chloride
SAS  statistical analysis system
SC   short cannulated fish
SD   synchronous displacement
SM   sporadic movement
TMS  tricaine methane sulfonate
1.0 GENERAL INTRODUCTION

Fishes are the most abundant and diverse of all vertebrate animals comprising approximately 23,500 - 28,500 species of a total of 45 - 50,000 extant species of jawed vertebrates (Jobling, 1995a). Figure 1.1 illustrates the general scheme of the phylogeny of fishes. Among the bony fish (Osteichthyes), the teleosts are by far the most numerous, comprising a total of approximately 22,000 species. One of the most primitive groups of the subdivision Teleostei is the superorder Protacanthopterygii, to which the salmonids belong. There are three subfamilies within the family Salmonidae, including the salmoninae; the subfamily that includes the Atlantic salmon (Salmo salar, which translates to "the leaping fish"). In general, salmoninae possess fine cycloid scales, large teeth on the maxillary bone of the upper jaw, and a short dorsal fin (Moyle and Cech, 1988). Atlantic salmon are distinguished from other salmoninae by several features, including the number of fin rays, the narrow caudal peduncle, the shallowly forked caudal fin, anteriorly decurved lateral line that straightens towards the operculum, and the extension of the jaw bone to the level of the rear of the eye (Mills, 1989; Scott and Scott, 1988).

Atlantic salmon is a diadromous species of fish and are referred to as anadromous, spawning in freshwater but migrating to a marine environment to grow and mature. The life history of Atlantic salmon is divided into seven stages; alevin, fry, parr, smolt, post-smolt, salmon, and kelt (Allan and Ritter, 1975). In freshwater, the juvenile salmon usually spends two to four years developing through the first four stages of its life cycle. The transformation to a smolt usually occurs during the late
Figure 1.1: General scheme of the phylogeny of fishes (Jobling, 1995a).
winter and early spring, after which time the fish migrates to the sea for its main feeding phase and subsequent rapid growth period. During migratory periods, some Canadian Atlantic salmon have been known to travel as far as Greenland, whereas others have remained in Canadian rivers throughout their lives (Scott and Scott, 1988). The post-smolt phase takes place from the time of departure from the river until onset of wide annulus formation on the scales which occurs at the end of the first winter in the sea. Beyond this stage, the fish is referred to as a salmon if it has spent two, three, or four years at sea before returning to freshwater. If the salmon has spent only one winter at sea before returning then it is referred to as a ’grilse’. Once in freshwater the salmon will migrate upstream to spawn in gravel covered nests or ’redds’. Canadian Atlantic salmon spawn in freshwater during late fall and early winter. Most males die after spawning but a small percentage of females return to spawn a second time. After spawning the fish is called a kelt until it reaches salt water to feed again.

1.1 Use of Atlantic salmon in Aquaculture

Aquaculture has been practiced for at least 4,000 years in China and almost as long in other parts of the world such as Egypt and Japan. The practice of aquaculture is defined as "the farming or husbandry of aquatic plants and animals, and implicit in the activity is some degree of human intervention" (Boghen, 1995). The first known treatise on aquaculture was written in 475 B.C., by the Chinese author Fan Li. In Europe, the practice of aquaculture was introduced almost 2,000 years ago with developments centering mostly around the culture of carp in ponds.

Salmon aquaculture or salmon farming refers to the rearing of salmon (with reference to the salmonid fishes in general) from the egg to the market (Monahan,
1993). The salmon are reared under controlled and supervised conditions to maintain
the well-being of the fish, minimize stress and promote efficient growth. Salmon
farming began in the late nineteenth century as an offshoot of the trout culture
developed in Denmark (Monahan, 1993). In the mid 1960’s, the Norwegians first
successfully cultured salmon in large sea enclosures, setting the pattern for salmon
culture practiced currently in many parts of the world today (Shearer, 1992). On a
global scale, the major producers of salmon include the UK, Norway, Scotland, Chile,
and Canada (Figure 1.2). Thousands of tonnes of Atlantic salmon are produced in these
countries (Figure 1.3) (Stamatopoulos, 1995). Salmon farming in Canada has grown
considerably over the years since the first successful production in the late 1970s in the
Fundy Isles region (Monahan, 1993). In Canada, the salmon industry generated 245
million dollars out of the total 300 million dollar aquaculture industry in 1993 (Figure
1.4) (Stamatopoulos, 1995). One of the most common salmonid species farmed in
Canada is Atlantic salmon. Atlantic salmon production accounts for approximately 78% 
of the total aquaculture production value in the Atlantic provinces alone, and
consequently, has an impact on the economy in certain parts of Atlantic Canada
(Boghen, 1995).
Figure 1.2: Annual production value (million $ US) for major salmonid producing countries. Values are for 1993. (Source: Fishery Information, Data and Statistic Service, FAO [1993]).
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Figure 1.4: Production value (millions of dollars - Canadian) of aquaculture species in Canada for 1993. (Source: Fishery Information, Data and Statistic Service, FAO [1993]).
1.2 Use of Salmonids in Research

The use of fish in biomedical research can be divided into three broad areas: aquaculture, basic biology and toxicology. For studies that require repeated physiological or pharmacokinetic measurements, fish models such as channel catfish, and rainbow trout are commonly used. Other fish such as the dogfish are employed for studying anatomy and smaller fish, like the swordtail, are used principally for toxicity studies (Bolon and Stoskopf, 1995).

As a common salmonid species in Canadian aquaculture, Atlantic salmon are used more frequently in experimental studies designed to facilitate the aquaculture industry. One of the main areas of aquaculture research focuses on the effects of stress on fish. Routine aquaculture practices such as handling and transport can evoke stress responses in fish resulting in mortality (Barton and Iwama, 1991). In many cases, fish are subjected to intensive culture conditions that impose environmental stresses which may have detrimental effects on the fish (Pickering, 1989, 1993). Understanding the responses of Atlantic salmon to changes in their environment is important to maintaining optimum health of the fish. Other aquaculture research areas such as nutrition, disease diagnosis and treatment, water quality, and developing better cages and nets, are vital to increasing productivity. Fish have also been described as excellent model systems for biomedical research in areas such as endocrinology (Sherwood and Parker, 1990; Chiappelli et al., 1993; Winborg and Nilsson, 1993) and neurobiology (Schwartz et al., 1985; Cohen et al., 1990). One of the most useful characteristics of fish is the regenerative capability of their central nervous system (CNS) (Bernstein, 1970). Accordingly, regeneration of the CNS in fish has had a
direct impact on neurobiological developments in mammals. For example, regenerating fish optic nerves have been shown to produce substances that induce regenerative responses in the CNS of mammals (Schwartz et al., 1985; Cohen et al., 1990).

Due to the increasing pollution of aquatic resources, fish are excellent models for the study of environmental toxicity (Beitinger and Freeman, 1983; Rand and Petrocelli, 1985; Doving, 1991), although Atlantic salmon are rarely the species of choice for such studies.

Fish are unique from most vertebrates in that they live their entire life in an aqueous environment. Consequently, when studying fish experimentaly, it is important to have a thorough understanding of the interaction of fish with their environment. Awareness of this relationship will help ensure that proper control of experimental conditions and appropriate husbandry practices are met.

1.2.1 Housing Considerations

Fundamental decisions to appropriately house fish include the design of the laboratory tank and the water supply. Holding systems for fish in a laboratory environment vary from a laboratory stream comprised of an oval shaped tank to rectangular aquaria. Most often, though, circular tanks are used for laboratory studies (Stickney and Kohler, 1990), and rectangular tanks and raceways are mostly employed for rearing small fry and fingerlings in the hatchery building (Piper et al., 1986a). In a circular tank, the main group of fish tends to congregate near the edge with only a few fish in the center. Fish housed in a rectangular tank, however, form a central "ball" moving in a counterclockwise direction and fewer fish move along the perimeter of the tank (Lapkin et al., 1989). The hydrodynamics involved in the two tanks indicate that
circular tanks provide the optimum water recirculation in a closed system (Piper et al., 1986a) and the most efficient use of a given volume of water (Rosenthal, 1987). If the flow is running along the edge of the tank, a vortex is created allowing good mixture of the inflow and resident water (Goodrich, 1992).

Sources of water supply to experimental tanks vary depending on the facility. Examples of different water sources include a well, deep spring, and domestic water line. In the case of housing saltwater species, such as Atlantic salmon, salt must be added to the system. Water exchange through the system can either be recirculated (closed) or flow through (open). The recirculating system is not as highly favoured as the flow through system because, in the latter, water is only used once. However, in facilities where water supply is limited or during experiments when fish are kept under quarantine conditions, or radioactive substances are employed, recycling water systems are more suitable.

The ergometrics of the fish must also be considered in the design of the experimental tank. In laboratory studies, fish are often housed in small aquaria under conditions of chronic confinement. Appropriate stocking density for salmonids is an important consideration when establishing optimum husbandry conditions. High stocking densities have been shown to affect hematocrit and plasma cortisol levels in both wild and hatchery reared chinook salmon (Mazur and Iwama, 1993). Salmonids, such as Atlantic salmon, display territorial behaviour in aquaria and are considered a very interactive species (Keenleyside and Yamamoto, 1962). Fish under these restrictive conditions establish stable, linear hierarchies (Noakes and Leatherland, 1977). However, social position has an effect on the physiological condition of the fish such
that the dominant fish are relatively devoid of socially imposed stress and the subordinates are stressed (Ejike and Schreck, 1980). Housing fish at certain stocking densities can result in weight loss and, in some cases, death of the more subordinate fish (Pottinger and Pickering, 1992). Therefore, adequate space for basic behavioural activities must be provided and appropriate stocking density determined in order to alleviate the possibility of stress in the fish.

Circular tanks provide the greatest carrying capacity as compared to troughs, rectangular tanks, and raceways, if sufficient water pressure for aeration is supplied (Piper et al., 1986b). The preferred stocking density or carrying capacity for fish held in restrictive holding facilities is defined as the animal load that an aquarium can support (Spotte, 1979). The formula for calculating the carrying capacity takes into account water flow, volume, exchange rate, temperature, oxygen content, pH, size and species of fish, and the accumulation of metabolic wastes (Piper et al., 1986b). There are two indices that are considered to determine carrying capacity, namely flow index and density index. The density index defines the spatial relationship of one fish with another and the flow index refers to the amount of oxygen available for life support and growth (Piper et al., 1986b). Both indices are critical for providing an adequate oxygen supply and preventing overcrowding. The carrying capacity is dependent on several factors including the type of system, whether it is flow through or recirculation, fresh or seawater, and the species of fish. The water flow rate into a tank should be reported as a volume over time rate (eg. liters per minute) and the volume of water the flow is going into (Goodrich, 1992). However, this is not always the case. Spotte (1979) stated that the flow rate of any aquarium system should be a minimum of 0.7
x 10^3 m·sec\(^{-1}\) and Goodrich (1992) found that the general consensus for the recommended water flow was 1 liter per minute per kilogram of biomass. Regardless of the method used, it is important to maintain the dissolved oxygen level near saturation at all temperatures and salinities (Spotte, 1979). Carrying capacity is generally measured as a volumetric density in lb·ft\(^3\)·l, kg·m\(^3\)·l or g·L\(^{-1}\) indicating the mass of the animals per volume of the rearing unit (Piper et al., 1986b).

### 1.2.2 Water Quality

Close monitoring of the water quality conditions is essential for maintaining optimum health of the fish in an experimental environment. Changes in water quality not only have an impact on the animals, but can also directly confound the experiment. Important aspects of water quality in a recirculating system include the un-ionized ammonia, dissolved nitrogenous oxygen, pH and alkalinity, salinity, temperature and particulates. In most systems, dissolved wastes are controlled using biological filters which facilitate the conversion of ammonia to nitrite and then to nitrate, thereby detoxifying nitrogen wastes. In addition, sandfilters or microscreen filters are essential to filter out the debris in the water before return to the tank.

To date, there are no official water quality standards established for salmonids in particular. However, there are recommendations for maintaining water quality parameters for freshwater (Piper et al., 1986a) and recommendations for seawater (Huguenin and Colt, 1989) systems. Generally for salmonids, dissolved oxygen concentrations should be maintained at 5.0 mg L\(^{-1}\) or higher, total ammonia concentrations up to 0.5 mg L\(^{-1}\) are considered safe (Stickney and Kohler, 1990), and temperature should be maintained between 6-15°C (Novotony and Nash, 1995). Water
temperature should not exceed 22°C or fall below -0.8°C as this can be lethal to the fish (Novotony and Nash, 1995).

Monitoring water quality for salmonids in research facilities or in any aquatic environment is essential in maintaining optimum health of the fish. Any drastic fluctuation in water quality can lead to serious repercussions resulting in stress to the fish and, in some cases, mortality.

1.2.3 Animal Welfare

As the aquaculture industry expands, the need for further research in areas such as housing facilities, water quality, and husbandry techniques for fish are fundamental to improving the quality of the industry and to increasing our knowledge of the biology of fish. With the use of any animal in research there are always concerns about the health and welfare of the animal (Needham and Lehman, 1991). In Canada, the Canadian Council on Animal Care (CCAC) is devoted to improving the care and use of animals in research, teaching, testing, and production, and provides appropriate guidelines for several different areas related to the use of fish, including transportation, water quality, and accommodation and management (CCAC, 1984). The Scientists Center for Animal Welfare (SCAW) is another association that promotes responsible treatment of laboratory and wild animals involved in research, testing and education (Schaeffer et al., 1992).

The main concern associated with the care of fish is the stress component (Kleinow, 1992). The biochemical response to stress in fish has been well documented for salmonids and other important aquaculture species in (Mazeaud et al., 1977; Donaldson, 1981; Mazeaud and Mazeaud, 1981) and for some new fish species that are
being introduced to the industry, such as the golden perch (*Macquaria ambigu*a) (Carragher and Rees, 1994). The knowledge gained from these studies has led to the development of practical guidelines for the management of the stress response (Davis, 1992; Pickering, 1992). Other components considered important in maintaining healthy fish include nutrition, water quality, housing, handling and experimental procedures (Kleinow, 1992). Ideally, the standards that are established for the use of fish in aquaculture and research should be uniform and should provide a good quality of life for the fish (Needham and Lehman, 1991).

1.3 Sensory Systems in Teleosts

The sensory systems of fish are known to involve photoreception, mechanoreception, and chemoreception (Hoar and Randall, 1971). These sensory modalities are in continuous interaction with the environment and enable the fish to both, respond immediately to a stimulus if necessary, and to encode information that is useful in the long term. Sensory systems in fish, as in all vertebrates, rely on external stimuli undergoing transduction and conveyance to the CNS which coordinates the behavioural and physiological responses of the fish.

1.3.1 Overview of Neural Systems in Fish

Fishes have a well developed nervous system which serves to coordinate the functions of all of their other systems. The fish nervous system is comprised of three closely related components; central, autonomic, and somatic (peripheral).

1.3.1.1 Central Nervous System

The CNS consists of the brain and the spinal cord and functions to regulate and modulate behavioural, hormonal, and immune responses of the fish. It contains neurons
and non-neuronal elements such as neuroglia, endothelia, and ependyma (Bernstein, 1970). One of the unique and intriguing properties of the CNS in fish is its ability to regenerate. Removal of part of the CNS in fish leads to the reconstitution of the vacuous area, often with the return of normal function (Bernstein, 1970). The ability of the fish CNS to regenerate immediately after injury has been attributed to the presence of soluble substances derived from regenerating nerves. Studies have shown that soluble substances, derived from regenerating optic nerves of fish, enable regenerative growth of the axons of injured adult rabbit optic nerves (Schwartz et al., 1985; Cohen et al., 1990). It has been postulated that the soluble substances contain inhibitory/cytotoxic factors that permit regeneration of the optic nerve, although, the mechanisms are not understood (Cohen et al., 1990).

All vertebrate brains have three major divisions: the hindbrain, midbrain and forebrain (Northcutt, 1988). The hindbrain is subdivided into the medulla (mylencephalon) and the metencephalon. The midbrain, or mesencephalon, consists of dorsal centers associated with the visual and octavolateralis systems and ventral centers that function as relay centers with other parts of the brain. In non-mammalian species, there is a dorsal enlargement of mesencephalic tissue called the optic tectum which is directly innervated by the ganglion cells of the retina. The caudal midbrain roof is comprised of the posterior colliculus in mammals and the torus semicircularis in non-mammals and receives information from the medullary centers associated with the octavolateralis nerves. The forebrain, or prosencephalon, is separated into the diencephalon and the telencephalon. The diencephalon is comprised of the hypothalamus, epithalamus and the thalamus. The hypothalamus in all vertebrates
consists of several nuclei that affect the activity of the preganglionic motor neurons of the autonomic nervous system and regulate endocrine glands. The telencephalon in all vertebrates appears as two laterally situated lobes, or telencephalic hemispheres, and rostrally paired olfactory lobes (Northcutt and Davis, 1983; Northcutt, 1988). Interestingly, however, the topography of the cell groups constituting the roof or pallium of the telencephalon in ray-finned fishes is different from that of other vertebrates (Northcutt, 1988). The functional significance of this difference is unknown.

1.3.1.2 Autonomic Nervous System

The autonomic nervous system (ANS) is primarily an efferent system that transmits impulses to the viscera and endocrine organs. However, there are autonomic nerves that contain afferent fibres from receptors of internal organs (Jobling, 1995b). The major site of integration of the ANS is the hypothalamus, which coordinates functions of the ANS and inputs from the somatic nervous system. There are two subdivisions of the peripheral ANS termed parasympathetic and sympathetic. Both divisions consist of preganglionic nerve fibres that synapse with postganglionic fibres in the nerve ganglia, but the anatomical features and function of each division are distinct, in that, the neurotransmitters are different and responses produced are often opposite (Jobling, 1995b).

The sympathetic nervous system outflow is from the spinal cord where short preganglionic nerve fibers synapse on postganglionic nerve fibers in ganglia that are linked together in a chain-like formation. The postganglionic nerve fibres extend from the ganglia and innervate various tissues and organs. In teleosts, the sympathetic chains are connected longitudinally, similar to what is found in higher vertebrates, although
in fish the sympathetic chain extends into the head and connects with several cranial nerves (Nilsson, 1984). Within the sympathetic system, the synapses within the ganglia are cholinergic and the postganglionic terminals employ catecholamines (epinephrine and norepinephrine) (Nilsson, 1984). The receptor sites for the catecholamines are referred to as adrenergic, and can be divided into α and β receptors. Both receptor types appear to be functionally diverse (Jobling, 1995b), although often the α-adrenoreceptor response is associated with excitation and the β-adrenoreceptor with inhibition (Nilsson, 1984). There are two types of adrenergic cells found in all vertebrates: the adrenergic neuron and the chromaffin cell. The main difference between the two is that chromaffin cells release catecholamines via the blood to the target organ, whereas, adrenergic neurons release the catecholamine neurotransmitter directly onto the effector cells (Nilsson, 1984). In teleosts, the chromaffin tissue is mainly found within the head kidney, lining the walls of the posterior cardinal veins (Nilsson, 1984). In addition to this, chromaffin tissue has also been located in small clusters closely associated with the sympathetic chain ganglia (Nilsson, 1984).

The parasympathetic preganglionic nerve fibres in fish extend from the brain to various regions of the head as part of cranial nerves III, V, VII, and IX. The majority of the outflow, though, is from the vagus nerve (cranial nerve X). The parasympathetic ganglia lie in close proximity to the organs they innervate. Therefore, the main distance between the CNS and the periphery is transversed by the preganglionic parasympathetic fibres (Jobling, 1995b). The neurotransmitter of the parasympathetic system is acetylcholine which is released at the ganglia and at the terminals of postganglionic
neurons on the target organs. Consequently, the transmission at both synapses, pre and postganglionic, is said to be cholinergic (Jobling, 1995b).

1.3.1.3 Somatic Nervous System

Fish, like other animals, are continuously receiving stimuli from their environment that provides information for either an immediate or long term response. The somatic nervous system is comprised of afferent nerves that transmit inputs from sense organs to the CNS and efferent nerves that carry information from the CNS to the skeletal muscles. Consequently, much of the activity of the somatic nervous system is under voluntary control (Jobling, 1995b). The following sense organs; the eye, ear and lateral line system, and chemoreception, are described briefly with respect to anatomy, organization of neuronal inputs to the CNS, and effects on behaviour.

1.3.1.3 (i) The Eye

The structural anatomy of the eye of a fish is similar to that found in terrestrial species. Fish eyes contain a cornea, an iris, a lens and a retina, however, differences within the fish eye are necessary to function properly in an aqueous environment. One principal difference is the shape of the eye. Fish have a highly refractive spherical lens that protrudes through the pupil. In order to focus properly, the lens is pulled back towards the retina by the retractor lentis muscle. Due to this structural difference in the fish eye, photomechanical movements are necessary to control light intensity at the retinal level (Brett, 1957; Munz, 1971). The retina of salmonidae is of the classical teleostean type (Ali and Anctil, 1976). The visual cells contain rods, single cones and equal double cones which are arranged in regular patterns and undergo retinomotor responses. Adaptation to light or dark conditions is accomplished by the synchronized
movements of the rods, cones, and pigment granules. The rates of adaptation to light and darkness vary among the salmonids. For example, the total time of light and dark adaptation for sockeye salmon is 20–25 minutes and one hour respectively (Brett and Ali, 1958). The retinal index for Atlantic salmon shows light-adaptation occurring in 60 minutes and dark adaption in 70 minutes (Ali et al., 1961). Ali (1962) found that the light intensity does influence the rate and state of adaptation, whereby, Atlantic salmon were reported to adapt more rapidly to light when exposed to high intensities and, following a preliminary exposure to bright light, take longer to adapt to the dark.

The retina responds to light and relays the message to several different areas in the brain via the optic nerve (Holmqvist et al., 1994). The major site of retinal input, however, is the optic tectum. The tectum opticum or mesencephalic roof, is the largest visual center and forms the bulk of the midbrain roof in fish (review by Ingle, 1988). Activation of the retinal map is through retinal input and descends by way of efferent pathways to the brain stem and spinal motor centers. The response types of the single ganglion cells of the retina are either 'on' or 'off' type creating several types of organization within the 'receptive field' of the retina.

The outputs of tectal neurons have an impact on the motor responses of the fish (review by Vanegas, 1983). Tectal stimulation can elicit body flexion, turning, and affect feeding and avoidance behaviour. A study by Springer et al. (1977), suggests that the tectum is important in the perception of form in fish. For example, removal of the bilateral tectum had resulted in the loss of attack behaviour in response to small black objects, and escape response to a net swept nearby.
1.3.1.3 (ii) Inner ear and Lateral line system

Sound detection in fish is achieved through the inner ear and the lateral line systems, referred to as the "octavolateralis". Fishes do not have an outer or middle ear. However, they do have an inner ear that is similar to higher vertebrates. The current understanding of the role of the inner ear is that many of the end organs are multifunctional such that there is no clear division between vestibular and auditory parts within the ear (Popper and Platt, 1993). The teleost ear has three semicircular canals which are arranged orthogonally. The semicircular canals respond to angular accelerations of the head and the otolith organs respond to linear acceleration. The otolith organs, utricleus, saccus, and lagena are located above a sensory membrane or macula containing many mechanoreceptive cells (Hawkins, 1986). All of the end organs within the inner ear are innervated by branches of cranial nerve VIII.

In general, hearing for fish is confined to the frequency range between 150 and 350 Hz (Fay, 1978). For salmon in particular, hearing is restricted to frequencies below about 380 Hz and above around 150 Hz (Hawkins and Johnstone, 1978). However, Knudsen et al. (1992), have shown that low frequency stimulation (5-10Hz) can evoke an awareness reaction in juvenile Atlantic salmon, indicating that such low frequencies may cause distress to the fish.

The function of the lateral line system has been under discussion for many years. Recently, however, there is increasing evidence to support the role of the lateral line as a 'hydrodynamic receiver' (Hassan, 1989) or a detector of low frequency motions. In comparison to the ear, the lateral line responds to relative movement between the fish and the surrounding water at very close distances, less than a few centimeters away.
These functions enable the fish to identify and localise stationary and moving objects as they swim. The lateral line plays a role in other behavioural responses including predator avoidance and capturing prey, intraspecific communication, and schooling (Bleckmann, 1986).

There are two classes of mechanosensory organs within the lateral line system in teleosts: superficial neuromasts and canal neuromasts. In fish, the mechanoreceptors of the ear and the neuromasts of the lateral line system are structurally similar (Flock, 1971). Each hair cell is comprised of a bundle of stereocilia and one asymmetrically placed kinocilium. In the lateral line system, the hair cell cilia is embedded in a gelatinous cupula that is situated at the bottom of a canal (canal neuromasts) or on the body surface (superficial neuromasts). Stimulation of the hair cell is by mechanical force applied to the distal end of the hair bundle. Depolarisation of the hair cell occurs when the hair bundle is displaced towards the kinocilium. Displacement in the opposite direction results in hyperpolarisation or an inhibitory response.

Anterior and posterior lateral line afferent nerves carry mechanoreceptive information to both the medulla and the cerebellum (McCormick, 1989). Several regions within the cerebellum receive input from fibers of the ascending mechanosensory bundles. The majority of such input is directed to a subdivision of the vestibulolateral lobe called the eminentia granularis (McCormick, 1989). As the lateral line fibers enter the medulla, they are separated into ascending and descending bundles. The dorsal portion of the medulla is involved in processing first-order information mainly from the lateral line system and the inner ear. The nucleus medialis or
intermedius comprises the bulk of this column. Ventrally, the octavus column receives information from the inner ear via the eighth nerve (McCormick, 1989).

1.3.1.3 (iii) Chemoreception

Chemoreception is important for survival of the fish in that it governs fish behaviours such as feeding, reproduction, predator avoidance, and migration. Detection of chemical stimuli in fish is through two major channels, olfaction (smell) and gustation (taste). The distinction between the two is determined by innervation to the CNS. Chemical information transmitted by the cranial nerve I is termed olfaction; information transmitted by the cranial nerves VII, IX, and X is termed gustation (Marui and Caprio, 1992). In addition, there is a differentiated epithelial sensory cell system composed of solitary chemosensory cells located in the external skin and oropharyngeal epithelium of teleosts. Innervation of these sensory cells is not fully known, however, they appear to be innervated by the facial nerve (VII) and/or the spinal nerves (review by Hara, 1993).

The function of the chemoreceptive system is to detect chemical stimuli and transform them into electrical signals that convey the information to the CNS. Olfactory and gustatory systems in fish are not easily distinguishable as both are activated by water soluble substances. However, the current understanding is that gustation is involved in detection, selection, and ingestion of food and protection against noxious substances, and olfaction is involved in a generalized alerting response and specific pheromonal responses (Marui and Caprio, 1992). It is generally accepted that chemoreception begins when a stimulus molecule binds to a specific membrane receptor site on the cilia or microvilli of the receptor cell and subsequently, the message is
passed on to the CNS (review by Hara, 1993). Initially, researchers studied the electrical activities of the olfactory tract in fish by exposing them to various chemicals that were odorous to humans (Hara, 1971). Today, studies have shown that the olfactory system of different species of fish are capable of detecting amino acids, steroids, and prostaglandins (review by Hara, 1993). The investigation into new chemostimulatory substances was prompted in part by the work of Sutterlin and Sutterlin (1971), who demonstrated that olfactory receptors in Atlantic salmon are highly sensitive to amino acids. Similarly, early studies of the gustatory system employed stimulants like salt, sugar, and acid that were used for humans (Hara, 1971). Following the work on amino acid stimulation of the olfactory system, amino acids and other chemical substances have since been found to have a stimulatory effect on the gustatory system of various fish species (review by Hara, 1993).

Chemosensory information is transmitted to the brain and is integrated into behavioural patterns. The three main chemosensory influenced behaviours include chemical signals, feeding, and homing migration in salmon (Hara, 1992). There are two hypotheses that account for homing ability of salmon. One is the imprinting hypothesis which postulates that the waters from home streams contain odours characteristic of the area to which the young salmon are conditioned or imprinted. Upon migration to spawn, they recognize these odours and orient themselves towards their spawning grounds (Stalbell, 1992). The other is the pheromone hypothesis that postulates that the homing of salmon to native spawning grounds is under genetic influence. This hypothesis is based on the assumption that a population of salmon will emit strain
specific substances or pheromones which are detected by the olfactory sense and produce an attraction behaviour in the recipient (Stalbell, 1992).

In salmonids, the paired olfactory organs are located on the dorsal side of the head. There is no direct contact between the olfactory and respiratory systems; as the fish swims, the water passes through the anterior and exits through the posterior naris (review by Hara, 1993). Within the nasal cavity is a series of mucosal folds or lamellae that extends from the floor of the cavity into a rosette formation. There is a secondary folding of lamallae in many species of salmoniformes. The lamellae are comprised of two layers of epithelium that are separated into two regions; sensory and non-sensory. The synaptic organization of the olfactory system is generally the same throughout the vertebrates (Oka et al., 1982). The receptor cells of the sensory epithelium, supporting, non-sensory, and basal cells, innervate the olfactory bulb. Information from the olfactory bulb is transferred to the telencephalic hemispheres through the olfactory tract which consists of lateral and medial bundles. Fibers from these bundles either project to the \textit{dorsalis telencephali} or the \textit{ventralis telencephali}, respectively, or cross in the anterior commissure to the contralateral terminal field (Oka et al., 1982).

Taste buds are located in the oral cavity and pharynx, on the gill rakers and gill arches in teleosts. There are three types of cells within the taste bud namely the gustatory (sensory), basal, and supporting cells. The main part of the taste bud is comprised of the sensory epithelium which consist of the gustatory and supporting cells. The basal cells are situated at the base of the taste bud and although their function is unclear, they are thought to be interneurons and may possess a mechanoreceptive function. The gustatory cells synapse with afferent nerve fibers and transmit information.
to the CNS through the facial (VII), glossopharyngeal (IX), and the vagal (X) nerve. Each nerve relays information from a different part of the body. The facial nerve transmits from the extraoral surface, the glossopharyngeal from the anterior part of the oral cavity, and the vagal nerve from the oropharynx (Kanwal and Finger, 1992). The facial and vagal nerves terminate in the medulla in the facial and vagal lobes and the glossopharyngeal nerve terminates in the dorsal medullary region between the two lobes (review by Hara, 1993). The extraoral part of the gustatory system is able to detect chemical stimuli at a distance serving as a food localizer. The oral and vagal parts have a sensorimotor function, providing the selective component of food ingestion (Kanwal and Finger, 1992).

1.4 Biological Stress

In an aquaculture or research environment, various stressors are encountered that can affect the well being of the fish. Stress in fish is not only caused by mechanical disturbances such as handling (Barton et al, 1980; Pickering et al., 1982; Patino et al., 1987) and netting (Specker and Schreck, 1980; Barton and Peter, 1982), but, can be a result of changes in the environment such as reduction in dissolved oxygen (Kramer, 1987) and tank lighting conditions (Malison and Held, 1992). The reaction of biological stress was first described by Hans Seyle in 1936 and subsequently became known as the "General Adaptation Syndrome" (GAS) (Seyle, 1973). Seyle (1973) defined stress to be "the nonspecific response of the body to any demand made upon it", a definition that is still widely accepted today. The first stage of the GAS is an alarm reaction that involves a rapid physiological response, followed by the second stage called resistance. Under conditions where the stress is too severe and homeostasis
is not achieved, the third stage is reached resulting in exhaustion. Although, the GAS was developed for humans and rodents, it can be applied to other vertebrates including fish (Pickering, 1981). One of the concerns with this type of response in fish was that it was too generalized and a reaction of this type would only be evident under some form of fright, discomfort or pain (Schreck, 1981). The more generally accepted stages of the response to stress in fish are: recognition of the stimulus as being stressful, the actual response to the stressor, and finally the consequences of the stress (Barton and Iwama, 1991). The response to stress can be divided into three main categories; primary, secondary, and tertiary. The primary response involves an immediate neuroendocrine change. Physiological, biochemical and immunological changes often occur as a secondary response and in the event of a chronic stress, such as prolonged confinement, the fish may undergo tertiary or "whole animal" changes including immunosuppression, reduced growth and reproductive dysfunction. Depending on the intensity and duration of the stressor the response may be adaptive or maladaptive (Pickering, 1993). An example of this is the elevation in cortisol in response to handling stress resulting in an adaptive response. This is beneficial in the short-term. However, under prolonged confinement cortisol levels remain elevated and may subsequently lead to immunosuppression (Pickering and Pottinger, 1989).

Considerable progress has been made in recent years in the understanding of the response of teleost fish to stressors encountered in the aquaculture environment (Pickering, 1981; Barton and Iwama, 1991). In response to a noxious or potentially threatening situation, adjustments at all levels of organization are required to maintain
homeostasis in the fish. The following is a brief description of the biochemical and behavioural changes associated with stress in fish and mammals.

1.4.1 Hormonal Response to Stress

The endocrine system involves the release of hormones from the hypothalamus. Input signals from peripheral sense organs are transmitted to centers in the brain that ultimately connect with the hypothalamus. Stimulation of the hypothalamus initiates the release of hypothalamic hormones and messenger substances, which in turn activate the release of hormones from the pituitary (Ball and Baker, 1969; Jobling, 1995b). There are two types of hormones released from the pituitary that are distinguished on the basis of their sites of synthesis and release; the neurohypophyseal peptides and the adenohypophyseal peptide hormones. Examples of the former include vasopressin and oxytocin and the latter include gonadotropins, growth hormone and adrenocorticotropic hormone (ACTH), and prolactin. The pituitary hormones not only function to release hormones from other endocrine tissue that act on target organs, but have direct action themselves and also have a direct feedback effect on the hypothalamus. This feedback system acts as a control mechanism for the release and synthesis of hormones into the system (Ball and Baker, 1969; Jobling, 1995b).

Environmental stimuli induce neuroendocrine responses that can lead to the activation of the hypothalamic-pituitary-adrenal (HPA) axis in mammals. Corticotropin-releasing hormone (CRH) has been identified, as a 41 amino-acid peptide located in the paraventricular nucleus. The release of CRH from the hypothalamus stimulates both the synthesis and secretion of ACTH from the pituitary. ACTH is transported through systemic circulation to the adrenal cortex and stimulates production and secretion of
glucocorticoids (GC) into the bloodstream. In humans, the primary GC secreted is cortisol, but in other species corticosterone may be more prevalent or, in some species, a mixture of both can be secreted (Malven, 1993a). The pituitary is highly responsive to circulating concentrations of GCs, whereby, elevated circulating concentrations of GC feedback to the hypothalamus and the pituitary to lower CRH and ACTH and subsequently GC secretion (Chiappelli et al., 1993). In mammals, cortisol is often released in response to stressful stimuli and is involved in carbohydrate metabolism and functions to counteract the hypoglycemic effects of insulin.

In addition to the HPA axis, the general sympathetic and the sympathomedullary systems also play a significant role in the physiological response of an animal to stressful stimuli (review by Johnson et al., 1992). In response to acute stress, epinephrine (E) and norepinephrine (NE) are released from the adrenal medulla, as well as from the sympathetic nerve terminals (NE only). More chronic stress results in changes in the adrenal medulla characterized by increased enzymatic activity involved in catecholamine biosynthesis, increased rates of synthesis and elevated tissue concentrations of catecholamines. Discharge of E and NE directly into the bloodstream allows for rapid actions on various organs throughout the body. An example of one of the responses to an emergency situation is glycogenolysis, whereby, glycogen stored in the liver and in the skeletal muscle is broken down to generate glucose. This process is mediated by adrenal-derived epinephrine (Malven, 1993b).

The hormonal response to stress in mammals and fish is similar. The diencephalon is a complex brain region that lies caudal to the telencephalon and rostral to the mesencephalon. It is the part of the brain that is directly involved in the primary
neuroendocrine stress response of teleosts. In teleosts, the hypothalamus appears to be
the major center for the union of information coming from the telencephalon and also
receives afferent input from the nerve fibers of the gustatory regions and the
octavolateralis system (Bernstein, 1970). Neurohormones secreted from the
hypothalamus function to regulate the endocrine activity of the adenohypophysis of the
pituitary. Activation of the Hypothalamic-Pituitary-Interrenal (HPI) axis (Figure 1.5)
is initiated by stimulation of the release of ACTH from the pituitary gland. The
stimulatory action of the hypothalamus on the pituitary is through CRH (Fryer and
Peter, 1977 a,b; Lederis et al., 1994). Following stimulation of the hypothalamus in
response to stress, a cascade of events takes place whereby the release of ACTH from
the pituitary activates the production and release of corticosteroids, such as cortisol,
from the interrenal tissue. Activation of the HPI-axis is paralleled by the activation of
the sympathetic nervous system, mediated as well through the hypothalamus.

The interrenal tissue of most teleosts is found in the pronephric part of the
kidney, or head kidney, lining the walls of the posterior cardinal veins (Nilsson, 1984).
Interrenal tissue in fishes is homologous to the adrenal gland in mammals and is
considered primarily as a steroid producing tissue. However, intermingled amongst the
interrenal cells are chromaffin cells that produce catecholamines and are homologous
to the adrenal medulla. Both cortisol and catecholamine secretion is stimulated in
response to many stressful stimuli from handling, confinement, and other physical
disturbances to toxicants and deteriorating water quality conditions (Barton and Iwama,
Figure 1.5: Overview of pathways involved in the stress response of fish to external stimuli.
The release of cortisol and catecholamines into blood circulation is referred to as the primary neuroendocrine responses to stress (Donaldson, 1981; Mazeaud and Mazeaud, 1981). Release of catecholamines into the blood stream can result in a systemic vasoconstriction and a branchial vasodilation (usually following a short acting vasoconstriction). As a result of this, an overall increase in surface area of the gill vasculature occurs. Catecholamine release can also modify blood flow in teleosts by increasing cardiac stroke volume, however, the effects vary depending on the species (Matty, 1985). The dominant stimulus for catecholamine mobilization is believed to be the lowering of blood O₂ content. Elevated plasma catecholamine concentrations play a role in improving blood oxygen transport by increasing the affinity of haemoglobin for oxygen (Thomas and Perry, 1992). During hypoxic conditions, cortisol also plays a role in increasing blood oxygen carrying capacity by increasing the number of erythrocyte β-adrenoreceptors, subsequently enhancing the response of the erythrocytes to catecholamines (Reid and Perry, 1991). Cortisol has also been implicated in mediating changes in hydromineral balance and carbohydrate metabolism (Mazeaud et al., 1977). However, the importance of cortisol in modifying carbohydrate metabolism in fish is not fully understood. Elevations in plasma glucose have been demonstrated by Barton et al. (1986), Brown et al. (1989), and Vijayan and Leatherland (1989), although not all studies were able to associate an increase in plasma glucose with elevated cortisol (Anderson et al., 1991; Soengas et al., 1992).

1.4.2 Behavioural Response to Stress

The behavioural response of mammals to stress involves several different hormones that respond to the stimulus as a means of maintaining homeostasis.
Hormones do not in actuality cause behavioural changes but they influence the sensory systems, CNS, and the effectors (muscles, organs, etc.) (Nelson, 1994). In mammals, intracerebroventricular injections of CRH in rats will induce dose dependent behavioural actions including grooming, sniffing and locomotion (review by Johnson et al., 1992). CRH has also been implicated as a mediator of stress-related suppression of appetite or food intake (Johnson et al., 1992). The release of ACTH from the pituitary is believed to enhance attention, motivation and learning in rats. However, ACTH's ability to increase activity is about 100 times less than CRH. ACTH has also been found to affect social behaviour by decreasing aggression and reducing social interaction (Johnson et al., 1992).

Behavioural or performance tests can be used as indicators of stress in fish (Schreck, 1990). Performance tests measure the ability of test fish to perform a behaviour in comparison to unstressed control fish. Sigismundi and Weber (1988) reported that previously stressed (30 sec handling) juvenile chinook salmon take longer to seek cover from a sudden overhead light stimulus than unstressed fish. They postulated that the increase in the fish's response time to the light stimulus is associated with their stressed state following handling. Another example of performance testing is avoidance-preference reactions of fish to toxic chemicals (Doving, 1990). Such behavioural tests are useful in indicating stress in fish, however, it is difficult to determine the severity of the stress because the effect on behaviour depends on several factors including genetic disposition, present environment, and development stage (Schreck, 1990).
The HPI axis has been implicated in modifying behaviour in fish. Strange and Cech (1992) reported that confinement stress reduced the swimming performance of striped bass during a swimming trial. To better define the stress level of the fish, Strange and Cech (1992) concurrently measured physiological parameters such as cortisol, glucose, and lactate and found that all three parameters were elevated immediately following the swimming trial (Strange and Cech, 1992).

ACTH has been said to facilitate memory and induce arousal in damselfish, and stimulate aggression in tilapia (review by Munro and Pitcher, 1983). As well, thyroid hormones and cortisol appear to be involved in the behaviour of juvenile salmon during smoltification (Hoar, 1976; Morin and Dodson, 1989).

The relationship between behavioural responses and the endocrine system are not as fully understood as in higher vertebrates. Ultimately, understanding the correlation between physiological changes and altered performance of fish in response to stress requires further research.

1.5 Statement of the Problem

The salmon industry, as stated earlier, is growing rapidly worldwide. In Canada, Atlantic salmon are one of the most commonly farmed salmonids and consequently have an impact on the economy of Atlantic Canada. Over the years considerable attention has been devoted to the physiological responses of salmonids to stresses encountered in an aquaculture environment (Barton and Iwama, 1991) and during the process, studies have shown that fish respond to numerous different stimuli. Although there is a general neuroendocrine response to most acute stressors, such that elevated concentrations of cortisol and catecholamines are released into the bloodstream, not all
stimuli appear to elicit an identical response. Further, the majority of studies measuring the physiological response of fish to stress usually employ only one neuroendocrine parameter. The discrepancies in the primary response to stress may be due to differing sampling techniques, housing conditions, fish species, duration and intensity of the stimulus or other experimental factors.

The response to a noxious stimulus (external) or potentially threatening situation involves activation of different levels of organization. One of the immediate adaptive mechanisms activated in a stressful situation is a behavioural response. In terms of response time, a fish can respond within seconds to avoid a threatening situation. Defining the stress level of fish by their actions alone, however, is not an easy task. Mammals have the advantage of expressing themselves with sounds as well as displaying overt behavioural responses to stressful situations. With respect to fish, however, we are far more limited in determining the severity of the stress.

Both the biochemical and behavioural responses of fish to stress have been well documented, but the correlation of the two parameters has not been dealt with in as much detail (Strange and Cech, 1992). Understanding the relationship between physiological and behavioural changes in response to stressful situations would provide a broader perspective on the effects of stress in fish.

1.6 Research Objectives

The objectives of this study were, firstly, to quantify the biochemical and behavioural responses of Atlantic salmon following exposure to either non-mechanical (light, formalin) or mechanical (pinch, probe) external stimuli and, secondly, to determine if different classes of stimuli elicit reproducibly distinct biochemical and
behavioural responses in Atlantic salmon. The ability to respond distinctly to different stimuli may suggest that Atlantic salmon possess some form of sensory discrimination.
2.0 BIOCHEMISTRY

2.1 Introduction

As the aquaculture industry expands, fish species such as channel catfish, rainbow trout, and salmon are used in research to promote increased productivity. These fish are often exposed to stressors such as confinement, handling and netting in both commercial and experimental environments. Identification of appropriate biochemical indicators of stress is critical to correctly assess the influence of these environments on the health of the fish. The stress response of fish to noxious external stimuli is categorized as primary, secondary and tertiary. These responses have been commonly used as quantitative indicators to measure physiological effects of stress in fish (Mazeaud et al., 1977; Donaldson, 1981; Mazeaud and Mazeaud, 1981; Barton and Iwama, 1991; Mazur and Iwama, 1993a).

The primary stress response involves an immediate neuroendocrine change in which components of the CNS initiate the synthesis and release of hormones into the blood stream (see Chapter 1). The neuroendocrine response to stress is initiated in the diencephalon. The diencephalon receives afferent fibers from the retina, olfactory bulb, optic tectum, cerebellum, telencephalon, and the facial lobe (Bradford and Northcutt, 1983). There are several different regions or zones within the diencephalon including the preoptic area, hypothalamus, epithalamus, and the thalamus. The hypothalamus is the major integrative center which controls the release of a range of hormones. In teleosts, there is a direct innervation of the pars distalis of the pituitary by the neurosecretory neurons of the hypothalamus (Ball and Baker, 1969). Activation of the pituitary following release of CRH from the hypothalamus (Figure 1.5 in previous
chapter) results in the subsequent release of hormones into the circulatory system including ACTH, melanotropin stimulating hormone, thyrotropin and prolactin (Jobling, 1995b). The function of these hormones is two-fold, whereby, they not only act on their target tissue but are also involved in a direct feedback effect upon the hypothalamus. This feedback serves to inhibit the release of the hormone from the pituitary (Jobling, 1995b). In addition to the connection between the hypothalamus and the pituitary, there are neuronal links from the hypothalamus to endocrine organs, or cells in the periphery. An example of this is the innervation of chromaffin tissue located in the head kidney of the fish. In the event of an emergency situation, catecholamines are rapidly released from the chromaffin cells into the circulation and consequently are distributed to all parts of the body in a relatively short period of time. Classically, the primary stress response in fish has been determined by measuring changes in circulating plasma concentrations of catecholamines (Mazeaud and Mazeaud, 1981) and corticosteroids (Donaldson, 1981).

Following the neuroendocrine response, secondary physiological, biochemical and immunological changes can also occur. These changes are categorized as metabolic, hematological, hydromineral, and structural (Barton and Iwama, 1991). Blood glucose as a secondary metabolic response is a reliable indicator of stress in fish (Chavin and Young, 1970; Mazeaud et al., 1977). However, in response to stress, the exact cause of elevated glucose is not fully understood. It has been postulated that the mobilization of glucose results from the action of a number of hormones including cortisol and catecholamines (Wedemeyer, 1972; Woodward and Smith, 1985; Saurez and Mommsen, 1987; Vijayan and Leatherland, 1989; Van Raaij et al., 1995). In
response to chronic stressors, such as prolonged confinement or crowding, tertiary, or whole animal responses can occur, including growth suppression, reproductive dysfunction and immunosuppression (Carragher et al., 1989; Pickering and Pottinger, 1989; Pickering, 1993).

2.1.1 Effect of stress on plasma cortisol

Cortisol is considered the major corticosteroid produced by fish (Donaldson, 1981). Synthesis and release of cortisol is regulated through stimulation of the hypothalamus and the subsequent release of CRH as described previously (Chapter 1, Section 1.4.1).

Elevation of plasma cortisol concentrations in response to stressful stimuli, is an important index of stress in salmonids. Changes in circulating cortisol concentrations in salmonids have been reported for different aquaculture-related stressors such as handling, confinement and transport (Barton and Iwama, 1991). The relationship between plasma cortisol and stressful stimuli has not been as well documented for Atlantic salmon as for other salmonids. However, studies have shown that Atlantic salmon exhibit elevated cortisol concentrations in response to various stressors (Kjartansson et al., 1988; Mazur and Iwama, 1993b). Generally, in response to an acute stressor, cortisol concentrations remain elevated for only a few hours and return to baseline within 24 hours in salmonid species (Barton and Peter, 1982; Pickering et al., 1982; Patino et al., 1987). This type of response is characterized as an adaptive response. However, in the case of a more chronic stressor, such as prolonged confinement or continuous intense handling, cortisol concentrations may remain elevated for several days and subsequently produce a maladaptive response (Barton et
al., 1980; Barton et al., 1986; Pickering and Pottinger, 1989). Resting concentrations of cortisol in unstressed salmonid fish have been reported to be as low as 0-5 ng·mL\(^{-1}\) (Pickering and Pottinger, 1989) but are more commonly reported as less than 30-40 ng·mL\(^{-1}\) (Barton and Iwama, 1991). The response of salmonids to stress can result in elevated plasma cortisol concentrations ranging from 40-200 ng·mL\(^{-1}\). Ultimately, the degree of elevation in plasma cortisol would be due to the particular stressor used and the duration and intensity applied to the fish. Cortisol is frequently measured as an indicator of stress because of its ease of measurement, responsiveness to stressors, and the adaptive importance of this hormone in fish (Barton and Iwama, 1991).

### 2.1.2 Effect of stress on plasma catecholamines

In addition to cortisol, another major hormonal response to stress in fish is the secretion of catecholamines, primarily NE and E, into the circulation (Mazeaud and Mazeaud, 1981). Control of catecholamine release is through stimulation of hypothalamic neurons which activate the sympathetic nervous system causing the release of catecholamines from the chromaffin cells. This has been summarized diagrammatically in Figure 1.5 in Chapter One. In teleosts, as in all vertebrates, the adrenergic neurons of the autonomic nervous system are postganglionic travelling in spinal autonomic pathways (Nilsson, 1984) (see Section 1.3.1.2 in previous chapter). It has been shown in Atlantic cod, *Gadus morhua*, that the chromaffin tissue is under sympathetic nervous control (Nilsson et al., 1976). The tissue appears to be innervated by preganglionic fibres passing through the sympathetic chain and entering the walls of the posterior cardinal veins. In response to electrical stimulation of the nervous supply to the left cardinal vein, a release of catecholamines from the chromaffin tissue
has been demonstrated (Nilsson et al., 1976). The main difference between the two sources of E and NE is that chromaffin cells release catecholamines via the blood to the target organ whereas adrenergic neurons release the catecholamine neurotransmitter directly onto the effector cells (Nilsson, 1984).

Reported baseline concentrations of catecholamines have varied greatly in the literature. Gingerich and Drottar (1989) reported that basal catecholamine concentrations from three studies using cannulated rainbow trout, ranged from 1.4 - 8.94 pmol mL\(^{-1}\) for E and 1.83 - 10.2 pmol mL\(^{-1}\) for NE. Plasma catecholamines have been shown to be increased in salmonids following either surgical intervention (Gingerich and Drottar, 1989) or stressors such as anoxia, thermal stress, violent exercise, acidosis and hypoxia (Mazeaud et al., 1977; Mazeaud and Mazeaud, 1981; Ristori and Laurent, 1985; Boutilier et al., 1986; Perry et al., 1991). Changes in circulating catecholamines, as indicators of stress in fish, have not been as widely studied as cortisol. This has been due to difficulties in accurately detecting low concentrations of catecholamines and the technical problems associated with obtaining valid resting plasma concentrations in fish (Gingerich and Drottar, 1989). In recent years, technical advances such as improved methods involving high performance liquid chromatography, have led to more sensitive methods for detecting plasma catecholamines and the use of chronic indwelling catheters has significantly reduced the stress of blood sampling (Woodward, 1982).

2.1.3 Effect of stress on plasma glucose

Resting concentrations of plasma glucose have been reported to vary from 46 - 123 mg·dL\(^{-1}\) in salmonids (Chavin and Young, 1970). This wide range of basal
concentrations can be due to several factors, including the developmental stage of the fish and diet (Wedemeyer, 1972; Specker and Schreck, 1980). Generally, resting plasma glucose concentrations have been reported to range from 50 - 85 mg·dL⁻¹ (Nichols and Weisbart, 1984a; Barton et al., 1986; Brown et al., 1989).

An elevation in blood glucose concentrations is well recognised as a secondary response to stress in fish (Chavin and Young, 1970; Mazeaud et al., 1977). Routine aquaculture procedures such as handling and transport (Wedemeyer, 1972; Specker and Schreck, 1980; Pickering et al., 1982; Barton et al., 1986; Barton et al., 1988) and exposure to acidic or hypoxic conditions (Mazeaud et al., 1977; Boutilier et al., 1986; Brown et al., 1989) often results in hyperglycaemia in fish. Glucose is an important metabolic fuel in all vertebrates and may originate as a result of glycogenolysis, through gluconeogenesis, or from the gut following ingestion of carbohydrates (Suarez and Mommsen, 1986). The elevation of glucose in a threatening situation provides the additional energy required to respond to the stress. The metabolic changes observed in response to stress have been considered to result from primary changes in catecholamines and cortisol (see Section 2.1). However, this direct cause and effect relationship is not fully understood.

The intensity and duration of the stressor can have a direct effect on the metabolic response of the fish. Post-stress plasma glucose concentrations have been reported to increase 2-4 fold (Wedemeyer, 1972; Mazeaud et al., 1977; Ristori and Laurent, 1985; Brown et al., 1989). In contrast, Barton and associates (1986) showed that while acute handling stress significantly elevated plasma glucose in juvenile chinook salmon, the changes were small. However, when exposed to multiple acute
handling stresses, the change in concentration of plasma blood glucose was much greater.

2.1.4 Objectives

The primary objective of the biochemical component of this study was to measure circulating concentrations of plasma cortisol, catecholamines (specifically NE and E), and plasma glucose during three time periods; post-surgery, baseline and experimental. A second objective was to determine if different classes of external stimuli elicit reproducibly distinct biochemical responses in any or all of these parameters.
2.2 Materials and Methods

2.2.1 Experimental Animals

Two groups of 18 second sea year Atlantic salmon, weighing between 0.9 and 1.5 kg, were employed in two separate experiments within the study. The first group was obtained from the New Brunswick Salmon Growers Association-Salmon Development and Demonstration Farm of St. George, N.B. The second group were from Brookvalley Marine Farms in Fortune, P.E.I. Fish were randomly selected from a stock tank; no distinction was made between males and females.

Each group of 18 fish was randomly distributed into the experimental tanks (see Section 2.2.2) such that each section of the tanks contained three fish. Cannulae were surgically implanted into the dorsal aorta (see Section 2.2.4.2) of 12 fish. These were chosen by randomly selecting two fish from each of the six sections. Of the two fish, one was designated the experimental subject and was additionally fitted with a 120 cm distal cannula which extended to the surface of the water. The other was referred to as the replacement fish and was fitted with a shorter distal cannula of 20 cm in length. The distal end of the shorter cannula was secured to the suture support previously attached caudal to the dorsal fin. The replacement fish were used in the event that the cannula of the experimental fish was no longer patent. The distal cannula of the replacement fish was further extended by the addition of an 100 cm distal cannula. The remaining fish was designated as the control. The purpose of the control was to provide comparisons of behavioural activities of the cannulated and non-cannulated (control) fish. In addition, housing fish in groups of three may alleviate some of the
social stresses commonly associated with maintaining fish in groups of two (Ejike and Schreck, 1980; Pottinger and Pickering, 1992).

2.2.2 Animal Housing

During the acclimation and experimental phases of the study the fish were housed in 1.5 m diameter by 90 cm deep, circular fiberglass reinforced plastic tanks (Waterline, Brookvale, Prince Edward Island). Each tank was divided into three equal sections by Vexar® screens that were tapered to fit the confines of the tank. The individual screens were constructed from 1" schedule 40 polyvinyl chloride (PVC) pipe, 90 cm in length, and contoured to span the width of the tank from top to bottom. A 1" mesh black Vexar® sheet was secured with short cable ties to the PVC frame. Each screen was attached with long cable ties to a 4" PVC pipe which slipped over the internal standpipe (see Figure 2.1).

Each tank contained approximately 1500 L of artificial seawater (Instant Ocean®, Aquarium systems, Mentor, Ohio, USA), maintained at a salinity of 28-30 parts per thousand. The stocking density in each tank was 7.5 kg/m³. Seawater at 11-12 °C was supplied to the tanks at ≥ 5 L min⁻¹ from a recirculating water system. System temperatures were monitored and alarmed by a Campbell CR7 Data Acquisition System. Dissolved oxygen concentration was maintained at ≥ 8 mg L⁻¹ and circulation was enhanced by an external airlift adjusted to recirculate the tank seawater at ≥ 5 L min⁻¹.

Components of the recirculating water system were an above floor seawater reservoir, a system water circulating pump, a biofilter circulating pump, a Triton sandfilter for the removal of particulates, titanium plate, warm and cold water heat exchangers (controlled by the Honeywell Controller), and warm and cold water header
Figure 2.1: Experimental tank with divider (A) and view of saltwater recirculating system (B), cameras are shown above each tank.
cold water header tanks. A trickle-tower biofilter (later replaced in the second experiment with an Aquacube™ biofilter (Advanced Aquaculture Systems, Inc., Brandon, FL)), was used to convert nitrogenous wastes to nitrates. A schematic diagram illustrating the components of the system is provided in Figure 2.2.

Lighting was provided by 4 cool white fluorescent 40 watt bulbs positioned above each tank. Photoperiod was 14 hour light-10 hour dark cycle with the lights turned on at 7:00 am and off at 9:00 pm. Environmental conditions were monitored manually on a daily basis and water quality parameters checked weekly according to "in-house" Good Laboratory Practice (GLP) Procedures (FHU S.O.P. # 404/02 and 401/02). Fish were manually fed to satiation once per day between 10:00 am and 11:00 am, except on surgery days, with Select extruded Atlantic salmon diet (Moore and Clark, St. Andrews, N.B.). Approximately half way through the study Moore and Clark terminated production of Select and for the remaining period fish were fed Royal pelleted dry feed from the same company.

2.2.3 Overview of Study Design

Two identical experiments were performed during the study. For each experiment, 18 Atlantic salmon were randomly assigned to two circular tanks using a list of randomly selected numbers generated from Minitab Statistical Package, Release 7.1. Each tank was divided into three equal sections with three fish per section. The fish were anaesthetized in 100 mg·mL⁻¹ of tricaine methane sulfonate (TMS) to be tagged, weighed and fork lengths measured prior to placement into the divided sections. The tagging procedure involved inserting a suture support (see section 2.2.4.2) through the superficial dorsal musculature just caudal from the dorsal fin. A 4 cm oval plastic
Figure 2.2: Schematic of the recirculating saltwater system for housing Atlantic salmon.
tag was attached to the support and served as identification for each individual fish.

Each experiment contained four time periods; Baseline 1, Post-surgery, Baseline 2, and Experimental (Figure 2.3). These periods are defined as follows:

(i) Baseline 1

Following a two week acclimation period, baseline behaviour was recorded, using a video-camera suspended above the tank, every second day for 11 days prior to cannulation surgery (Figure 3.1 in following chapter).

(ii) Post-Surgery

Immediately following baseline observations, the experimental and replacement fish from each section were fitted with an indwelling aortic catheter. Blood samples were collected immediately prior to surgery (from the caudal sinus, see Section 2.2.5) and subsequently over a 14 day period (Figure 2.4). Behaviour was not recorded during this period.

(iii) Baseline 2

Fish were acclimated for a two week period following completion of post surgery sampling. Baseline biochemical (Figure 2.4) and behavioural (Figure 3.1 in following chapter) observations were conducted every second day for eleven days prior to application of the experimental stimuli.

(iv) Experiment

Following baseline biochemical and behavioural observations, the experimental fish were exposed to non-mechanical and mechanical stimuli as described in Table 2.1. The experiment was conducted over 5 weeks with each experimental fish receiving four different stimuli with a minimum of two days between successive stimuli. During the
Figure 2.3: Overview of study design for monitoring biochemical (•) and behavioural (▲) activity of Atlantic salmon.
Figure 2.4: Blood sampling schedule for Atlantic salmon during Post-surgery (A), Baseline 2 (B) and Experimental (C) period. * indicates blood sample collection.
experiment both biochemical (see Figure 2.4) and behavioural data (see Figure 3.1. in following chapter) were collected.

During the course of each experiment one experimental fish died, therefore only five sections were included in the statistical analysis for Experiment 1 and 2. In addition, replacement fish were employed during Experiment 1 and 2. One experimental fish was replaced in Experiment 1, during the course of the Experimental period. The replacement fish received two of the four experimental stimuli. In Experiment 2, two experimental fish were replaced prior to the Baseline 2 observational period and the replacement fish were employed throughout the remainder of the study. Also during Experiment 1, an additional experimental fish was replaced immediately prior to the Experimental period.

2.2.3.1 Experimental Stimuli

Each of the experimental fish was randomly exposed to four different stimuli employed in the experiment: light, formalin, probe and pinch. The first two were categorized as non-mechanical and the latter two were referred to as mechanical stimuli. A description of each stimulus is provided in Table 2.1.

2.2.4 Surgical Procedures

2.2.4.1 Preparation of Cannulae

The cannula (PE50, Clay Adams Intramedic™, I.D. 0.58 mm (0.023"), O.D. 0.965 mm (0.038"), used for chronic cannulation of the dorsal aorta, was constructed from an 18 cm proximal section and an additional distal portion of either 20 cm or 120 cm in length. Prior to surgery a 1 cm reinforcement collar of PE190 tubing was glued (Krazy Glue®, The Borden Co. Ltd., Ontario) onto the proximal cannula at the mid-
Table 2.1: Description of the stimuli employed during the Experimental period.

<table>
<thead>
<tr>
<th>CATEGORY</th>
<th>STIMULUS</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>NON-PHYSICAL</td>
<td>Light</td>
<td>Light stimulation was created by switching off the lights (4 F40 watt cool white bulbs) for one minute.</td>
</tr>
<tr>
<td></td>
<td>Formalin</td>
<td>Formalin (37% formaldehyde) was added to the oxygenated tanks to a concentration of 250 ppm. A total volume of 312 mL was equally divided into 3 parts and added to each section of the tanks. The saltwater circulation was shut off 15 minutes prior to addition of the formalin and remained off for an additional hour and 15 minutes.</td>
</tr>
<tr>
<td>PHYSICAL</td>
<td>Pinch</td>
<td>Pinching involved quickly applying a sharpened surface at both sides of the fish just caudal to the dorsal fin. The stimulus was applied without penetration of the skin. The pinchers consisted of tongs with two white plastic moldings, holding modified microcentrifuge tubes.</td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>A clear plastic hollow rod with a rubber stopper at the end was utilized as the probing device. The probe was tapped gently on the dorsal surface, caudal to the dorsal fin.</td>
</tr>
</tbody>
</table>
point. A 15 cm length of suture material (Supramid White, USP2/0, Serag -Weissner, Germany) was tied around the collar and glued in place. The proximal end of the distal section of the cannula was fitted with a 2 cm section of 23 gauge stainless steel needle and secured with glue. A blunt 23 gauge needle and syringe, filled with 0.5 mL sterile heparinized saline (140 IU heparin·mL⁻¹ of 0.85% saline), was attached to the distal end of the distal section of the cannula. The proximal cannula was flushed and the distal cannula was filled with sterile heparinized saline (280 IU heparin·mL⁻¹ of 0.85% saline). During surgery the cannula was assembled by inserting the section of stainless steel needle of the proximal end of the distal cannula into the distal end of the proximal section of the cannula. This connection was covered by a 3 cm collar of PE190 tubing and attached with glue. The distal cannula was secured by suture supports consisting of 24 cm of suture material inserted into a 8 cm length of PE50 tubing. Upon completion of surgery, the distal portion of the cannula was capped with a PRN adapter cap (Deseret®, Becton Dickson, Mexico). The design of the cannulae and the suture supports required for securing the cannulae to the fish are illustrated in Figure 2.5.

2.2.4.2 Protocol for Cannulation of the Dorsal Aorta

Atlantic salmon were fitted with chronic indwelling cannulae into the dorsal aorta according to established procedures (modified from Pye-MacSwain, 1992). Fish were anaesthetized with TMS (Sigma Chemical Company, St. Louis, Miss.) prior to surgery and a small submersible recirculating pump (Aquaresearch Ltd., QC.) was employed to maintain anaesthesia throughout surgery. The fish were initially anaesthetized in TMS at a concentration of 100 mg·L⁻¹ and a maintenance dose of 50
Fig. 2.5: Catheter assembly used in the cannulation of Atlantic salmon  
(adapted from Pye-MacSwain J.K., MSc Thesis, University of Prince Edward Island, 1992)
mg·L⁻¹ was used during surgery. Following induction of anaesthesia the fish were placed onto a surgery table in a dorsoventral position. A 14 gauge needle was inserted through the cartilage of the snout with the needle point positioned approximately 1 cm into the mouth cavity. The fish was then repositioned into a ventrodorsal position. An internal 0.016" diameter trocar (GHS Strings, Battle Creek, Michigan) was inserted into the 18 cm proximal portion of the cannula such that the sharpened tip of the trocar was exposed. The cannula was inserted at a 45° angle through the mid-line of the palate between the second and third gill arches. At this time, the cannula was levelled and advanced slowly until such time as the tip of the trocar penetrated the dorsal aorta. As the trocar was withdrawn, the cannula was held firmly and advanced approximately 7 cm into the dorsal aorta. Patency of the proximal cannula was confirmed at this point by flushing the cannula with 0.1 mL of sterile heparinized saline (280 IU heparin·mL⁻¹ of 0.85% saline) and observing ease of blood withdrawal. The proximal cannula was further flushed with heparinized saline solution and passed through the 14 gauge needle, previously inserted through the cartilage of the snout, and attached to the distal cannula. A protective collar was positioned over the junction of the two cannulae and glued in place. The proximal cannula was sutured to the hard palate and the distal portion secured to two suture supports, one just caudal from the head and another just caudal from the dorsal fin. The suture supports were attached previously by passing the suture through a 14 gauge needle inserted into the superficial dorsal musculature. A 1.0 mL blood sample was collected from the dorsal aorta and the distal end of the cannula was closed with a PRN adapter cap. Recovery of each fish took place within the individual section where they would be housed during the remainder of the experiment.
2.2.5 Blood Sampling

Prior to surgery, 1.0 mL blood samples were withdrawn from the caudal vessels of both the experimental and replacement fish using an 18 gauge needle on a 1.0 mL sterile syringe. All remaining blood samples (1.0 mL) were collected from the needle attached to the distal end of the cannula of the experimental fish. Initially, 0.5 mL of sterile heparinized saline (140 IU heparin·mL⁻¹ of 0.85% saline) was slowly injected into the cannula through a sterile syringe. An initial volume, containing 0.3 mL of blood plus the previously injected heparin saline solution, was withdrawn back into the syringe. The blood sample (1.0 mL) was collected in a second sterile 1.0 mL syringe and immediately transferred into a test-tube (Vacutainer® containing sodium heparin) (Becton Dickinson, Rutherford, New Jersey). The first syringe, containing the blood and heparinized saline solution, was flushed back into the cannula, followed by 1.0 mL of sterile heparinized saline (140 IU heparin·mL⁻¹ of 0.85% saline) and the cannula was sealed. Samples were kept on ice until centrifugation (Beckman, TJ-6R Tabletop Centrifuge) at 1500 g for 20 minutes (-4°C). Plasma was removed using Nichiryo pipettes (Model 5000, Fisher Scientific, Nepean, Ontario) and divided into three aliquots (0.3 mL, 0.1 mL, and 0.05 mL). The aliquots were stored in 1.5 mL microcentrifuge tubes (Fisher Scientific) for the eventual assay of catecholamines, cortisol and blood glucose, respectively (see section 2.2.6). The storage conditions and additives required for each blood chemical were varied. Catecholamine samples (0.3 mL) received an additional 30 µL of heparinized saline (1330 IU heparin·mL⁻¹ of 0.85% saline) and 20 µL of 5 mM sodium bisulphite to prevent oxidation. The samples were initially frozen in liquid nitrogen for one minute then stored at -80°C for future analysis. Cortisol
samples (0.1 mL) were stored at -20°C for two months with no additives required. Blood glucose samples (0.05 mL) were kept on ice for a maximum of five hours before analysis.

Blood samples were collected from the experimental fish for the three phases during each experiment as listed in Figure 2.4. Replacement fish were sampled immediately before and after surgery only. In the event that a replacement fish was required, an extension was attached to the existing distal catheter of the replacement fish and blood sampling was resumed.

2.2.6 Assays

*Catecholamines*

Circulating plasma concentrations of E and NE were measured using reversed phase high performance liquid chromatography (HPLC) with electrochemical detection (0.55 volts) according to the method of Woodward (1982).

Prior to extraction, stock solutions were prepared as follows: 60.55 g Trizma base (Sigma T-1503) and 5 g EDTA (Sigma ED2SS) was added to 250 mL of ultrapure water, stirred and heated to 40-50°C until Trizma and EDTA dissolved. The Trizma/EDTA solution (2 mol·L⁻¹) was adjusted to pH 8.7 and designated Reagent A. Reagent B was prepared by diluting Reagent A to a final concentration of 0.2 mol·L⁻¹ and adjusting the pH to 8.1. An additional solution (solution A) was prepared by adding 50 µL of 10% sodium disulfite, 50 µL of 5% EDTA, 100 µL of glacial acetic acid, and ultrapure water to make up a volume of 10 mL. Catecholamine standards at concentrations of 12 nmol·L⁻¹ noradrenaline, 6 nmol·L⁻¹ adrenaline, and 14 nmol·L⁻¹ DHBA (3,4-dihydroxybenzylamine), were prepared as follows: 9.5 mg noradrenaline
(bitrate salt) and 4.995 mg adrenaline (bitrate salt) were added to 500 mL of 0.1 N HCl (made in ultrapure water), diluted 5,000 X and stored at -80°C. DHBA (7.704 mg) was added to 500 ml of 0.1 N HCl (made in ultrapure water), diluted 5,000 X and also stored at -80°C.

The procedure for extracting catecholamines from the plasma involved the following steps: In a 1.5 mL microcentrifuge tube, 400 μL of reagent A, 10 mg of alumina (aluminum oxide (Sigma 3A-1772; Type WA-4 acid, Activity grade super 1)), 200 μL of plasma, and 40 μL of DHBA solution (Sigma 3A 9512) (internal standard) were added sequentially. Standards for calibrating the HPLC were prepared using the same method except in place of the plasma, 200 μL of the adrenaline/noradrenaline standard was added. All samples were shaken for 20 minutes and centrifuged for one minute, followed by aspiration of the buffer/plasma from the alumina. The samples were washed by adding 1.0 mL of reagent B, shaking for one minute, centrifuging for one minute, and aspirating the wash buffer from the alumina. The wash was repeated three times. Following this, 125 μl of solution A was added to each sample, the samples were shaken for 20 minutes and centrifuged for one minute. Samples that were not analysed on the HPLC immediately were stored at -20°C. Samples were injected into a 5 μm C18 column (ultratechsphere) (Waters Chromatography Division, Millipore (Canada) 'td.) with a Waters mobile phase for catecholamines delivered at 1.0 mL·min⁻¹.

All plasma catecholamine samples were extracted and analysed in Dr. Perry's laboratory at the University of Ottawa (Department of Biology). The plasma samples
from the first experiment were analysed by Dr. Perry's staff. All subsequent samples were analysed by the author.

**Cortisol**

Cortisol concentrations were determined using a commercially available radioimmunoassay (Coat-A-Count®, Intermedico, Diagnostic Products Corporation, Markham, Ontario). The Coat-A-Count kits had been previously verified for specificity of cortisol in fish plasma and were stated to have a detection limit of approximately 0.2 μg·dL⁻¹. The inter-assay and intra-assay variability was approximately 1.5% and 5.0%, respectively, during analysis of the plasma samples in this study.

Blood samples (25 μL) were pipetted into antibody coated plastic tubes in duplicate and brought to room temperature. Controls were placed at the beginning, middle, and end of each run. In addition, two uncoated (plain) tubes and two uncoated tubes containing a standard concentration of cortisol were included during each analysis to determine the total count (TC) and non-specific binding of the tubes, respectively. Six standards of increasing concentration were also used to generate a standard curve prior to each analysis of the samples.

The reagent (1.0 mL volume), ¹²⁵I-cortisol was added to all tubes. Following an incubation period (37°C) of 45 minutes (excluding the TC tubes), all reagents were decanted from the tubes and the residual binding of ¹²⁵I-cortisol was measured in a gamma counter (Packard, Multi-Prias™ 2, Canada) for determination of cortisol concentration.
**Glucose**

Circulating concentrations of plasma glucose were measured using standard protocols as provided by the Beckman Glucose Analyser 2 operating manual (Beckman Instruments Inc., 1987). The plasma samples were kept on ice, for a maximum period of five hours, and mixed thoroughly (Vortex mixer) prior to injection (10 μL) into the analyser. The results of a pilot study on Atlantic salmon blood indicated that values were stable up to 12 hours using this protocol (data not presented). All samples and controls were measured in duplicate.

### 2.2.7 Data Analysis

Statistical analysis of the data was performed by General Linear Models Procedure (Proc GLM), followed by a post hoc Scheffes test using the Statistical Analysis System Inc. (SAS Institute, Inc., 1985) software program. Data were analysed using the repeated measures design to account for the variability between the fish and subsequently transformed to increase homogeneity of variance. Data were transformed using \( \log_{10} \), \( \sqrt{\text{square root}} \), \( x^2 \) where applicable. Analysis of post-surgery and experimental data involved comparisons at each time point between 0 and 24 hours with the corresponding pre-stimulus values. All comparisons were considered significant at the 5% probability level. Baseline data was analysed for differences between days by Proc GLM and further analysed in the Minitab statistical package, Release 7.1, using a t-test \( (p \leq 0.05) \) to determine statistical differences between sampling times (Before and After videotaping, see Section 2.4) within each day. In addition, a comparison between 'Before' baseline data and
Post-Surgery sampling period (Day 2 to Day 14) was performed using Proc GLM. The probability values presented in this chapter are derived from the transformed data with the exception of the baseline data analysed in Minitab. All data are given as arithmetic means ± SEM. Statistically, most data obtained in Experiment 1 were not significantly different from those obtained in Experiment 2, therefore, means (± SEM) represent pooled data from Experiments 1 and 2 unless stated otherwise.

2.3 Results

2.3.1 Plasma Cortisol Concentrations

*Post - Surgery*

Changes in plasma cortisol at different time points following surgery are presented in Figure 2.6. The data point B in Figure 2.6 represents the mean ± SEM baseline cortisol concentration immediately prior to surgery. When compared with the basal concentration of 113.49 ± 30.83 nmol·L⁻¹, plasma cortisol increased significantly (p = 0.0001) to 359.87 ± 39.58 nmol·L⁻¹ at the first sampling time following surgery and continued to increase over 30 minutes to 429.33 ± 17.11 nmol·L⁻¹ (p = 0.0001) (Figure 2.6). Within six hours plasma cortisol concentrations had fallen to basal concentrations and remained low after 24 hours following surgery. Over the remaining course of the Post-Surgery sampling period (14 days), plasmacortisol concentrations were consistently low, fluctuating near initial baseline concentrations (B). *Baseline 2*

Baseline concentrations exhibited a marked decrease over the 11 day interim from 101.04 ± 35.68 to 13.95 ± 3.34 nmol·L⁻¹ and 100.84 ± 5.63 to 25.73 ± 7.79 nmol·L⁻¹ for Before and After data, respectively (p = 0.0085) (Table 2.2). This
Figure 2.6: Mean (± SEM) plasma cortisol concentrations of Atlantic salmon before (B) and following catheterization of the dorsal aorta. Each value represents 7-10 fish. 'B' denotes blood sample collected from the caudal sinus prior to surgery. Blood sample collected immediately after surgery is indicated by the arrow. 'a' indicates significant difference from B (p < 0.05).
fluctuation in plasma cortisol concentrations observed during the Baseline 2 period was also evident during the latter sampling period (Day 2 - Day 14) following surgery (Figure 2.6). Because no significant difference was found between Before and After data, only the Before data was used for the comparison between baseline and Post-Surgery data. However, there was a significant difference between the patterns of response between baseline cortisol concentrations (Table 2.2, 'Before' ) and plasma cortisol concentrations observed during the latter Post-surgery sampling period (Figure 2.6) (p = 0.035) and between experiments (p = 0.0078). Plasma cortisol concentrations observed during both sampling regimes, Post-Surgery and Baseline 2 (Before), were generally lower than 50 nmol·L⁻¹.

**Experimental**

The response profile of plasma cortisol concentrations during the experimental period is shown in Figure 2.7. Plasma cortisol concentrations ranged from 12.48 ± 1.20 to 38.95 ± 13.81 nmol·L⁻¹ prior to introduction of the stimuli. The pattern of response in the first three hours following stimulation was significantly different for the four stimuli employed during the experiment (p = 0.0373). All stimuli, with the exception of the probe, elicited a significant increase in mean plasma cortisol concentrations immediately after stimulation in comparison to baseline cortisol concentrations. Probing, as compared to the other stimuli, did not elicit a significant change in plasma cortisol within the first three hours following stimulation. Introduction of the pinch stimulus produced the greatest response in plasma cortisol concentrations with an increase to 164.41 ± 37.26 nmol·L⁻¹. Exposure to the light and formalin stimulus did not produce as great a response as the pinch stimulus, however, significant increases to
Table 2.2: Mean (± SEM) plasma cortisol concentrations in Atlantic salmon over the eleven day baseline period. Blood samples were collected between 1:30 -2:30 pm ('Before' videotaping) and between 4:30 - 5:30 pm ('After' videotaping).

<table>
<thead>
<tr>
<th>CORTISOL (nmol·L⁻¹)</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Before</td>
<td>101.04</td>
</tr>
<tr>
<td></td>
<td>(±35.68)</td>
</tr>
<tr>
<td>After</td>
<td>100.84</td>
</tr>
<tr>
<td></td>
<td>(±16.9)</td>
</tr>
</tbody>
</table>
Figure 2.7: Mean (± SEM) plasma cortisol concentrations of Atlantic salmon before (B) and following introduction of four external stimuli. Values for light, formalin, and pinch represent 8-9 fish. Values for probe represent 6-7 fish. 'B' denotes blood samples collected 30 minutes prior to stimulation. Introduction of the stimulus (3:00 pm) is indicated by the arrow. 'a' indicates significant difference (p ≤ 0.05) in comparison to B at time 0 (light, formalin, and pinch), time 1 h (formalin and pinch), time 3 h (formalin), and time 6 h (all stimuli).
108.12 ± 31.15 and 102.52 ± 15.84 nmoi·L⁻¹, respectively, were observed. One hour following stimulation, plasma cortisol concentrations in response to the pinch and formalin stimulus remained elevated, but in the case of light stimulation, cortisol concentrations had returned to baseline. By three hours, plasma cortisol concentrations were similar to baseline cortisol concentrations for both the light and pinch stimulus; however, cortisol concentrations still remained elevated following introduction of formalin. In fact, plasma cortisol concentrations increased steadily over the three hour period following exposure to the formalin stimulus.

Following the variable changes in plasma cortisol concentrations within the first three hours, a significant peak (approximately 10-20 fold increase) in cortisol concentrations was observed six hours after all four stimuli. It is important to note that at this time an additional stimulus was introduced to all fish, because the lights were turned off during the regular photoperiod. Although the lights were off at the six hour sampling time, the fish were sampled under indirect light from the anteroom adjacent to the holding module. Due to the possible interference of the photoperiodon plasma cortisol concentrations, this increase may not be a direct effect of the previously introduced stimuli. Plasma cortisol concentrations following introduction of all four stimuli, had returned to baseline within 24 hours.

2.3.2 Plasma Catecholamine (NE and E) Concentrations

Post-Surgery

The response patterns of plasma catecholamine concentrations following surgical intervention are shown in Figure 2.8. Over the 14 day Post-Surgery sampling period, plasma NE (Figure 2.8A) and E (Figure 2.8B) concentrations were not significantly
Figure 2.8: Mean (± SEM) plasma norepinephrine (A) and epinephrine (B) concentrations of Atlantic salmon before (B) and following catheterization of the dorsal aorta. Each value represents 7-10 fish. 'B' denotes blood sample collected from the caudal sinus prior to surgery. Blood sample collected immediately after surgery is indicated by the arrow. 'a' indicates significant difference from B (p ≤ 0.05).
elevated after surgery at the times sampled. Plasma NE concentrations were significantly greater (p = 0.0078) in Experiment 1 in comparison to Experiment 2.

There was a slight increase in plasma E concentration to 48.37 ± 32.90 nmol·L⁻¹ immediately following surgery. However, due to considerable variability among the fish this increase was not statistically different from the mean pre-surgery concentration of 40.05 ± 27.95 nmol·L⁻¹. The large variability in E concentrations among the fish may have been due to consistently high plasma E concentrations exhibited by two fish in the first experiment (see data in Appendix 1).

Baseline 2

The results of the baseline concentrations of plasma catecholamines are summarized in Table 2.3. Baseline data for plasma catecholamines includes only the 'Before' data. Resting concentrations of plasma E and NE were invariably low, ranging from 0 - 5 nmol·L⁻¹, averaging around 0.78 nmol·L⁻¹ for both NE and E. Basal concentrations of plasma E were significantly lower than E concentrations observed during the latter post-surgery sampling period (p = 0.0001). The large variability among the fish shown in the post-surgery data may account for the significant difference in plasma E concentrations between the latter Post-Surgery samples and baseline.

Experimental

As shown in Figure 2.9A, plasma NE did not change following any of the four stimuli employed in the experiment. Similarly, no significant elevations were observed in plasma E concentrations in response to the four stimuli (Figure 2.9B). Overall, mean plasma concentrations observed over the 24 hour experimental period remained low.
Table 2.3: Mean (± SEM) plasma catecholamine baseline concentrations of Atlantic salmon for six days over an eleven day period. Blood samples were collected between 1:30-2:30 pm ('Before' videotaping). Samples collected after videotaping were not analyzed. ND = not detected.

<table>
<thead>
<tr>
<th>CATECHOLAMINE</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>(nmol L⁻¹)</td>
<td>1</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>1.29 (±1.01)</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>1.31 (±0.66)</td>
</tr>
</tbody>
</table>
Figure 2.9: Mean (± SEM) plasma norepinephrine (A) and epinephrine (B) concentrations of Atlantic salmon before (b) and following introduction of four external stimuli. Values for light, pinch, and formalin represent 8-9 fish. Values for probe represent 6-7 fish. ‘b’ denotes blood sample collected 30 minutes prior to stimulation. Introduction of the stimulus (3:00 pm) is indicated by the arrow.
(<5 nmol·L⁻¹) with respect to both catecholamines measured. These low values shown in Figures 2.9 A&B are comparable with basal concentrations during the 11 day baseline period (Table 2.3).

2.3.3 Plasma Glucose Concentrations

Post-Surgery

The response pattern of plasma glucose concentrations following surgery are presented in Figure 2.10. There was a significant difference between experiments (p = 0.0033), however for ease of presentation, pooled data is represented in Figure 2.10. Similar to the plasma cortisol Post-Surgery results, plasma glucose concentrations were also significantly elevated immediately after surgery in comparison to mean basal concentrations of 104.80 ± 3.64 mg·dL⁻¹ (Expt. 1) and 71.90 ± 2.84 mg·dL⁻¹ (Expt. 2). Plasma glucose concentrations had risen to 145.63 ± 11.58 mg·dL⁻¹ (Expt. 1) and 98.37 ± 7.38 mg·dL⁻¹ (Expt. 2) and continued to increase after 30 minutes to 170.00 ± 17.03 mg·dL⁻¹ (Expt. 1) and 115.75 ± 15.51 mg·dL⁻¹ (p = 0.0001) (Figure 2.10). Elevated plasma glucose concentrations were still evident after six hours (148.13 ± 17.65 mg·dL⁻¹ (Expt. 1) and 121.10 ± 14.37 mg·dL⁻¹ (Expt. 2)) but returned to baseline within 24 hours. Plasma glucose concentrations remained low during the latter Post-Surgery sampling period (Day 2- Day 11) and were consistent with basal concentrations collected prior to surgery during both experiments.

Baseline 2

Plasma glucose concentrations observed during Baseline 2 observations (Table 2.4) were comparable to plasma glucose concentrations shown in the latter Post-Surgery period (Figure 2.10). There was a significant difference between the sample days
Figure 2.10: Mean (± SEM) plasma glucose concentrations of Atlantic salmon before (B) and following catheterization of the dorsal aorta. Each value represents 7-10 fish. 'B' denotes blood sample collected from the caudal sinus prior to surgery. Blood sample collected immediately after surgery is indicated by the arrow. 'a' indicates significant difference from B (p ≤ 0.05).
Table 2.4: Mean (± SEM) plasma glucose baseline concentrations of Atlantic salmon for six days over an eleven day period. Blood samples were collected between 1:30 - 2:30 pm ('Before' videotaping), and between 4:30 - 5:30 pm ('After' videotaping).

<table>
<thead>
<tr>
<th>GLUCOSE (mg dl⁻¹)</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>9</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before</strong></td>
<td>65.78</td>
<td>60.81</td>
<td>69.9</td>
<td>65.81</td>
<td>62.78</td>
<td>69.28</td>
</tr>
<tr>
<td></td>
<td>(+1.42)</td>
<td>(+2.23)</td>
<td>(+3.21)</td>
<td>(+2.46)</td>
<td>(+3.17)</td>
<td>(+2.61)</td>
</tr>
<tr>
<td><strong>After</strong></td>
<td>69.69</td>
<td>60.33</td>
<td>70.67</td>
<td>70.13</td>
<td>60.39</td>
<td>68.28</td>
</tr>
<tr>
<td></td>
<td>(+2.19)</td>
<td>(+1.78)</td>
<td>(+3.57)</td>
<td>(+2.73)</td>
<td>(+1.42)</td>
<td>(+2.88)</td>
</tr>
</tbody>
</table>
during baseline observations 'Before' and 'After' (p = 0.0008). Baseline 2 glucose concentrations ranged from 60.33 ± 1.42 mg·dL⁻¹ to 70.67 ± 3.57 mg·dL⁻¹.

Experimental

Following introduction of the four different stimuli, plasma glucose concentrations were not elevated significantly over the 24 hour sampling period (Figure 2.11). The glucose concentrations shown in Figure 2.11 are consistent with baseline plasma glucose concentrations (Table 2.4) and are similar to concentrations shown in Figure 2.10 with respect to the latter samples.

In summary, from the physiological parameters investigated in the present study, distinct evidence of the stressful effects of surgery were observed. Both plasma cortisol and glucose were significantly elevated following cannulation surgery. Plasma cortisol concentrations continued to increase after 30 minutes and dropped back to baseline within six hours. The elevated concentration of glucose in the blood was prolonged, remaining elevated after six hours, but returned to baseline within 24 hours. Although the release of catecholamines into the blood stream is recognised as a major hormonal response to stress, there was no evidence to support a significant elevation in either plasma E or NE following surgery. However, there was a slight elevation in E immediately following surgery but due to the large variability among fish this change was not significant. In comparison with the severe stress of surgery, the four stressors, light, formalin, pinch, and probe, did not elicit as great a response in the physiological parameters measured. Virtually no response was observed in glucose or E and NE in response to any of the stimuli. Cortisol, on the other hand, did respond to three of the stimuli. In response to light, pinch, and the introduction of formalin, plasma cortisol
Figure 2.11: Mean (± SEM) plasma glucose concentrations of Atlantic salmon before (B) and following introduction of four external stimuli. Values for light, formalin, and pinch represent 8-9 fish. Values for probe represent 6-7 fish. 'B' denotes blood samples collected 30 minutes prior to stimulation. Introduction of the stimulus (3:00 pm) is indicated by the arrow.
concentrations significantly increased immediately following stimulation. The probe stimulus did not appear to significantly change plasma cortisol concentrations. Plasma cortisol had returned to baseline within three hours following the light and pinch stimulus, but remained elevated in response to the formalin. At the six hour time, a peak in plasma cortisol concentrations was observed, irrespective of the stimulus. Concentrations had increased approximately 10-20 fold for each stimulus at six hours but returned to basal concentrations within 24 hours.

2.4 Discussion

Cortisol

The significant elevation in plasma cortisol concentrations immediately after surgery, is consistent with previously reported findings for chronically catheterized rainbow trout (Bry and Zohar, 1980). Plasma cortisol fell rapidly within six hours and fluctuated between 5.28 ± 2.72 and 70.4 ± 27.62 nmol·L⁻¹ for the remaining post-surgery sampling period (Figure 2.6). Statistical analysis showed a significant difference between basal cortisol concentrations recorded during baseline observations (Table 2.2 'Before') and the concentrations reported after 48 hours post surgery. The high cortisol concentrations reported during the first two days of baseline observations may be a result of a few fish that exhibited unusually high plasma cortisol concentrations (Appendix 2). Nonetheless, the concentrations during both sampling periods generally ranged between 20-50 nmol·L⁻¹. These values closely resemble resting values previously reported for cannulated salmonids (Bry and Zohar, 1980; Nichols and Weisbart, 1984b).
Changes in plasma cortisol concentrations in response to the four stimuli exhibited different patterns over the first three hours following stimulation (Figure 2.7). At the onset of the light, formalin, or pinch stimulus, plasma cortisol concentrations were elevated significantly in comparison to basal concentrations (B). With the exception of the formalin stimulus, plasma cortisol concentrations had returned to baseline within three hours. These results are comparable to previous findings which reported a similar pattern of events in cortisol response for salmonids subjected to acute handling and confinement stress (Barton et al., 1980; Pickering et al., 1982; Patino et al., 1987; Pickering and Pottinger, 1989). The formalin stimulus produced significantly higher plasma cortisol concentrations at the three hour sampling time in comparison to the other stimuli. Previous studies examining the effects of formalin treatment on pituitary activation, have shown that interrenal vitamin C depletes significantly in rainbow trout after one hour exposure to 200 ppm formalin (Wedemeyer, 1971). This depletion in interrenal vitamin C, as a measure of ACTH release, indicates the stress imposed on the fish. The effects of formalin, nevertheless, may be species specific among salmonids. A further study examining the effects of 200 ppm formalin treatment on steelhead trout and coho salmon reported that plasma cortisol concentrations and vitamin C concentrations were not affected by a one hour treatment (Wedemeyer and Yasutake, 1974). However, during prolonged treatment (six hours), plasma cortisol concentrations were significantly elevated at three and six hours in steelhead trout. In the present study, complete replacement of the tank water volume, taking in to account volume and flowrate through the tank, should occur within approximately six hours.
There is a possibility that prolonged elevation in plasma cortisol was in response to residual formalin in the water before complete replacement of the tank occurred.

Probing did not elicit a change in plasma cortisol, immediately following, or within the first three hours after onset of the stimulus. This lack of increase in plasma cortisol illustrated the varying responses of plasma cortisol to different intensities and duration of stimuli. The duration of the probe stimulus was comparable to the pinching stimulus, however, the intensity may not have been great enough to elicit a response.

Following the low cortisol concentrations at three hours (with the exception of formalin), a six hour peak in plasma cortisol concentration was observed in response to all four stimuli. At this time, a common stimulus was introduced as the lights were turned off at 9:00 pm during the regular photoperiod. The basis of this marked increase in plasma cortisol has not been determined. However, an episodic peak may have occurred during the circadian rhythm of the fish. Diurnal variations in plasma cortisol have been previously described in some salmonids including Atlantic salmon (Nichols and Weisbart, 1984a), brown trout (Pickering and Pottinger, 1983), and rainbow trout (Bry, 1982; Rance et al., 1982), although no rhythm could be detected in fingerling rainbow trout (Barton et al., 1980). Nichols and Weisbart (1984a) reported a significant diurnal variation in catheterized Atlantic salmon with highest values occurring during the evening at 9:00 pm. Bry (1982) also reported a peak in plasma cortisol beginning at 8:30 pm in two out of four catheterized rainbow trout. In addition, Rance et al. (1982) found that plasma cortisol concentrations in rainbow trout were elevated significantly at 8:00 pm. Considering the similar pattern in elevation of cortisol at 9:00 pm in the present study, this may be evidence of a circadian rhythm in the Atlantic
salmon. However, this relationship may be spurious since the onset of darkness occurred at approximately the same time during each experiment (Bry, 1982; Rance et al., 1982; Pickering and Pottinger, 1983; Nichols and Weisbart, 1984a). Therefore, the elevation in plasma cortisol may be related to the change in photoperiod as opposed to a naturally occurring circadian rhythm.

Alternatively, if the sudden change in lighting was the cause of the increase in cortisol, then the light stimulus at time 0 should have elicited a similar response. The degree in elevation of plasma cortisol at time 0, however, was not as pronounced as at the six hour sample time. Therefore, elevated cortisol concentrations at six hours could also be explained as a possible conditioning mechanism whereby cortisol is secreted in anticipation of the lights turning off since the same photoperiod was used throughout the study. Plasma cortisol concentrations had returned to baseline within 24 hours following introduction of each stimulus.

**Catecholamines**

Plasma catecholamine concentrations observed after cannulation surgery, did not elevate significantly in comparison to the basal concentrations (B) (Figure 2.8). Both NE and E have been reported to increase significantly immediately after surgery in eels (Le Bras, 1982) and in rainbow trout (Gingerich and Drottar, 1989). In comparison to plasma NE levels prior to surgery, there was a slight increase in NE after 30 minutes following surgery in the present study (Figure 2.8A). However, the response in plasma NE concentrations reported by Gingerich and Drottar (1989) following surgery was much greater (approximately 30 nmol·L⁻¹). The lack of a significant change in NE in this study is not known. Overall, plasma NE levels during each sampling time, pre
surgery and all subsequent samples afterwards were consistent with basal levels (Table 2.3) and were comparable with previously reported resting values in rainbow trout (Perry et al., 1989).

The elevation in plasma E concentration following surgery, 48.37 ± 32.90 nmol·L⁻¹ in the current study, is comparable to that reported by Gingerich and Drottar (1989). Although, in comparison to the mean pre-surgery value of 40.06 ± 27.96 nmol·L⁻¹, the increase was not significant. This result was probably due to the large individual variation among the fish (Appendix 1) and the limited number of experimental animals. Variability in plasma E, however, is not uncommon among fish. Previous studies have also indicated large individual variations in catecholamine response to various stressors in rainbow trout (Ristori and Luarent, 1985; Gingerich and Drottar, 1989). Nonetheless, the mean pre surgery level of E ('B' - Figure 2.8B) in comparison to basal levels (Table 2.3) may indicate that the fish were stressed before undergoing cannulation surgery. Gingerich and Drottar (1989) report that plasma catecholamine levels in rainbow trout during anaesthesia with TMS (100 mg·L⁻¹) were elevated. At stage II of anaesthesia, plasma NE and E were elevated significantly and remained elevated after righting and during the extended 12 h sampling period. The marked elevation in pre-surgery values of plasma E may, therefore, have been due to the effects of TMS.

TMS has been previously shown to produce stress effects in rainbow trout (Sovio et al., 1977) and other salmonids (Strange and Schreck, 1978). Iwama et al. (1989) reported that adrenaline levels in rainbow trout increased considerably during stage III of anaesthesia with buffered TMS (100 mg·L⁻¹) and continue to increase
during stage I of recovery but returned to baseline by stage II of recovery. The marked reduction in plasma E to 15.08 ± 7.71 nmol·L⁻¹ after 30 minutes (Figure 2.8B) could be due to the clearance of the anaesthetic within the fish. Houston and Woods (1972) report that the blood anaesthetic level of brook trout, following a 15 minute exposure to TMS (100 mg L⁻¹) and a subsequent 15 minute irrigation at the same concentration, were reduced 50 and 90% after 20 and 55 minutes respectively, following transfer to the recovery tank. In the present study, the fish were returned to the experimental tank immediately following completion of the surgery (approximately 15-30 minutes for total surgical procedure) and sampled 30 minutes afterwards. This may have been sufficient time for adequate clearance of the anaesthetic and subsequent depletion of E in the blood circulation to occur.

The variability among experimental fish appeared to be greatest during the extended sampling period following surgery (Figure 2.8B). Again, there were two fish that exhibited high E levels throughout this period. The levels for one experimental fish ranged from 65.5 to 539.1 nmol·L⁻¹ over the extended sampling period and E levels for the second experimental fish ranged from 20.8 to 260.5 nmol·L⁻¹ (Appendix 1). Relative to the other experimental fish, these values were extremely high. An explanation for the continued elevation of E has not been determined. Although the data is not presented in this thesis, hematocrits of the experimental fish were recorded for each sample time throughout the study. Perry et al. (1989) found that acutely anaemic rainbow trout display elevated plasma E concentrations under normoxic conditions. However, the hematocrit of the two outliers over the course of the extended sampling period were comparable to the other experimental fish during both
experiments. Also, there were no signs of reduced activity or overt behavioural activities as an indication of stress or discomfort that distinguished these fish from the other cannulated fish and controls. Nonetheless, the variability among the fish may simply be a result of individual variation such that the severe stress of surgery including adjusting to the catheter and repeated blood sampling may affect the physiology of each fish in a different manner.

Baseline concentrations of plasma catecholamines (Table 2.3) were comparable to previously reported baseline plasma catecholamine concentrations for chronically cannulated rainbow trout (Gingerich and Drottar, 1989).

Results for plasma catecholamine changes during the experiment, indicated that NE and E concentrations were not significantly elevated in response to any of the stimuli employed (Figure 2.9). Previous studies have shown that acute stress induces an elevation of plasma catecholamine in several species of fish (Nakona and Tomlinson, 1967; Mazeaud and Mazeaud, 1981; Gingerich and Drottar, 1989; Perry et al., 1989). The lack of catecholamine response of the Atlantic salmon to the acute stressors employed in this study is not known. Thomas and Perry (1992) postulated that the dominant stimulus for catecholamine release is related to the oxygen requirements of the animal. Studies have demonstrated that intense bursts of activity (Butler et al., 1986; Ristori and Laurent, 1985) and hypoxia (Perry et al., 1991) increased catecholamines in salmonids. Alternatively, Woodward and Smith (1985) reported that NE and E were not significantly increased following a mild agitation in untrained cannulated rainbow trout. The lack of response in catecholamines in this study may
have been due the intensity and/or duration of the stimuli such that the stimuli were not adequate to elicit a response in catecholamines.

Glucose

Plasma glucose concentrations were elevated significantly, in comparison with basal concentrations (B), immediately following surgery and at one and six hours afterwards (Figure 2.10). Glucose concentrations had returned to baseline within 24 hours and concentrations remained consistently low over the latter part of the 14 day sampling period. The pattern of response of plasma glucose to surgery was consistent with previous reports of elevated plasma glucose concentrations in salmonids when subjected to different kinds of stress (Wedemeyer, 1972; Mazeaud et al., 1977; Specker and Schreck, 1980; Barton et al., 1988; Brown et al., 1989). Results for resting plasma glucose concentrations collected during baseline observations were not significantly different from basal concentrations observed in the latter sampling period after surgery. Resting concentrations of plasma glucose in chronically cannulated Atlantic salmon have been reported to range generally from 50-70 mg·dL⁻¹ (Nichols and Weisbart, 1984a). These values are consistent with basal concentrations of plasma glucose observed in this study (see Figure 2.10 and Table 2.4).

Exposure to the four different experimental stimuli employed did not elicit a response in plasma glucose concentrations (Figure 2.11). An elevation in plasma glucose has been reported in response to handling and transport (Wedemeyer, 1972; Specker and Schreck, 1980; Pickering et al., 1982; Barton et al., 1986; Barton et al., 1987) and during acute acidosis (Boutilier et al., 1986). In the present study, plasma glucose was elevated following cannulation surgery but not in response to experimental
stimuli. The dissimilarity in response may imply that the experimental stimuli were not severe enough to elicit a change in plasma glucose.

In summary, plasma E and NE did not respond to the four stressors used in this study nor were they significantly elevated after surgery. A slight increase in catecholamine concentrations was observed after surgery; however, the increase was not statistically significant due to the large variability among the fish. Plasma glucose was significantly elevated after surgery but did not respond to any of the experimental stressors. These findings would imply that a higher intensity and/or longer duration stimulus is required to elicit a glucose response. Plasma cortisol was elevated significantly after surgery and in response to the experimental stressors, suggesting that plasma cortisol may be the most sensitive indicator, among the parameters examined, of stress in Atlantic salmon. Also, the varying patterns of response of cortisol to the different stimuli implied that Atlantic salmon may have some rudimentary form of sensory discrimination. Overall, the results showed that plasma cortisol as previously reported (Barton and Iwama, 1991) is a reliable biochemical indicator to use when evaluating husbandry techniques and housing conditions for Atlantic salmon in laboratory or aquaculture environments.
3.0 BEHAVIOUR

3.1 Introduction

Considerable attention has been directed towards the neuro-endocrine and biochemical changes that occur in fish in response to stressful stimuli (Barton and Iwama, 1991). These changes are categorized as primary and secondary effects, respectively. In response to a chronic stressor such as prolonged confinement or poor water quality conditions, fish can exhibit tertiary or 'whole animal' responses such as immunological changes (Pickering, 1989; Pickering and Pottinger, 1989) and growth suppression (Pickering, 1993). Concurrently, behavioural changes can also occur when a fish is exposed to stressful stimuli. These changes are also referred to as 'whole animal' responses. In terms of response time, behavioural responses comprise the first line of defence whereby the fish can respond within seconds to avoid an aversive situation. It is important then to recognise that different levels of organization, such as biochemical and behavioural, are all involved in the response of fish to (external) stressful stimuli.

In a commercial or laboratory environment, fish are continuously receiving and responding to various kinds of external stimuli. Examples of stimuli are light, such as artificial light cycles (photoperiods), temperature, physical disturbances, including handling and transport, and chemicals employed for the treatment of infection. Over the years, behavioural changes associated with external stimuli, have been used as indicators of stress in fish. A prime example is the use of behavioural patterns in fish as an indication of environmental toxicity. The phrase 'behavioural toxicity' has been used to describe a situation where a change in the environment produced a behavioural
change but not a corresponding anatomical or physiological response (Marcucella and Abramson, 1978). Fishes have been shown to exhibit direct behavioural responses to various chemicals introduced in a laboratory environment. Traditionally, behavioural patterns such as locomotion, avoidance reaction, attraction, and feeding behaviour have been assessed as indicators of environmental toxicity (Steele, 1983; Rand and Petrocelli, 1985; Hadjinicolou, and LaRoche, 1988; Doving, 1991). There is overwhelming evidence that fishes possess the ability to respond behaviourally to water-borne chemicals. Beitingler and Freeman (1983) reported that fish made avoidance and/or selection behaviour responses following exposure to 46 out of 75 chemicals. Less specific behavioural observations have been reported for fish exposed to chemicals used to treat infection. Behaviours such as changes in general activity within the tank, ventilatory movements and skin colouration have been observed in rainbow trout (Powell et al., 1994).

A commonly used chemical toxin in salmonid aquaculture is formalin (37% formaldehyde). Formalin is an organic chemical that is often used as a bath treatment for the control of ectoparasites such as *Ichthyobodo necator* and *Trichodina* species (Piper et al., 1986). The stress involved with the use of this treatment has been of some concern to the aquaculture industry. Studies have been conducted to assess the effects on both growth (Kristjansson et al., 1995) and physiological stress responses (Wedemeyer, 1971; Wedemeyer and Yasutake, 1974) associated with formalin use. Treatment with 200 ppm formalin significantly elevated cortisol levels in steelhead trout, three and six hours following exposure (Wedemeyer and Yasutake, 1974). Behavioural responses of fish exposed to formalin, however, are not as well
documented as physiological responses. Summerfelt and Lewis (1967) reported that
green sunfish, *Lepomis cyanellus*, did not exhibit overt behavioural avoidance to
concentrations of formalin as high as 1000 mg·L⁻¹. Despite this fact, the reported
physiological changes in different salmonid species indicate that the fish experience
some stress (Wedemeyer, 1971; Wedemeyer and Yasutake, 1974). It is, therefore,
possible that behavioural changes may also occur in salmonids following exposure to
chemicals such as formalin.

Fishes are generally characterized as 'photonegative' (photophobic or
scotophilic) or 'photopositive' (photophilic) in response to light. In teleosts,
adaptation of the eye to changes in light conditions occurs by way of the retino-motor
movements where visual cells and pigments move within the retina (see Chapter
Section 1.3.1.3(i)). Categorization of the response of fish to different light intensities
can be determined by their preference for light or dark areas within the environment.
Juvenile Atlantic salmon have been described as photonegative in response to a high
light stimulus and have exhibited a negative phototaxis to light intensities ranging from
0.2 to 200 foot candles (Pinhorn and Andrews, 1963). However, Atlantic salmon have
also been shown to be predominantly photopositive in the case where shelter, such as
pebbles and rocks, is available (Gibson and Keenleyside, 1966). These opposing views
could be attributed to differences in experimental design whereby housing conditions
and illumination sources varied. A study by Ferno and associates (1995) indicated that
Atlantic salmon reared in net pens, migrate downwards in response to increased light
levels. This photonegative response can be attributed to an evolutionary response to the
risk factor of predation in connection with increased visibility of the fish (Levy, 1990).
Researchers examining the effects of natural and artificial photoperiods on fish often monitor locomotor activity as a measure of behavioural response. Behavioural patterns of salmonids under different lighting conditions have provided insight into the mechanisms for seaward migration (Hoar et al., 1957), the transformation from parr to smolt stage of development (Pinhorn and Andrews, 1963), fish distribution (Ferno et al., 1995) and daily motor activity rhythms (Varanelli and McCleave, 1974; Richardson and McCleave, 1974). As with other animals, fish experience daily variations in locomotor activity with fluctuating light levels during a 24 hour period. Ali (1964) reported that yearling Atlantic salmon generally exhibit diurnal (light active) locomotor activity. Subsequent studies also suggested a daily locomotor periodicity in juvenile Atlantic salmon (Varanelli and McCleave, 1974; Richardson and McCleave, 1974). These studies reported locomotor behaviour to be mainly diurnal in nature; however, a significant number of fish exhibited nocturnal and light-change-active (ie. active when lighting changes) behaviour.

In addition to chemical toxins and the effects of artificial light, fish are often exposed to several other stressors in a laboratory environment. For experimental purposes, fish are routinely housed in an artificial environment where optimum water quality conditions are difficult to maintain. Behavioural responses such as orientation and movement, changes in activity, respiratory rate, and interactivity within the group may be important indicators of the status of the fish. Circular tanks are often used to accommodate fish in research facilities. In circular tanks the fish tend to crowd to the outer perimeter creating a localized stocking density much greater than calculated for the tank (Rosenthal, 1987). Crowded conditions for salmon fry and parr have been
shown to lead to frequent and often prolonged bouts of fighting during the defence of territories within an aquarium (Keenleyside and Yamamoto, 1962). Studies have shown that chronic confinement stress can result in reduced swimming performance (Strange and Cech, 1992) and affect the formation of a social structure within the group (Pottinger and Pickering, 1992). In addition, behavioural responses of fish at high stocking densities in aquaria have also been shown to provide information about migratory behaviour. Lapkin et al. (1989) postulate that the circular movement of fish in aquaria at high stocking densities can be used as a test to determine the species migration period in nature.

Studies on fish behaviour have not been limited to laboratory conditions. In recent years, several studies relating to salmonid fish farming have focused on the behavioural responses of fish in enclosed environments. Effects of lighting have been shown to influence the vertical distribution of fish within a net pen. This was demonstrated in Atlantic salmon, whereby surface light avoidance was exhibited in response to increased light intensities (Ferno et al., 1995). This photonegative response could be an important indicator of the proper lighting required for Atlantic salmon in a restricted environment. Flow rate of water into an enclosure can also affect the behaviour of fish. For example, in cage structures the flow of water is often hindered by factors such as the framework and netting. The distribution and distance between rainbow trout was significantly influenced by wave frequencies and height (Srivastava et al., 1989). Dissolved oxygen also has a direct effect on behaviour. Kramer (1987) reviewed the behavioural responses of fishes to reduced levels of dissolved oxygen and reported that the principal categories of behavioural response include changes in
activity, vertical or horizontal habitat changes, and increased use of aquatic surface respiration. Additional behaviours such as increased surface activity can indicate the status of the fish. Furevik et al. (1993) reported that increased surface activity in Atlantic salmon, such as rolling and leaping, indicated recent acute stress or present acute stress.

The role of behaviour as an indicator of the status of the fish is an important component in determining optimal conditions for fish in aquaculture and in experimental studies. This reasoning is supported by Mello (1975) who stated that "The behaviour of the organism is the endpoint of the functional integration of the nervous system encompassing sensory, motor, and cognitive aspects. The functional capacity of the central nervous system cannot be determined by histological or even physiological studies independent of behavioural analysis".

The main objective of the following study was to develop a method of quantifying Atlantic salmon behaviour and to determine if different stimuli elicit behavioral changes in comparison to 'normal’ or baseline behaviour in a laboratory setting. Biochemical data collected concurrently with the behavioural recordings would allow for comparison of the physiological state with the corresponding behaviour (see Chapter 2). If different stimuli elicit distinct behavioural responses, then monitoring behavioural changes could become a useful tool in determining stress in fish housed in laboratory environments.
3.2 Materials and Methods

Description of the experimental animals, animal housing, and overview of study design have been previously described in Chapter 2 in Section 2.2.1, 2.2.2, and 2.2.3, respectively.

3.2.1 Video-taping

A video camera (Panasonic, WV-BL 200, CCTV Camera) was positioned directly above the center of each tank (see Figure 2.1 in previous chapter). The cameras were attached to a threaded rod and bracket which was mounted to the ceiling such that the camera was positioned 60 cm above the surface of the tank. A wide angle lens (Cosmicar TV Lens, 3.7mm, 1:1.6) was used to provide full view of all three sections within each tank. The cameras were connected to two separate video cassette recorders in an adjoining room, allowing for continuous recording of each tank and to a common monitor for viewing the tanks without disturbing the fish. The video-taping regime employed in both Experiment 1 and 2 is outlined in Table 3.1.

3.2.2 Ethogram

A standard ethogram was developed based on personal observation of a group (N=9) of Atlantic salmon in a free swimming state within a circular tank where the fish were able to interact with one another without the confines of the dividers. A qualitative assessment of the fish formed a basis for classification of the most common salmonid behaviours that could be easily observed. The same fish were then observed in a partially confined state, whereby the tank was divided into three equal sections with three fish per section. Following the qualitative observations in the confined state, fish were monitored via videocamera to test if the observed behaviours were recognizable
Table 3.1: Videotaping schedule for monitoring Atlantic salmon behaviour.

<table>
<thead>
<tr>
<th>RECORDING PERIOD</th>
<th>TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline 1</td>
<td>3:00-4:00 pm (Every second day for eleven days)</td>
</tr>
<tr>
<td>(Pre Surgery)</td>
<td></td>
</tr>
<tr>
<td>Baseline 2</td>
<td>3:00-4:00 pm (Every second day for eleven days)</td>
</tr>
<tr>
<td>(Post Surgery)</td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>2:30-4:30 pm (Each experimental day)</td>
</tr>
</tbody>
</table>

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and quantifiable on video-tape. The camera lens included the circumference of the tank, but an overhead view of the fish resulted in limitations on the number of behaviours that could be clearly identified on video-tape. Based on both the qualitative and quantitative analysis of behaviours of Atlantic salmon on videotape, an ethogram was created consisting of six behaviours categorized in two main groups: movement and interaction. A qualitative description of each behaviour is presented in Table 3.2. Although the ethogram used in the current study was derived from personal observation, similar behaviours have been previously described for Atlantic salmon in a laboratory environment (Keenleyside and Yamamoto, 1962).

3.2.3 Data Collection

Atlantic salmon were videotaped during three time periods as described in Table 3.1. A computer program was developed specifically for the quantification of the behaviours described previously in the ethogram (Table 3.2). The program was designed to allow recording of the six behaviours for each of the three fish simultaneously over each fifteen minute period. This design permitted concurrent behavioural data collection of all fish within one section of the tank. Duration of each movement was measured in seconds with a corresponding frequency of movements to determine the average length of time spent in continuous and sporadic motion. Interactive behaviours were measured as frequencies of events.

Six days of video-recordings were accumulated over an eleven day period for Baseline 1 and Baseline 2 (Table 3.1). Behavioural data was collected from Day 3 and Day 11 as a representation of baseline behaviours during both the pre and post surgery
Table 3.2: Ethogram for the quantitative analysis of Atlantic Salmon behaviour.

<table>
<thead>
<tr>
<th>BEHAVIOUR</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOVEMENT</td>
<td></td>
</tr>
<tr>
<td>Continuous (CM)</td>
<td>Any movement from a position of rest or ‘station’ that continues for more than 10 seconds.</td>
</tr>
<tr>
<td>Sporadic (SM)</td>
<td>Any movement from a position of rest or ‘station’ that continues for 10 seconds or less.</td>
</tr>
<tr>
<td>INTERACTION</td>
<td></td>
</tr>
<tr>
<td>Attack</td>
<td>Attack behaviour consisting of repeated quick charges towards an opponent.</td>
</tr>
<tr>
<td>Retreat</td>
<td>Avoidance or fleeing from attack.</td>
</tr>
<tr>
<td>Displacement</td>
<td></td>
</tr>
<tr>
<td><em>Asynchronous</em></td>
<td>One fish displaces another individual from its ‘station’.</td>
</tr>
<tr>
<td>(ASD)</td>
<td></td>
</tr>
<tr>
<td><em>Synchronous</em></td>
<td>One fish displaces all other members of the group from their respective ‘stations’.</td>
</tr>
<tr>
<td>(SD)</td>
<td></td>
</tr>
</tbody>
</table>
periods. Experimental data was collected over a two hour period (Table 3.1). Details of the videotaping regime for both baseline and the experimental period are provided in Figure 3.1.

3.2.4 Data Analysis

Movement

Movement behaviour was divided into 4 different components: continuous, sporadic, total time, and total frequency, for statistical analysis. Continuous movement was calculated by dividing the time spent in continuous movement by the frequency of continuous movements. Sporadic movement was calculated in a similar manner whereby time spent in sporadic movement was divided by the frequency of sporadic movement. Total time and total frequency represented the combined data of time spent in continuous and sporadic motion and combined data of continuous and sporadic frequency of movement, respectively.

Statistical analysis of the movement (continuous, sporadic, total time, and total frequency) data was performed using the SAS statistical software package (SAS Institute, Inc., 1985). Baseline and experimental data were statistically analysed using a General Linear Models Procedure (Proc GLM) with a repeated measures design to account for the variability between the fish. Data for each behaviour were analysed for three treatment groups: long cannula or experimental (LC), short cannula or replacement (SC), and control (C) (see Section 2.2.2 in previous chapter). The fish were subsequently nested within the treatment groups for analysis. Details of the comparison tests performed for baseline and experimental periods are provided in Table 3.3. All comparisons were considered significant at the 5% probability level. Data
Figure 3.1: Data collection schedule for analysis of Atlantic salmon behaviour during Baseline 1 and 2 (A) and Experimental (B) period. Shaded area indicates 15 minute periods of data collection for analysis. Arrow indicates introduction of stimulus. * indicates videorecording day.
Table 3.3: Data analysis of movement behaviour of Atlantic salmon during baseline and experimental observations (see Figure 3.1). TG = treatment group, Pre = before cannulation surgery, Post = after cannulation surgery.

<table>
<thead>
<tr>
<th>BEHAVIOUR</th>
<th>RECORDING PERIOD</th>
<th>COMPARISON</th>
<th>METHOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous, Sporadic, Total time and Total frequency</td>
<td>Baseline (TG)</td>
<td>Day 3 and Day 11</td>
<td>Proc General Linear Models Procedure (SAS):</td>
</tr>
<tr>
<td></td>
<td>Pre</td>
<td></td>
<td>• Fish nested within treatment group</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td></td>
<td>• Repeated measures design with post hoc Scheffes test</td>
</tr>
<tr>
<td></td>
<td>Pre/Post</td>
<td>Pre (Day 3 + 11) and Post(Day 3 + 11)</td>
<td></td>
</tr>
<tr>
<td>Experimental (TG)</td>
<td>Light</td>
<td>2:30, 3:00, 3:30, and 4:00 pm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pinch</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Formalin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
obtained from Experiment 1 and 2 were not statistically different and were therefore pooled for analysis. Means (± SEM) represent pooled data. Data were transformed using square root and log_{10} where applicable to increase homogeneity of variances.

**Interactive Behaviour**

Details of the statistical analysis of ASD and SD behaviours are provided in Table 3.4. Analysis of attack and retreat behaviours was not performed because the observed frequencies were too low to allow for meaningful analysis. ASD behaviour during baseline observations was analysed using a McNemar's test at the 5% probability level. Analysis of the ASD experimental data was performed by comparing the patterns of occurrence (PO) of the three treatment groups using a Chi-square test (p ≤ 0.05). PO was determined by comparing the number of fish that displayed ASD behaviour before (B) and after (times 0, 1, and 3 hours) introduction of the stimuli. The four most common patterns of response displayed over the course of the experimental period were: no change, fluctuate, increase, and decrease, therefore these four patterns were used in the analysis. Further analysis of the experimental data was performed by combining the three treatment groups to determine the difference between stimuli using a Chi-square test (p ≤ 0.05). Analysis of SD behaviour was performed by McNemar's tests (p ≤ 0.05) for both the baseline and experimental recording periods. Frequency of SD behaviour was analysed for each separate section of the tanks because no distinction could be made between treatment groups.
Table 3.4  Data analysis of interactive behaviour of Atlantic salmon during baseline and experimental observations (see Figure 3.1). TG = treatment group, PO = pattern of occurrence, Pre = before cannulation surgery, Post = after cannulation surgery.

<table>
<thead>
<tr>
<th>BEHAVIOUR</th>
<th>RECORDING PERIOD</th>
<th>COMPARISON</th>
<th>METHOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asynchronous Displacement</td>
<td>Baseline (TG)</td>
<td>Pre (Day 3 + 11) and Post (Day 3 + 11)</td>
<td>McNemar’s Test</td>
</tr>
<tr>
<td></td>
<td>Pre/Post</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental (TG)</td>
<td>Light</td>
<td>PO: no change, fluctuates increase, and decrease</td>
<td>Chi-square (Minitab)</td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pinch</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Formalin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental (Stimuli)</td>
<td></td>
<td>PO: no change, fluctuates, increase, and decrease</td>
<td>Chi-square (Minitab)</td>
</tr>
<tr>
<td>Synchronous Displacement</td>
<td>Baseline (Section)</td>
<td>Pre (Day 3 + 11) and Post (Day 3 + 11)</td>
<td>McNemar’s Test</td>
</tr>
<tr>
<td></td>
<td>Pre/Post</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental (Section)</td>
<td>Light</td>
<td>Before (2:30 pm) and after (3:00 + 3:30 + 4:00 pm)</td>
<td>McNemar’s Test</td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pinch</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Formalin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.3 Results

The results obtained in each major behavioural category (movement and interaction) are summarized according to sub-category for both the surgical (Pre and Post) and Experimental time periods.

3.3.1 Movement

3.3.1.1 Continuous Movement

*Pre (Baseline 1) and Post Surgery (Baseline 2)*

The results of baseline continuous movement (CM) behaviour for the three treatment groups; LC, SC, and C, are presented in Figure 3.2. There was no significant difference in average time spent in CM between the treatment groups nor was there any significant change in behaviour over the two days of Baseline 1 observations. The average time that the fish (including all treatment groups) engaged in CM behaviour ranged from 16.48 ± 7.74 to 39.24 ± 19.72 seconds.

This behaviour was significantly altered (p = 0.0480) in all groups after surgical cannulation of the SC and LC fish. Figure 3.2 shows the decrease in time spent in CM during baseline observations following a two week acclimation period after surgery. Following surgical intervention, the time spent in CM was reduced in all three treatment groups with a range of CM activity from 7.60 ± 3.52 to 16.15 ± 6.35 seconds. No significant difference in CM following surgery was found within or between the two days during Baseline 2 observations or between treatment groups (Figure 3.2). Table 3.5 shows the frequency of CM behaviour prior to and after surgery. The data indicate that the frequency of CM behaviour decreased considerably after surgery in relation to the decrease in time spent in CM.
Figure 3.2: Mean (± SEM) time (sec) spent in continuous movement for each treatment group recorded at two times on each of two days prior to and after surgery. ** indicates significant difference from corresponding pre-surgery value (p ≤ 0.05). Values represent 8-10 fish.
Table 3.5: Frequency of movement behaviour during baseline observations for three treatment groups before (PRE) and after (POST) catheterization of the dorsal aorta of Atlantic salmon. C = control, LC = long cannula, SC = short cannula. N = 8-10 fish.

<table>
<thead>
<tr>
<th>MOVEMENT</th>
<th>DAY</th>
<th>PRE</th>
<th>POST</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>LC</td>
</tr>
<tr>
<td>Continuous</td>
<td>3</td>
<td>110</td>
<td>198</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>133</td>
<td>147</td>
</tr>
<tr>
<td>Sporadic</td>
<td>3</td>
<td>86</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>154</td>
<td>142</td>
</tr>
</tbody>
</table>
**Experimental**

Exposure to the light stimulus did not significantly alter CM behaviour of the fish in comparison to the recording period before (B) stimulation (Figure 3.3). There was, however, a significant decrease ($p = 0.0265$) in CM 30 minutes after stimulation was observed in the controls and the LCs. Activity level of the LCs remained low after one hour. The control fish appeared to increase the average time spent in CM after one hour, but due to the large variability among fish this increase was not statistically significant (Figure 3.3). There was no significant change in CM activity among the SC fish.

Introduction of formalin did not produce a significant change in CM behaviour within the treatment groups in comparison to 'B' (Table 3.6). There was, however, considerable variation in activity levels of each group over the course of the experimental period. The LCs and controls appeared more active with respect to CM behaviour than the SCs. Because of the large variability within each treatment group the increase was not statistically significant.

With respect to the mechanical stimuli, pinch and probe, only the experimental fish were exposed to the stimulus directly. Following the pinch stimulus, CM activity changed among all three treatment groups (Figure 3.4A). The overall change resulted in a significant increase in the amount of time spent in CM ($p = 0.0036$) immediately after stimulation. The controls at this time appeared to exhibit the greatest response. However, closer examination of the data revealed that this increase appeared to be due to an outlier whereby one fish exhibited an extended time in CM (132.00 seconds). The activity levels decreased after 30 minutes ($p = 0.0036$) among all treatment groups,
Figure 3.3: Mean (± SEM) time (sec) spent in continuous movement for each treatment group prior to (B) and following introduction of the light stimulus. Values represent 7-9 fish. 'B' denotes video-recording period 30 minutes prior to stimulation. Introduction of the stimulus (3:00 pm) is indicated by the arrow. 'a' indicates significant difference from B (p ≤ 0.05), 'b' indicates significant difference from time 0 (p ≤ 0.05).
Table 3.6: Mean (± SEM) time (sec) spent in continuous movement for three treatment groups of Atlantic salmon during the experimental period for formalin stimulus. LC = long cannula, SC = short cannula. ‘B’ denotes video-recording period at 2:30 pm. Introduction of stimulus (3:00 pm) is indicated by the arrow.

<table>
<thead>
<tr>
<th>TREATMENT GROUP</th>
<th>ELAPSED TIME</th>
<th>N</th>
<th>MEAN</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>B</td>
<td>8</td>
<td>37.21</td>
<td>12.84</td>
</tr>
<tr>
<td></td>
<td>→ 0</td>
<td>8</td>
<td>37.28</td>
<td>4.86</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>7</td>
<td>60.77</td>
<td>30.64</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>8</td>
<td>51.82</td>
<td>11.35</td>
</tr>
<tr>
<td>LC</td>
<td>B</td>
<td>9</td>
<td>48.95</td>
<td>29.04</td>
</tr>
<tr>
<td></td>
<td>→ 0</td>
<td>9</td>
<td>38.73</td>
<td>10.90</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>8</td>
<td>41.92</td>
<td>15.48</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>9</td>
<td>28.91</td>
<td>9.04</td>
</tr>
<tr>
<td>SC</td>
<td>B</td>
<td>8</td>
<td>16.3</td>
<td>3.95</td>
</tr>
<tr>
<td></td>
<td>→ 0</td>
<td>8</td>
<td>21.90</td>
<td>4.02</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>7</td>
<td>20.16</td>
<td>5.88</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>8</td>
<td>20.27</td>
<td>4.97</td>
</tr>
</tbody>
</table>
Figure 3.4: Mean (± SEM; 'time (sec) spent in continuous movement for each treatment group prior to (B) and following introduction of the pinch (A) and probe (B) stimulus. Values represent 7-9 fish. 'B' denotes video-recording period 30 minutes prior to stimulation. Introduction of the stimulus (3.00 pm) is indicated by the arrow. 'a' indicates significant difference from B, 'b' indicates significant difference from time 0, 'c' indicates significant difference from time 0.5, (p ≤ 0.05).
but remained low for only the cannulated fish at one hour. The total increase in time spent in CM of the control fish at one hour was again the result of one fish swimming continuously for a prolonged period of time (253.43 seconds) (Figure 3.4A).

Probing did not result in a significant change in CM of the LC or alter the CM behaviour of the other fish (Figure 3.4B). Introduction of the probe stimulus appeared to increase activity in each group at time 0, however, the elevation was not significantly greater in comparison to the recording time before stimulation. There was a significant decrease in the time spent in CM for all groups at the later recording times following stimulation (30 minutes and one hour) in comparison to time 0 (p = 0.0070) (Figure 3.4B).

3.3.1.2 Sporadic Movement

Pre (Baseline 1) and Post (Baseline 2) Surgery

There was no significant change in average time spent in SM between Day 3 and 11 during baseline observations prior to surgery nor was there any difference between the treatment groups (Table 3.7). Similar results were found following surgery (Table 3.7) whereby no significant change in SM behaviour was found between the pre and post surgery baseline observational periods or between treatment groups. There was some variability in frequency of SM behaviour between the baseline days before surgery but after surgery, frequency of SM was relatively consistent for the SCs and the controls (Table 3.5).
Table 3.7: Mean (± SEM) time (sec) spent in sporadic movement for three treatment groups of Atlantic salmon before (PRE) and after (POST) catheterization of the dorsal aorta. LC = long cannula, SC = short cannula. N = 8-10 fish.

<table>
<thead>
<tr>
<th>TREATMENT GROUP</th>
<th>DAY</th>
<th>TIME (pm)</th>
<th>PRE</th>
<th>POST</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MEAN</td>
<td>SEM</td>
<td>MEAN</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>3:00</td>
<td>2.88</td>
<td>3.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3:30</td>
<td>4.04</td>
<td>3.67</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>3:00</td>
<td>4.44</td>
<td>4.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3:30</td>
<td>4.40</td>
<td>3.34</td>
</tr>
<tr>
<td>LC</td>
<td>3</td>
<td>3:00</td>
<td>3.27</td>
<td>4.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3:30</td>
<td>3.03</td>
<td>4.42</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>3:00</td>
<td>4.79</td>
<td>2.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3:30</td>
<td>4.84</td>
<td>4.50</td>
</tr>
<tr>
<td>SC</td>
<td>3</td>
<td>3:00</td>
<td>3.69</td>
<td>3.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3:30</td>
<td>4.48</td>
<td>3.92</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>3:00</td>
<td>4.25</td>
<td>4.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3:30</td>
<td>5.07</td>
<td>4.15</td>
</tr>
</tbody>
</table>
Experimental

Only the light stimulus produced a significant change \((p = 0.0349)\) in average time spent in SM after introduction of the stimulus (Table 3.8). The average time spent in SM ranged from 1.0 to 5.2 seconds for all four stimuli (Table 3.8).

3.3.1.3 Total Time in Motion

Pre (Baseline 1) and Post (Baseline 2) Surgery

Baseline locomotor behaviour prior to surgery (Figure 3.5) was found to be significantly greater \((p = 0.0001)\) with respect to total time moving than after surgery (Figure 3.5). Also, there was a significant difference \((p = 0.0017)\) between the controls and the cannulated groups whereby the control fish, on average, spent more time in a stationary position after surgery. Total frequency of movement was also found to be significantly greater \((p = 0.0001)\) prior to surgery than afterwards (Table 3.9).

Experimental

Following the light stimulus total time in motion of each treatment group did not change significantly over the course of the experimental period (Table 3.10). However, a marked decrease in total time in motion 30 minutes after stimulation was observed in comparison to pre-stimulus locomotor activity. There was a significant difference between the treatment groups \((p = 0.0002)\) whereby the control fish were generally more active than the cannulated fish during each recording period.

Locomotor activity of the treatment groups prior to and after introduction of formalin did not significantly change over the length of the experiment (Table 3.11). There was a difference between the three treatment groups. The control fish were
Table 3.8: Mean (± SEM) time (sec) spent in sporadic movement for three treatment groups of Atlantic salmon during the experimental period. LC = long cannula, SC = short cannula. B denotes video-recording period at 2:30 pm. Introduction of stimulus (3:00 pm) is indicated by the arrow. a indicates significant difference from B (p ≤ 0.05).

<table>
<thead>
<tr>
<th>TREATMENT GROUP</th>
<th>ELAPSED TIME</th>
<th>N</th>
<th>LIGHT</th>
<th>FORMALIN</th>
<th>PINCH</th>
<th>PROBE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>B</td>
<td>6-8</td>
<td>3.13 ± 0.72</td>
<td>3.45 ± 0.95</td>
<td>4.22 ± 0.91</td>
<td>3.44 ± 1.41</td>
</tr>
<tr>
<td></td>
<td>→ 0</td>
<td>6-8</td>
<td>4.78 ± 0.81a</td>
<td>4.21 ± 0.97</td>
<td>3.73 ± 0.73</td>
<td>1.72 ± 0.70</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>5-7</td>
<td>3.65 ± 0.89</td>
<td>3.92 ± 0.76</td>
<td>2.51 ± 0.68</td>
<td>2.36 ± 1.05</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6-8</td>
<td>4.91 ± 1.02</td>
<td>2.69 ± 0.63</td>
<td>2.75 ± 0.92</td>
<td>2.48 ± 1.01</td>
</tr>
<tr>
<td>LC</td>
<td>B</td>
<td>7-9</td>
<td>3.21 ± 0.53</td>
<td>2.51 ± 0.84</td>
<td>4.27 ± 0.93</td>
<td>2.62 ± 0.99</td>
</tr>
<tr>
<td></td>
<td>→ 0</td>
<td>7-9</td>
<td>4.58 ± 0.96a</td>
<td>4.42 ± 0.65</td>
<td>4.42 ± 0.50</td>
<td>2.67 ± 1.01</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>6-8</td>
<td>3.24 ± 0.83</td>
<td>3.54 ± 0.56</td>
<td>2.98 ± 0.89</td>
<td>1.97 ± 0.80</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>7-9</td>
<td>2.64 ± 0.89</td>
<td>3.86 ± 0.87</td>
<td>2.87 ± 1.18</td>
<td>3.02 ± 1.14</td>
</tr>
<tr>
<td>SC</td>
<td>B</td>
<td>6-8</td>
<td>2.52 ± 0.79</td>
<td>2.93 ± 0.49</td>
<td>4.31 ± 1.01</td>
<td>2.13 ± 0.87</td>
</tr>
<tr>
<td></td>
<td>→ 0</td>
<td>6-8</td>
<td>3.56 ± 0.76a</td>
<td>2.78 ± 0.55</td>
<td>3.42 ± 0.84</td>
<td>1.53 ± 0.62</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>5-7</td>
<td>3.39 ± 0.83</td>
<td>3.95 ± 0.58</td>
<td>3.02 ± 0.80</td>
<td>1.63 ± 0.73</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6-8</td>
<td>3.21 ± 1.01</td>
<td>3.42 ± 0.65</td>
<td>3.32 ± 1.11</td>
<td>1.81 ± 0.74</td>
</tr>
</tbody>
</table>
Figure 3.5: Mean (± SEM) total time (sec) in motion for each treatment group recorded at two times on each of two days prior to and after surgery. ‘*’ indicates significant difference from corresponding pre-surgery value (p ≤ 0.05). Values represent 8-10 fish.
Table 3.9: Mean (± SEM) total frequency of movement for three treatment groups of Atlantic salmon before (PRE) and after (POST) catheterization of the dorsal aorta. LC = long cannula, SC = short cannula. N = 8-10 fish.

<table>
<thead>
<tr>
<th>TREATMENT GROUP</th>
<th>DAY</th>
<th>TIME (pm)</th>
<th>PRE MEAN</th>
<th>PRE SEM</th>
<th>POST MEAN</th>
<th>POST SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>3:00</td>
<td>10.00</td>
<td>3.82</td>
<td>11.11</td>
<td>3.54</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3:30</td>
<td>9.63</td>
<td>3.30</td>
<td>11.44</td>
<td>3.61</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>3:00</td>
<td>15.38</td>
<td>2.33</td>
<td>10.33</td>
<td>2.55</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>3:30</td>
<td>14.00</td>
<td>2.51</td>
<td>9.89</td>
<td>2.77</td>
</tr>
<tr>
<td>LC</td>
<td>3</td>
<td>3:00</td>
<td>16.80</td>
<td>2.76</td>
<td>11.90</td>
<td>3.14</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3:30</td>
<td>13.50</td>
<td>2.70</td>
<td>12.70</td>
<td>2.41</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>3:00</td>
<td>15.10</td>
<td>1.52</td>
<td>4.70</td>
<td>1.77</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>3:30</td>
<td>13.80</td>
<td>2.08</td>
<td>7.30</td>
<td>2.44</td>
</tr>
<tr>
<td>SC</td>
<td>3</td>
<td>3:00</td>
<td>13.00</td>
<td>2.60</td>
<td>9.80</td>
<td>3.07</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3:30</td>
<td>10.13</td>
<td>1.72</td>
<td>9.30</td>
<td>3.04</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>3:00</td>
<td>16.12</td>
<td>2.20</td>
<td>9.70</td>
<td>2.89</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>3:30</td>
<td>13.38</td>
<td>2.67</td>
<td>8.90</td>
<td>2.61</td>
</tr>
</tbody>
</table>
Table 3.10: Mean (± SEM) time (sec) spent in continuous movement for three treatment groups of Atlantic salmon during the experimental period for light stimulus. LC = long cannula, SC = short cannula. B denotes video-recording period at 2:30 pm. Introduction of stimulus (3:00 pm) is indicated by the arrow. a indicates significant difference from LC and SC (p ≤ 0.05), b indicates significant difference from SC (p ≤ 0.05).

<table>
<thead>
<tr>
<th>TREATMENT GROUP</th>
<th>ELAPSED TIME</th>
<th>N</th>
<th>MEAN</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>B</td>
<td>8</td>
<td>322.98(^b)</td>
<td>110.31</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>8</td>
<td>299.03(^a)</td>
<td>125.39</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>7</td>
<td>143.82(^a)</td>
<td>102.88</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>7</td>
<td>352.06(^a)</td>
<td>141.34</td>
</tr>
<tr>
<td>LC</td>
<td>B</td>
<td>9</td>
<td>265.04</td>
<td>102.17</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>9</td>
<td>160.96</td>
<td>49.21</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>8</td>
<td>82.82</td>
<td>45.54</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>8</td>
<td>115.51</td>
<td>52.38</td>
</tr>
<tr>
<td>SC</td>
<td>B</td>
<td>8</td>
<td>126.01</td>
<td>40.92</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>8</td>
<td>77.53</td>
<td>25.39</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>7</td>
<td>88.44</td>
<td>51.22</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>7</td>
<td>74.30</td>
<td>30.93</td>
</tr>
</tbody>
</table>
Table 3.11: Mean (± SEM) total time (sec) in motion for three treatment groups of Atlantic salmon during the experimental period for formalin stimulus. LC = long cannula, SC = short cannula. ‘a’ indicates significant difference from LC and SC (p ≤ 0.05). ‘B’ denotes video-recording period at 2:30 pm. Introduction of stimulus (3:00 pm) is indicated by the arrow.

<table>
<thead>
<tr>
<th>TREATMENT GROUP</th>
<th>ELAPSED TIME</th>
<th>N</th>
<th>MEAN</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>B</td>
<td>8</td>
<td>423.14^a</td>
<td>127.99</td>
</tr>
<tr>
<td></td>
<td>→ 0</td>
<td>8</td>
<td>441.53^a</td>
<td>90.00</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>7</td>
<td>485.04^a</td>
<td>134.97</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>9</td>
<td>282.55</td>
<td>119.12</td>
</tr>
<tr>
<td>LC</td>
<td>B</td>
<td>9</td>
<td>178.77</td>
<td>88.86</td>
</tr>
<tr>
<td></td>
<td>→ 0</td>
<td>9</td>
<td>253.95</td>
<td>78.08</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>8</td>
<td>187.17</td>
<td>105.48</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>7</td>
<td>281.87</td>
<td>105.56</td>
</tr>
<tr>
<td>SC</td>
<td>B</td>
<td>8</td>
<td>56.05</td>
<td>16.38</td>
</tr>
<tr>
<td></td>
<td>→ 0</td>
<td>8</td>
<td>142.88</td>
<td>42.63</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>7</td>
<td>186.96</td>
<td>118.53</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>9</td>
<td>212.33</td>
<td>65.81</td>
</tr>
</tbody>
</table>
significantly more active \((p = 0.0023)\) than the cannulated fish before, immediately following, and 30 minutes after application of the stimulus. Activity of the control fish started to decline within one hour following introduction of the formalin.

Pinching the experimental fish was the only stimulus that significantly \((p = 0.0058)\) increased total time spent moving immediately after introduction of the stimulus (Figure 3.6A). During the experimental period the control fish were repeatedly more active than the cannulated fish as demonstrated with the formalin and light stimulus \((p = 0.0012)\). After 30 minutes, total time in motion was reduced significantly \((p = 0.0058)\), in all treatment groups, in comparison to time 0, returning to pre-stimulus values at the one hour recording time.

Probing the experimental fish slightly increased the total time in motion of each treatment group but not significantly in comparison to the pre-stimulus recording time. There was a marked decrease \((p = 0.0018)\) in locomotor activity after 30 minutes in all groups which was maintained at the one hour recording period (Figure 3.6B).

Probing the fish was the only stimulus that produced an increase in frequency of movement \((p = 0.0120)\) which occurred immediately following stimulation (Table 3.12) but an increase was evident in only the cannulated fish. The SCs were found to move more frequently \((p = 0.0001)\) in comparison to the other treatment groups after the probe stimulus. Although there was no significant change in frequency of SM behaviour, there was a significant difference between treatment groups following the formalin and pinch stimulus. Following the formalin and pinch stimulus, the controls moved more frequently \((\text{pinch} \cdot p = 0.003; \text{formalin} \cdot p = 0.0402)\) in comparison to the LCs and the SCs, respectively.
Figure 3.6: Mean (± SEM) total time (sec) in motion prior to (B) and following introduction of the pinch (A) and probe (B) stimulus. Values represent 5-9 fish. 'a' denotes video-recording period 30 minutes prior to stimulation. Introduction of the stimulus (3:00 pm) is indicated by the arrow. 'a' indicates significant difference from B (p ≤ 0.05), 'b' indicates significant difference from time 0 (p ≤ 0.05).
Table 3.12: Mean (± sem) total frequency of movement for three treatment groups of Atlantic salmon in response to the different experimental stimuli. LC = long cannula. SC = short cannula. 'B' denotes video-recording period at 2:30 pm. Introduction of the stimulus (3:00 pm) is indicated by the arrow. 'a' indicates significant difference from B (p ≤ 0.05).

<table>
<thead>
<tr>
<th>TREATMENT GROUP</th>
<th>ELAPSED TIME</th>
<th>N</th>
<th>LIGHT</th>
<th>FORMALIN</th>
<th>PINCH</th>
<th>PROBE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>B</td>
<td>6-8</td>
<td>10.88(± 1.92)</td>
<td>14.63(± 3.99)</td>
<td>5.86(± 1.94)</td>
<td>6.50(± 2.74)</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>6-8</td>
<td>10.88(± 3.25)</td>
<td>14.5(± 2.18)</td>
<td>9.71(± 2.11)</td>
<td>6.83(± 2.24)</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>5-7</td>
<td>11.71(± 4.70)</td>
<td>15.57(± 2.32)</td>
<td>8.14(± 2.57)</td>
<td>4.00(± 1.30)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6-9</td>
<td>8.29(± 2.70)</td>
<td>7.11(± 1.57)</td>
<td>10.00(± 2.85)</td>
<td>6.17(± 2.44)</td>
</tr>
<tr>
<td>LC</td>
<td>B</td>
<td>7-9</td>
<td>9.44(± 2.20)</td>
<td>6.78(± 2.93)</td>
<td>5.57(± 1.91)</td>
<td>5.00(± 1.11)</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>7-9</td>
<td>8.67(± 2.92)</td>
<td>9.44(± 2.37)</td>
<td>6.00(± 1.60)</td>
<td>8.00(± 2.64)*</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>6-8</td>
<td>10.00(± 4.16)</td>
<td>6.00(± 0.85)</td>
<td>6.14(± 2.21)</td>
<td>6.33(± 3.08)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>7-8</td>
<td>10.00(± 3.43)</td>
<td>11.43(± 3.99)</td>
<td>5.71(± 2.49)</td>
<td>5.00(± 2.51)</td>
</tr>
<tr>
<td>SC</td>
<td>B</td>
<td>6-8</td>
<td>7.87(± 2.67)</td>
<td>7.37(± 2.26)</td>
<td>6.43(± 1.69)</td>
<td>9.67(± 3.05)</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>6-8</td>
<td>8.38(± 2.45)</td>
<td>9.63(± 2.34)</td>
<td>5.297(±2.08)</td>
<td>13.17(± 3.12)*</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>5-7</td>
<td>10.29(± 6.01)</td>
<td>11.29(± 4.59)</td>
<td>4.86(± 1.86)</td>
<td>7.40(± 4.96)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6-9</td>
<td>6.14(± 2.31)</td>
<td>12.22(± 2.61)</td>
<td>3.29(± 1.19)</td>
<td>12.67(± 4.87)</td>
</tr>
</tbody>
</table>
3.3.2 Interactive Behaviour

*Pre (Baseline 1) and Post (Baseline 2) Surgery*

Surgery did not appear to alter aggressive behaviour since the frequency of attack behaviour displayed was relatively low before and after surgery (data not presented). Three fish exhibited attack behaviour prior to surgery and only one attack was observed following surgery. In the latter case the attacker had been previously fitted with a short cannula. A subsequent retreat from the recipient of the attacks was observed following each of the attacks from the aggressive fish pre and post surgery. The number of attacks displayed by each of the four attackers was generally 1-2 and often directed at one particular fish.

Frequencies of ASD among the fish appeared to be greater then those of attacks and retreats both prior to and after surgery. Table 3.13 shows the comparison of the three treatment groups prior to and following surgery. There was no significant change in the number of fish that engaged in ASD behaviour following surgery in comparison to before surgery. It does appear, however, that a higher percentage of the cannulated fish were engaging in ASD behaviour as compared to the controls after surgery. The number of ASD behaviours exhibited by each fish ranged from 1-13 and, generally, those fish that were active prior to surgery, also engaged in ASD behaviour afterwards. Although, the number of fish displaying ASD behaviour did not change following surgery, there was a considerable decrease in frequency of ASDs (Table 3.13).

SD behaviour prior to surgery was observed in eight out of the 10 sections (five sections from each experiment) ranging from one to four times per section (Table 3.14).
Table 3.13: Frequency of asynchronous displacement for three treatment groups of Atlantic salmon prior to (PRE) and after (POST) catheterization of the dorsal aorta. C = control, LC = long cannula, SC = short cannula. NA = Not available.

<table>
<thead>
<tr>
<th>EXPeriment</th>
<th>SECTION</th>
<th>PRE</th>
<th>POST</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>C</td>
<td>LC</td>
<td>SC</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
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<td>13</td>
</tr>
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<td>1</td>
<td>NA</td>
</tr>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>NA</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 3.14: Frequency of synchronous displacement behaviour of Atlantic salmon, confined in groups of three in ten different sections, prior to (PRE) and after (POST) catheterization of the dorsal aorta.

<table>
<thead>
<tr>
<th>EXPERIMENT</th>
<th>SECTION</th>
<th>PRE</th>
<th>POST</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
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<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Following surgery, the number of SDs declined significantly \((p = 0.0100)\) whereby SD behaviour was observed only once throughout the post surgery recording period.

**Experimental**

Attack behaviour was observed during the experimental periods for the light, formalin, and probe stimulus (Table 3.15). The number of attacks ranged from one to four attacks for each fish and often attacks occurred prior to introduction of the stimulus. One fish attacked during the recording period for the light and formalin stimulus and two fish attacked following application of the probe. In all instances the attacks and retreats were displayed by either the long cannulated fish or the control fish. No attacks were recorded in fish receiving the pinch stimulus.

There was no significant variation in the patterns of occurrence of ASD behaviour to each of the experimental stimuli (Table 3.16). The three treatment groups were then combined to determine the variance between the patterns of response for the different stimuli. The analysis showed no significant difference between the stimuli. Overall, the patterns of occurrence that occurred most often included no change in ASD activity, or a decrease in activity over time. Details of the number of fish displaying ASD behaviour during the experimental period for each stimulus are provided in Table 3.16. One to two ASD behaviours were exhibited on average by each ASD active fish.

The frequency of SD behaviour prior to and after introduction of each stimulus is provided in Table 3.17. Introduction of the light, pinch, and probe stimulus did not invoke a change in the occurrence of SD behaviour in comparison with the pre-stimulus sampling time for all ten sections. Formalin, however significantly increased the occurrence of SD behaviour \((p = 0.0500)\) whereby fish within seven out of a total of
Table 3.15: Frequency of attacks for three treatment groups of Atlantic salmon during the experimental period. LC = long cannula, SC = short cannula. B denotes video-recording period at 2:30 pm. Introduction of stimulus (3:00 pm) is indicated by the arrow. Data in parentheses indicates retreat. N = 8-10 fish.

<table>
<thead>
<tr>
<th>STIMULUS</th>
<th>ELAPSED TIME</th>
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Table 3.16: Number of Atlantic salmon (three treatment groups) displaying asynchronous displacement behaviour during the experimental period. C = control, LC = long cannula, SC = short cannula. 'B' denotes video-recording period at 2:30 pm. Introduction of stimulus (3:00 pm) is indicated by the arrow. N = 8-10 fish.

<table>
<thead>
<tr>
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<th>ELAPSED TIME</th>
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Table 3.17: Frequency of synchronous displacement behaviour displayed by Atlantic salmon prior to (PRE - B) and following (POST - 0, 0.5, 1h) introduction of four experimental stimuli. Fish were confined in groups of three in ten separate sections.

<table>
<thead>
<tr>
<th>STIMULUS</th>
<th>Light</th>
<th>Formalin</th>
<th>Pinch</th>
<th>Probe</th>
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<tbody>
<tr>
<td>EXPERIMENT</td>
<td>SECTION</td>
<td>PRE</td>
<td>POST</td>
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10 sections were SD active. The data reveals that the number of sections where SD behaviour was displayed reduced over time such that the peak of SD activity occurred immediately after introduction of formalin (Table 3.18).

In summary, CM, frequency of CM, and total time in motion decreased following cannulation surgery. There was no significant change in SM behaviour and a slight variability in frequency of SM during Baseline 1 and Baseline 2 observations. Following the light stimulus, CM activity was reduced 30 minutes following stimulation. CM activity and total time in motion was increased significantly after introduction of the pinch stimulus, followed by a decline after 30 minutes. An increase in frequency of motion was observed in the cannulated fish following the probe stimulus. After 30 minutes, CM activity and total time in motion was significantly reduced in comparison to time of stimulation. With respect to the formalin stimulus, there was no significant change in any aspect of movement behaviour.

The frequency of attack and retreat behaviour was minimal during the baseline observations, before and after surgery, and during the experimental recording periods. The fish engaging in attack behaviour during the experimental phase were either the controls or the LC fish and interestingly, the recipient of the attacks was always a control or a LC fish. ASD activity was observed in many of the fish prior to and following surgery and during the experimental period. There was no effect of surgery on ASD behaviour nor was there a significant difference between the patterns of ASD occurrence following the four stimuli. The predominant pattern of occurrence in response to the stimuli was no change in ASD behaviour or a decrease in activity over time. However, overall, the number of fish engaging in ASD behaviour was minimal.
Table 3.18: Frequency of synchronous displacement behaviour displayed by Atlantic salmon prior to (B) and following (0, 0.5, 1h) introduction of formalin stimulus. The fish were confined in groups of three in ten different sections. 'B' denotes video-recording period at 2:30 pm. Introduction of formalin (3:00 pm) is indicated by the arrow.

<table>
<thead>
<tr>
<th>EXPERIMENT</th>
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With respect to the SD behavioural response, there was a significant decline in the frequency of SD behaviour following surgery. SD behaviour was observed in eight out of ten sections within the tanks prior to surgery, but only one section was SD active afterwards. Only formalin resulted in an increase in SD activity which subsequently declined over time following introduction of the stimulus.

Overall, the behaviour that appeared to be most affected by a disturbance such as surgical intervention and introduction of a stimulus were ASD and SD behaviour.

3.4 Discussion

Behaviour is defined as "the observable action of a living organism, either instigated by the organism or imposed by the external circumstances" (Hurnik et al., 1985). In research and commercial aquaculture, fish are housed in artificial environments that impose limits on their habitat boundaries. Also, disturbances such as transport and handling of the fish are common occurrences in the aquaculture industry. It would seem reasonable then to monitor behaviour of fish as an indicator of their stressed condition. In assessing the well-being of the fish, researchers have performed extensive studies on the biochemical and physiological responses of fish to stressors that occur in laboratory and commercial environments (Barton and Iwama, 1991). Biochemical assays provide an objective measure of stress in fish, but often require an invasive procedure which is itself a stressor. Observations of behavioural responses, which are inherently non-invasive, should also be considered in combination with biochemical measurements as a means of gaining a broader perspective in the area. A problem with this approach is that fish may not display overt behavioural changes.
except under extreme conditions. It is important then to understand the basic or baseline behaviours of the fish in order to recognise more subtle changes.

The purpose of this study was to determine if there were observable and quantifiable behaviours that Atlantic salmon exhibited when exposed to different external stimuli. If the fish responded behaviourally in a different manner to various stimuli, then there may be a possibility that Atlantic salmon are capable of discriminating between different classes of stimuli.

*Pre (Baseline 1) and Post (Baseline 2) Surgery*

Baseline CM behaviours of the fish established prior to surgery were compared to baseline behaviours following aortic cannulation of the experimental fish. The results indicated that, following an acclimation period of two weeks after surgery, the average time spent in CM significantly decreased in all treatment groups in comparison to presurgery baseline values. Also, the total time spent moving and the total frequency of movement behaviour was significantly less following surgery. Unlike CM, however, there was a significant difference between the control fish and the cannulated fish. The control group was less active overall with respect to total time swimming than were the cannulated fish. The dissimilarity in activity among treatment groups may suggest that the cannulated fish had not fully adjusted to the indwelling cannula at that time. Another possibility could be that the cannulated fish were displaying dominant behaviour over the controls (discussed later). This would be consistent with the decrease in CM time in the control group following cannulation of the experimental fish. Nonetheless, the significant reduction in activity, regardless of the parameter used (CM, Total Time or Total Frequency) in each treatment group following surgery.
probably indicated that the fish were either depressed from the stress of prolonged
confinement or that they had further acclimated to their environment.

Pottinger and Pickering (1992) report that an acclimation period of 2-4 weeks
was required following transfer of rainbow trout from stock tanks to holding tanks of
reduced volume for complete acclimation to occur. In the current study, Atlantic
salmon were transferred from a stock tank to the confined conditions of the
experimental tanks. The time lapse from pre-surgery baseline to post-surgery baseline
was approximately six weeks for both Experiment 1 and 2. This duration may have
been sufficient for complete acclimation of the fish.

Alternatively, the reduction in swimming activity post-surgery among the
treatment groups may have been an indication of the stress imposed by confinement
resulting in a decrease in swimming activity. Sigismondi and Weber (1988) reported
that the avoidance response time of previously stressed (30 sec handling) juvenile
Chinook salmon to a sudden overhead light was delayed in comparison to non-stressed
fish. They postulated that acute stress not only increased the response time of Chinook
salmon to avoid an additional stressors (overhead light) but also elicited general
lethargic behaviour. The reduction in swimming activity displayed by all treatment
groups in this study may represent lethargic behaviour brought on by prolonged
confinement stress.

The average time for SM activity was not altered after surgery nor was there a
notable change in frequency of SM among all treatment groups (Table 3.7 and 3.5,
respectively). The average time spent in sporadic movement was approximately 3-4
seconds in duration for each treatment group. Since sporadic activity was not altered
over time, this may have been an indication of daily locomotor activity of the fish. There have been considerable inconsistencies in the literature with respect to locomotor activity rhythms in salmonids. Ali (1964) reported that yearling Atlantic salmon generally exhibited diurnal locomotor activity, however, subsequent studies have also indicated a nocturnal tendency in Atlantic salmon parr (Varanelli and McCleave, 1974; Richardson and McCleave, 1974). The differences in locomotor activity rhythm have been attributed to the varying photoperiods, light intensity, and temperature employed in each study. The significant reduction in total time spent in motion following surgery in the current study and the fact that sporadic activity was not affected, suggested that the latter may be an indication of diurnal locomotor activity in these Atlantic salmon.

The frequency of attack behaviour was low before and after surgery. There were only three attacks observed prior to surgery among all the fish and one attack observed following acclimation to cannulation surgery. Keenleyside and Yamamoto (1962) report that crowded conditions (8-12 fish) coupled with the confinement of an aquarium (100 cm long x 30 cm wide x 25 cm deep at 70-350 L capacity) can lead to frequent and often prolonged bouts of fighting among Atlantic salmon fry and parr during the defence of territories. Such aggressive behaviour would undoubtedly create a stressful environment for the more subordinate fish in each group. The consequences of social position have been shown to affect physiological parameters in Coho salmon such that the dominant fish are generally devoid of socially imposed stress and subordinates are stressed (Ejike and Schreck, 1980). Kjartansson and associates (1988) found that physiological parameters were not affected in adult Atlantic salmon at low to high stocking densities in laboratory tanks. They suggested that the territorial behaviour of
parr, reported by Keenleyside and Yamamoto (1962), may be needed to increase the survival capabilities of salmon during the early stages of life. However, as adults, the territorial behaviour may not exist within a population. This may explain the infrequent attacks within the groups of Atlantic salmon in the current study. Another possibility might be that the fish had acclimated to their confined environment and consequently a social structure had been established.

A reduction in both ASD (Table 3.13) and SD (Table 3.14) behaviour of Atlantic salmon following a two week acclimation period after surgery was shown in all treatment groups within the current study. The reduction in interactive behaviour (ASD and SD) over time may provide further evidence of acclimation of the fish to their confined environment. However, the reduction in interactive behaviour (ASD and SD) may have also indicated phlegmatic behaviour among the treatment groups from the stress of confinement (Sigismondi and Weber, 1988). Another possibility for the decrease in interactive behaviour may be related to the limited space within each section. Aggressive behaviour displayed in defence of territory may become futile if the territory is continually invaded because of the restricted environment.

Although there was a reduction in ASD behaviour in all treatment groups, ASD was still evident during post-surgery baseline observations (Table 3.13). This suggested the possibility of an established social structure within each section of the tanks. The number of fish displaying ASD behaviour over time remained relatively consistent for the cannulated groups, whereas the number of ASD active control fish was reduced. Furthermore, the control fish did not display ASD behaviour as frequently and their swimming activity (total time in motion) was significantly less than
the cannulated fish following surgery. It has been documented that fish confined in small numbers are able to establish social hierarchies. Social position has been determined by physiological parameters such as plasma cortisol concentrations (Ejike and Schreck, 1980) such that cortisol levels are lowest in the dominant fish and highest in the submissive fish. Pickering and Pottinger (1992) reported, on the basis of physiological parameters including plasma cortisol, that rainbow trout confined in pairs and groups of five are capable of forming social hierarchies. Also through behavioural observations, Scott and Currie (1980) found that swordtail (6.2 - 7.2 cm) maintained in groups of three in 60 x 30 x 30 cm aquaria, established straight-line hierarchies with one clearly dominant (α) fish, one clearly subordinant (γ) fish, and one intermediate (β) fish.

Atlantic salmon in the present study were confined in groups of three. Behavioural observations were recorded on all three fish, but only the experimental fish were sampled for biochemical parameters (see Chapter 2, Section 2.2.1). ASD behaviour was displayed by experimental and replacement fish in six out of ten sections and by the control fish in three out of nine sections. Results from the biochemical analysis showed that plasma cortisol and catecholamine levels of the experimental fish were consistent with baseline concentrations reported for salmonids (Nichols and Weisbart, 1984a; Gingerich and Drottar, 1989). Consequently, behavioural observations (ASD behaviour, Table 3.13) and physiological analysis (Table 2.2 and 2.3 in Chapter 2) may have indicated the formation of an established social structure with the experimental fish as a more dominant (α) fish, and the control as the more subordinate (β) fish. However, the evidence provided from this study is not conclusive because the
physiological data was limited to only the experimental fish. Nonetheless, the
behavioural data with respect to swimming activity (total time in motion - Figure 3.5)
indicate that the control fish were less active than the cannulated fish. The reason for
the elevated swimming activity of the cannulated fish is not known. The cannulated
fish may not have adjusted fully to the indwelling cannula and therefore moved around
more to attempt to avoid the discomfort of the cannula. Another possibility may be that
the cannulated fish held a more subordinate position within the group. The increased
swimming time may have been an indication that the cannulated fish had not
established a territory within the section. Keenleyside and Yamamoto (1962) reported
that aggressive acts, as demonstrated by the experimental and replacement fish, are
often performed during competition for territories. The control fish, devoid of the insult
from the indwelling cannula, may have been able to establish a territory before the
cannulated fish, thus establishing a dominant position within the group.

By monitoring the behaviours of the fish prior to and following surgical
intervention, we were able to obtain baseline behaviours of Atlantic salmon housed in
the divided circular tanks. These behaviours were used as a basis for monitoring the
behavioural responses of the fish to four different stimuli.

Experimental

The results from the experimental period for the light stimulus showed no
significant change in CM behaviour immediately following the stimulus in comparison
to the pre-stimulus recording period nor was there a significant change in total time
spent swimming or total frequency of movement. However, there was a significant
increase in average time spent in SM (Table 3.8). Hardenon and McCleave (1974)
reported that juvenile Atlantic salmon showed a consistently faster response time to seek cover following a light to dark transition than a dark to light change. The sudden transition from light to dark in this experiment did not invoke a significant change in behaviour with respect to time or frequency of movement but the increase in average time spent in SM may have indicated that the fish were responding to the stimulus. There was a significant decrease in CM and total time in motion after 30 minutes in comparison to time of stimulation. The light stimulus may have been stressful and consequently the fish became lethargic in response to the stress (Sigismondi and Weber, 1988).

Introduction of formalin into the tanks did not invoke a change in either average time in CM or SM or total time or frequency of movement. The SM data following the formalin stimulus indicated a slight increase in time spent in SM but due to the variability among fish, this difference was not statistically significant. There was a significant difference between treatment groups whereby the control fish were more active than the cannulated fish with respect to total time and frequency of movement. Formalin affected interactive behaviour of the fish by significantly increasing the occurrence of SDs immediately after introduction of the stimulus. This would suggest that the formalin caused a uniform effect on the fish such that all fish within a section were equally disturbed by the stimulus. Formalin was the only stimulus that produced a change in SD behaviour. Previous studies have shown that fishes possess the ability to respond behaviourally to water-borne chemicals (Beitinger and Freeman, 1983). It would appear that the increase in SD behaviour could be paralleled to avoidance behaviour responses whereby the fish were attempting to avoid the chemical toxin.
behaviour occurs when the swimming activity of one fish initiates the displacement of the other fish within the section. During the formalin stimulus, the inflow to the tank was shut off which may have created an oxygen deficit within the tank. Alternatively, the increase in SD behaviour may have been due to the fish attempting to settle near the water flow from the airlift and ultimately invading the territory or space occupied by the other fish. Although the pattern of occurrence for ASD behaviour was not significantly different among the treatment groups there did appear to be an increase in the number of experimental and control fish engaging in ASD behaviour. ASD behaviour could be associated with the need to reposition closer to the airlift. The sudden increase in SD behaviour may not have been solely due to the foreign chemical introduced into the tank or a reduction in dissolved oxygen but rather a combination of the two. Frequency of SD behaviour did, however, decrease after 30 minutes following introduction of the formalin (Table 3.18).

Both the light and formalin stimulus were considered as non-mechanical stimuli and therefore introduction of each would equally affect all the fish. Only the experimental fish directly received the mechanical stimuli (ie. pinch and probe). However, behaviours of the other fish, controls and replacement fish, were also considered in the assessment of the behavioural response. In comparison to the pre-stimulus recording period, swimming behaviour changed following the pinch stimulus. Time spent in CM and total time spent swimming were significantly increased immediately after onset of the pinch stimulus. This activity was reduced significantly within 30 minutes after stimulation. Total time and frequency of movement varied among the treatment groups whereby the control fish were significantly more active

135
than the cannulated fish. Pinching the experimental fish produced an increase in locomotor activity (CM and total time in mo. . . . ) yet one would expect that this increase would be greater than that for the controls and the replacement fish. The increased activity of all treatment groups may be a result of SD whereby the replacement and control fish were responding to the increased activity of the experimental fish. However, there was no significant change in SD behaviour prior to and after stimulation in comparison to pre-stimulus SD behaviour.

Another explanation for the uniform increase in locomotor activity among the treatment groups may be that the fish were responding to the pinchers whereby introduction of the pinchers into the tank may have prompted a startle response among the fish. The pinchers were introduced five minutes before actual stimulation to allow the fish to become accustomed to the new object in the tank. This length of time may not have been sufficient for the fish to adapt. Therefore, the increase in swimming activity may have been due to apprehension towards the pinchers as opposed to the pinch stimulus. Nevertheless, introduction of the pinchers was the only stimulus that elicited a significant increase in locomotor activity immediately after stimulation. Probing the experimental fish did not produce a significant change in locomotor activity, however, there was a slight increase in activity following stimulation. The short cannulated fish were moving around more frequently then the other treatment groups. This increase was probably due to two fish that displayed unusually high frequencies of movement at this time. After 30 minutes, average time in CM and total time and frequency of movement dropped significantly among all treatment groups in comparison to pre-stimulus activity. Similar to introduction of the pinch stimulus, the
probe was introduced to the tank five minutes prior to stimulation. The initial reaction to the probe was not as great as that for the pinchers but an increase in locomotor activity observed following stimulation in each treatment group may suggest an alarm response to the probe.

In summary, locomotor activity of all treatment groups - controls, short and long cannulated fish - had declined significantly following surgery. A reduction in interactive behaviour, ASD and SD, was also apparent following surgery. Overall, each stimulus produced a significant change in behaviour in the treatment groups, although, different behaviours were affected by different stimuli. Light produced a decrease in average time in CM, pinching resulted in an increase in average time in CM and total time in motion, formalin produced a significant increase in SD behaviour, and probing increased the total frequency of movement in the cannulated fish. The varying behavioural responses implied that the fish were responding distinctly to different stimuli, although the differences are subtle and require validation in subsequent studies. Nonetheless, these data suggest that Atlantic salmon may be capable of discriminating between different types of external stimuli.

Monitoring behavioural changes as indicators of stress in fish due to unfavourable conditions or physical disturbance can be difficult and time consuming. Before an accurate assessment of behavioural changes can be made, a basic understanding of particular fish behaviours within their environment is required. Although further research is required, it seems possible to employ behavioural responses as an indication of the well-being of the fish.
4.0 GENERAL DISCUSSION

The salmon industry is growing rapidly worldwide (Novotony and Nash, 1995). The most common salmonid species farmed in Canada is Atlantic salmon which accounts for approximately 78% of the total value of aquaculture production in the Atlantic provinces (Boghen, 1995). Consequently, the increasing economic importance of this species requires frequent use of Atlantic salmon in experimental studies designed to benefit the aquaculture and/or aquaculture support industries. In an aquaculture or research environment fish are often exposed to stressors such as handling, confinement, and social stresses. In response to a stressful stimulus, there are different levels of organization including biochemical, physiological, and behavioural, that are involved in maintaining homeostasis in fish. A change in behaviour in response to an external stimulus can be an immediate indicator that the stimulus has produced an effect. Concurrent determination of a biochemical change such as an elevation in plasma cortisol or catecholamines can further characterise the stimulus as being stressful. Over the years, considerable attention has been devoted to understanding the physiological response to stress in fish (Barton and Iwama, 1991). Behavioural responses of fish to stress have also been examined (see Chapter 2, Section 3.1), but not as extensively as the physiological response. The objectives of this study were (1) to examine the biochemical and behavioural responses of Atlantic Salmon to different classes of external stimuli and (2) to determine if different classes of stimuli elicit reproducibly distinct biochemical and behavioural responses in Atlantic salmon.
To concurrently measure both biochemical and behavioural responses to external stressors employed in this study, the experimental and replacement fish were surgically implanted with a chronic indwelling cannula in the dorsal aorta. Following surgery, the biochemical parameters were monitored for 14 days. Behavioural observations were not recorded at this time. The biochemical results showed a significant elevation in plasma cortisol and plasma glucose following cannulation surgery with plasma concentrations returning to baseline within 24 hours (Figures 2.6 and 2.10). Plasma NE concentrations were less responsive to the surgical stressor and no significant change occurred during the post-surgery sampling period (Figure 2.8A). Plasma E, on the other hand, appeared to respond to the stressors of surgery, but the large variability among the fish and the limited number of experimental animals confounded the statistical analysis such that no significant differences were found (Figure 2.8B).

There have been inconsistencies in the literature with regard to individual fish variation and pattern of catecholamine release following physical and environmental disturbances. Thomas and Perry (1992) postulated that the discrepancies among the different studies could be due to several factors including; the amount of catecholamines stored in the head kidney, metabolic degradation of catecholamines, tissue accumulation, species variability of response, and differences in analytical methodology. The ratio of NE and E release in response to a stressor has been shown to vary considerably. Generally, plasma E concentrations tend to rise to a greater extent than NE during most stresses (Ristori and Laurent, 1985; Boutillier et al., 1986; Perry et al., 1989), although, not all studies have demonstrated the same trend (Butler et al., 1986; Fritsche and Nilsson, 1990). Perry et al. (1991) demonstrated two distinctly different patterns of
catecholamine release in Atlantic cod during hypoxia. The pattern of catecholamine release was dependent upon the time to achieve final $P_{w_{o_{2}}}$ (water $P_{o_{2}}$). A gradual reduction in $P_{w_{o_{2}}}$ caused significant elevations in plasma E only, while a more rapid reduction resulted in elevation of both catecholamines. Alternately, Fritsche and Nilsson (1990) found that stress from increasing external hypoxia produced an elevation in NE several minutes before the appearance of E into the blood circulation in Atlantic cod. Consequently, the variable responses of NE and E in the present study may be due to the intensity and/or duration of the stimulus (ie., stress from anaesthesia and cannulation surgery), although intra-species biological variability may also be an important factor. Perry et al. (1991) postulated that NE and E are released in variable quantities from the chromaffin tissue within the head kidney depending upon the nature of the stimulus and the existing concentrations in the blood. Ultimately, the pattern of catecholamine elevation and the ratio of NE to E release during any particular stress is not fully understood.

Prior to surgery, baseline observations (Baseline 1) of movement and interactive behaviour were recorded for comparison of post-surgery baseline (Baseline 1) behaviour. Post-surgery movement and interactive behaviours were monitored following a two week post-cannulation recovery. The results indicated that the general activity of the fish (all treatment groups) was significantly reduced after surgery in all aspects of behaviour measured with the exception of SM. SM, including mean time in motion and frequency of sporadic bursts, did not change significantly in the baseline observations before surgery and after. This persistent behaviour may have been an indication of daily locomotor activity of the fish (see Chapter 3, Section 3.4).
The reduction in locomotor activity (CM, total time and frequency) among the three treatment groups following cannulation surgery suggested that either surgery had affected the fish equally or that the fish may have further acclimated to their confined environment. Both assumptions were supported by the reduction in interactive behaviour with respect to ASD and SD behaviour displayed after surgery (Table 3.13 and 3.14, respectively). Determination of the cause of reduced locomotor activity however, would require further examination of the progressive changes following surgery and during each baseline day. For purposes of this study only two days were analysed following post-cannulation recovery for baseline behaviour. Nonetheless, the behavioural data suggest that the cannulated fish may have moved around less to avoid further insult from the catheter thus maintaining homeostasis or that locomotor activity may have decreased gradually over time suggesting further adjustment to the confined environment.

Concurrent blood samples were collected prior to and after videotaping to correlate post-surgical biochemical and behavioural baseline activity in the experimental fish. Baseline observations of the biochemical parameters, plasma cortisol, catecholamines, and glucose, further indicated that the fish may have acclimated to their environment. Plasma concentrations of cortisol collected during the baseline sampling period closely approximate resting values previously reported for cannulated Atlantic salmon (Nichols and Weisbart, 1984a) and rainbow trout (Bry and Zohar, 1980). Resting concentrations of E and NE were within the range reported for cannulated rainbow trout (Butler et al., 1986; Gingerich and Drottar, 1989) and eel (Le Bras, 1982). In addition, plasma glucose concentrations in the present study were comparable to
resting concentrations of plasma glucose previously reported in Atlantic salmon (Nichols and Weisbart, 1984a). The basal concentrations of the biochemical parameters may provide evidence that the experimental fish had adjusted to their confined environment.

Consistent with the reduced locomotor (CM and total time) and interactive behaviour (ASD and SD), low cortisol concentrations and the suggestion of further acclimation to confinement would be the interpretation that a social structure had been established, with the experimental fish as a dominant individual within the groups. Fish confined in small numbers are able to establish social hierarchies. Assessment of hierarchial position in previous studies have been assessed by physiological parameters such as plasma cortisol concentrations (Ejike and Schreck, 1980) and behavioural observations (Scott and Currie, 1980). In the present study, the low concentrations of plasma cortisol observed in the experimental fish during baseline observations may suggest a dominant hierarchial position within the group. In accordance with this are the behavioural observations of the group as a whole. With respect to movement behaviour (total time), the experimental and replacement fish were significantly more active than the controls following cannulation surgery (Figure 3.5). Also, ASD behaviour exhibited after surgery was displayed by cannulated fish in all but one section whereas ASD behaviour by control fish was observed in only three out of nine sections (Table 3.13).

Agonistic behaviour such as ASD behaviour has been reported to be common among young salmon who compete for territory within an aquarium (Keenleyside and Yamamoto, 1962). In the present study, the fish displaying ASD behaviour (aggressor)
would chase the displaced fish for a brief period (approximately 5-30 seconds) and the aggressor would then return to its former resting spot. The displaced fish would often rest in a new position away from the aggressor. Keenleyside and Yamamoto (1962) found that agonistic behaviour is more pronounced when young Atlantic salmon are initially placed in an aquarium, but after several days territories become well established and aggressive behaviour becomes less common. This may explain the decrease in ASD and SD behaviour following cannulation surgery in this study. Keenleyside and Yamamoto (1962) also report that dominant fish perform a greater number of aggressive acts per observation than the subordinate fish. In the current study, both the experimental and replacement fish were more active (total time, Figure 3.5) and displayed ASD behaviour more frequently then the controls (Table 3.13). Scott and Currie (1980), through behavioural observations, found that swordtail maintained in groups of three established a stable social hierarchy. On the basis of the behavioural observations of the three treatment groups in the present study, there is evidence to support the establishment of a stable social structure within most sections, with the assumption that the cannulated fish are dominant over the controls. As part of this study positioning of the fish within each section was monitored in combination with the movement and interactive behaviour. Further analysis of the behavioural data may provide a clearer understanding of the exact social structure within each section.

The statement that the experimental fish held a dominant (α) hierarchical position on the basis of low cortisol concentrations is not conclusive since the plasma cortisol data for the replacement and control fish were not available. Also, the dominant position of the cannulated fish within the groups was based on the assumption that
increased movement and interactive activity was indicative of a dominant social status. Alternately, the increased swimming activity and ASD behaviour of the cannulated fish, in comparison to the controls, may not have been evidence of a dominant hierarchical position but rather, an indication of pain induced or frustration aggression. Another possibility could be that the cannulated fish were displaying avoidance behaviour to attempt to reduce the discomfort of the cannula. The reason for the difference between movement and interactive behaviour of the cannulated fish and the controls, following cannulation surgery, is not known. There may have been several factors involved including adjustment to the catheter, avoidance behaviour to reduce the discomfort of the cannula, or social status that caused the dissimilarity between the treatment groups.

Following baseline biochemical and behavioural observations the experimental fish were exposed to four different external stimuli; light, probe, pinch, and formalin, over a five week period. During the experimental period for each stimulus, blood samples were collected from the experimental fish and the behaviour of the group within the specific section was monitored. The blood sampling times (B, 0, 1, and 3 hours) correspond to behavioural observations recorded at 2:30, 3:00, 3:30, and 4:00 pm respectively. In summary, there was no response of plasma catecholamine or plasma glucose concentrations to any of the stimuli. However, introduction of the light, formalin, and pinch stimulus elicited changes in plasma cortisol concentrations.

The release of catecholamines into the circulation has been established as a primary neuroendocrine response to stress (Mazeaud and Mazeaud, 1981). Previous studies have reported an elevation in catecholamines in response to stressors such as severe physical disturbance (Nakano and Tomlinson, 1967), acute acidosis (Boutilier
et al., 1986), and intense bursts of swimming activity (Ristori and Laurent, 1985; Butler et al., 1986). Hyperglycaemia is one of the most frequently used indicators of a secondary response to stress in teleost fishes (Mazeaud et al., 1977). Plasma glucose concentrations in fish have been reported to increase in response to acute handling stress (Pickering et al., 1982; Barton et al., 1986), acid stress (Brown et al., 1989), severe physical disturbance (Nakano and Tomlinson, 1967), and transportation (Wedemeyer, 1972; Specker and Schreck, 1980). Although plasma catecholamines and glucose have been shown to increase in response to various stressors, the intensity and duration of the stressor may have an impact on the response. Woodward and Smith (1985), report that untrained cannulated rainbow trout had no significant increase in plasma NE, E, or plasma glucose following two minutes of mild agitation. It may be possible that the duration and intensity of the stimuli employed in this study, were not adequate to elicit a response in plasma catecholamines or glucose.

During the experimental period for the light stimulus plasma cortisol concentrations were elevated significantly following introduction of the stimulus in comparison to pre-stimulus baseline values (B) (Figure 2.7). Plasma cortisol returned to basal concentrations after one hour and remained low over 3 hours. There was a corresponding significant increase in the average time spent in SM in all treatment groups immediately following the light stimulus. The increase in time spent in sporadic movement may suggest that the fish were responding behaviourally to the light change with longer bursts of sporadic activity. However, the increase in sporadic activity was minimal. Average time spent in CM, and total time in motion, decreased at 0.5 h in comparison to pre-stimulus swimming activity. After one hour, swimming time of the
experimental fish (CM and total time) and the replacement fish (CM) increased slightly in comparison to the 0.5 h recording period and CM and total time in motion of the control group had returned to pre-stimulus values. The reduction in CM and total time at 0.5 h may have been related to the increase in plasma cortisol concentrations, or vice versa, after one hour following introduction of the light stimulus. As a means of determining the relationship between plasma cortisol and movement behaviour an analysis of covariance (SAS Institute, Inc., 1985) was performed on the experimental data. The regression analysis revealed no relationship at time B, 0, and 1 h between plasma cortisol (cort) and swimming time for CM: \( y = -0.451\text{cort} + 5.124; r^2 = 0.333 \), slope was not significantly different from 0 (\( p = 0.869 \)), and total time: \( y = -0.189\text{cort} + 14.910; r^2 = 0.358 \), slope was not significantly different from 0 (\( p = 0.936 \)) during any of the experimental stimuli. Sigismondi and Weber (1988) reported that acute handling stress increased the response time of chinook salmon to a light stimulus. The timing of stresses and the recovery times of the study by Sigismondi and Weber (1988) were chosen to coincide with another study (Barton et al., 1986) examining the physiological responses of chinook salmon to acute handling stress. Comparison of the plasma cortisol concentrations and the behavioural response times to a sudden overhead light indicated that the longest behavioural response time occurred before the peak plasma cortisol stress responses would be expected. Nonetheless, the significant reduction in CM and total time in motion may have been a result of stress imposed from the sudden light change as suggested by Sigismondi and Weber (1988).

Following introduction of formalin into the tank, plasma cortisol was elevated significantly at time 0, and remained elevated over a three hour period (Figure 2.7).
Similar results have been shown in steelhead trout following prolonged treatment with 200 ppm formalin. Locomotor behaviour (CM, SM total time and frequency of motion) did not change significantly among the three treatment groups during the formalin experimental period but there was a change in interactive behaviour. Immediately following introduction of formalin SD behaviour increased significantly within most sections (Table 3.17). Beitingler and Freeman (1983) report that fishes possess the ability to respond behaviourally to water-borne chemicals. One possibility for the increase in SD may be that the fish were attempting to avoid the formalin and in doing so disrupted the group. Alternatively, the increase in SD behaviour may have been due to the fish attempting to settle near the water flow from the airlift and ultimately invading the territory or space occupied by the other fish. Although, both explanations appear plausible, the sudden increase in SD behaviour may have been a result of a combination of the two. Frequency of SD behaviour decreased 30 minutes after introduction of the formalin (Table 3.18).

Following the pinch stimulus, plasma cortisol concentrations of the experimental fish were elevated significantly at time 0 in comparison to pre-stimulus cortisol concentrations (Figure 2.7). Time spent in CM and total time moving was significantly increased immediately after application of the stimulus for all treatment groups. This increase in locomotor activity coincided with the increase in plasma cortisol concentrations. Locomotor activity (CM and total time in motion) also declined with the concurrent decrease in plasma cortisol after one hour. Although the stimulus was applied to the experimental fish only, an increase in locomotor activity was observed in all treatment groups. The increase in locomotor behaviour of each treatment group
may have been an alarm response to the pinchers being placed into the tank. Therefore, the increase in swimming time of the experimental fish may not have been a direct response of the pinch stimulus but rather a behavioural response to the presence of the pinchers. Probing the experimental fish did not elicit a behavioural or a biochemical change. Plasma cortisol concentrations were not elevated over the three hour period in response to probing and there was no significant change in locomotor behaviour. However, there was an increase in CM activity immediately following stimulation that occurred in all treatment groups. The similar increase in locomotor activity at time 0 among the treatment groups may have been an alarm response to the probe as previously suggested with the pinchers. Another possibility might be that increased activity of the experimental fish in response to the pinch and probe stimulus resulted in the disturbance of the other fish within the section. However, SD behaviour did not appear to increase during the experimental period for either the pinch or probe stimulus.

A peak in plasma cortisol was observed at six hours following stimulation. The increase in plasma cortisol could be attributed to the effects of repetitive blood sampling. Mazik et al. (1994) reported that cortisol was elevated significantly after three hours following repeated blood sampling prior to, during and one hour after introduction of a stressor. Basal concentrations of plasma cortisol observed after three hours in the present study implied that blood sampling was not imposing stress upon the fish. In addition, behaviour (movement and interactive) of the experimental fish did not appear to be altered during blood sampling.

Alternatively, the marked elevation in plasma cortisol at six hours may have been an indication of a peak occurring during the circadian rhythm of the fish. A
similar pattern of elevated plasma cortisol, with the highest values occurring between 20:00 and 21:00 hours, has been attributed to diurnal variation in catheterized Atlantic salmon and rainbow trout (Bry, 1982; Nichols and Weisbart, 1984a). The uniform increases of plasma cortisol at six hours following introduction of each stimulus in the current study may provide further evidence of a circadian rhythm in the Atlantic salmon. However, the exact cause of the plasma cortisol peak at that particular time is not known and further research is required. One approach to consider is establishing baseline behavioural activity and corresponding biochemical profiles of Atlantic salmon over 24 hours during different photoperiods.

The cortisol peak in this study occurred at the time when the lights were turned off during the regular photoperiod. An elevation in plasma cortisol also occurred near the time when the lights were turned off during previous studies (Bry, 1982; Nichols and Weisbart, 1980a). A more plausible explanation for the six hour peak could be that plasma cortisol was elevated as a conditioned response to the light change. The photoperiod in the present study was instantaneous such that the fish experienced a sudden change from light to dark at 21:00 h. Previous studies have shown that locomotor activity is greatest in Atlantic salmon during a light to dark change as opposed to the reverse. An increase in plasma cortisol concentrations at the time when the lights were turned off in this study may suggest a relationship between locomotor activity and elevated plasma cortisol concentrations although further research is required. Nonetheless, the elevation in plasma cortisol may act as a conditioned response to the sudden change in lighting and assist in maintaining homeostasis following the light to dark transition.
In summary, cannulation surgery significantly elevated plasma cortisol and glucose concentrations, thus indicating the severe stress associated with an invasive surgical procedure. Plasma catecholamines, however, were not significantly influenced by cannulation surgery, but E concentrations, although not statistically significant, were elevated following surgery. Behaviourally, locomotor activity and interaction among the fish decreased considerably following surgery with the exception of sporadic activity. As discussed previously, sporadic activity may have been an indication of daily activity in Atlantic salmon.

Plasma cortisol was the only biochemical parameter that changed significantly following introduction of the four different stimuli. The patterns of response of cortisol to the stimuli were significantly different during the three hour sample period. Overall, the significant difference in cortisol response and the varying behavioural responses to the stimuli supports the assumption that Atlantic salmon are capable of sensory discrimination, however, the differences were subtle and require validation in subsequent studies. The absence of significant changes in plasma catecholamines and glucose may suggest that a higher intensity and/or longer duration stimulus is required to elicit a response in these biochemical parameters. Therefore, among the parameters examined, plasma cortisol appears to be the most sensitive biochemical indicator of stress in Atlantic salmon. The behavioural response to the four stimuli were also varied such that different behaviours were seen following each stimulus. Changes in locomotor activity were observed in response to the light, pinch, and probe stimulus, and SD behaviour was displayed following introduction of formalin.
The relationship between the behavioural observations and biochemical data was not apparent from the results in this study. The regression analysis revealed no relationship between plasma cortisol and movement behaviour. This may have been due to the small sample size and the large variability among the fish. Previous studies relating biochemical and behavioural responses to stress have found similar results. Strange and Cech (1992) reported that the length of confinement in a dip net significantly reduced the average swimming time of striped bass but, concurrent physiological measurements indicated that plasma cortisol was elevated only slightly with increasing time in confinement. Behavioural observations of chinook salmon by Sigismondi and Weber (1988), in comparison with physiological responses of the same species, indicated that the behavioural response to stress occurred before the peak plasma cortisol stress response. Similarly, behavioural activity did not appear to coincide with plasma cortisol concentrations in the present study. Ultimately, understanding the behavioural changes in relation to biochemical observations following exposure to stressful stimuli may provide a fast and practical method of evaluating the status of fish in a commercial or research environment. The behavioural responses to the external stimuli observed in the present study supports the idea of monitoring fish behaviour as an indicator of stress.

Monitoring horizontal positioning of fish in combination with vertical distribution could also be considered in the evaluation of the status of individuals and groups of fish. Fish housed in small groups, as is often the situation in research facilities, are able to establish stable social hierarchies. One confounding problem is the stress imposed on the subordinate fish. Determining the stress level of each fish
within the group thus depends upon the initial status of the fish within the social hierarchy (Pickering and Pottinger, 1992) if in fact a social structure is established. As well, behavioural characteristics may differ according to the status of the fish as indicated by the varying interactive behaviours of Atlantic salmon in this study. Often, the dominant fish express more aggressive behaviour towards the subordinates. Also, after prolonged residence the dominant fish tend to increase the size of their territory and all other fish are forced into loose groups (Keenleyside and Yamamoto, 1962). Consequently, there are several factors to consider when evaluating the biochemical and behavioural stress response of fish in confinement.

Behavioural observations of each treatment group and biochemical analysis of the experimental fish did not provide conclusive evidence of a social structure among the fish in this study. Further analysis of the positioning data may provide an indication of a social hierarchy within individual sections of the tanks.

The purpose of this study was to evaluate the biochemical and behavioural responses of Atlantic salmon to different classes of external stimuli and to determine if Atlantic salmon are able of discriminating between external stimuli. The results of the biochemical analysis and the corresponding behavioural observations demonstrate that Atlantic salmon probably possess some rudimentary form of discrimination between different classes of sensory stimuli. Evidence supporting this assumption, however, is not conclusive from these results and further research is required in this area. Nonetheless, the implications of possessing a rudimentary form of sensory discrimination suggests the possibility that Atlantic salmon may experience the more abstract cognitive functions such as pain and fear. This would have obvious
implications for issues of animal welfare in these species. Establishing criteria for the study of biochemical and behavioural responses to stress and pain may provide further insight into optimum husbandry techniques and housing facilities for maintaining the well-being of fish in confinement.
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Appendix 1: Plasma epinephrine concentrations (nmol·L⁻¹) of Atlantic salmon during the Post-Surgery sampling period for Experiment 1 and 2. NS indicates missing data. ND = not detected. Pre represents blood sample collected from the caudal sinus prior to surgery.

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Appendix 2: Plasma cortisol concentrations (mg·dL⁻¹) of Atlantic salmon during baseline observations before (B) and after (A) videotaping for Experiment 1 and 2. NS indicates missing data.

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