

**ASSESSING THE WELFARE STATUS OF FARMED RAINBOW TROUT
(ONCORHYNCHUS MYKISS) WITH ELECTROMYOGRAM TELEMETRY**

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of

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by

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ABSTRACT

ASSESSING THE WELFARE STATUS OF FARMED RAINBOW TROUT (ONCORHYNCHUS MYKISS) WITH ELECTROMYOGRAM TELEMETRY

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University of Guelph, 2000.

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Fish are sentient animals that may experience stress, fear and pain. It is therefore appropriate to recognize and study the welfare of farmed fish. The utility of electromyogram telemetry (EMT) for assessing the physiological and behavioural responses of rainbow trout to various aquacultural rearing environments was investigated. Video observations of the 'startle response' of rainbow trout, induced by 'instant-on' lighting, were analyzed and could be correlated with EMT signals, indicating that telemetry data were an accurate measure of swimming activity. EMT signals from fish monitored during a transportation episode indicated that vigorous swimming was induced, and subsided during a post-transportation resting period. Both 'instant-on' and 'natural, phased-in' artificial lighting regimens affected the behaviour of rainbow trout, as evaluated by EMT data. When interpreted within an appropriate context, the information obtained by EMT, in combination with other biological indicators, may be used to evaluate the welfare status of farmed fish.

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1. General Introduction

1.1 Overview

There is a paucity of scientific information on the welfare of fish raised under intensive aquacultural conditions. This indicates, in some respects, a misunderstanding of the sensory capabilities of fish, and an adherence to popular misconceptions that these animals are non-sentient. Published articles relevant to the welfare of fish are sparse, and the inherent complexity of the animal welfare issues in relation to fish make it especially difficult for investigators to interpret their work within a welfare context. This thesis, therefore, includes an account of the social, philosophical, and biological components associated with animal welfare. This is required if there is to be any understanding of how animal welfare concepts will apply to farmed fish, and to guide the scientific investigations that should follow.

1.2 Animal Agriculture And Animal Welfare

During the second half of the twentieth century, considerable effort was put forth by the farming industry to improve the efficiency of livestock production. The new technologies that have allowed for intensive agricultural practices, have in many regards, achieved the economic goals for the industry. In the course of this development, however, a number of unintended, and sometimes problematic consequences occurred, especially with respect to the physical and "mental condition" of the farmed animals. A societal concern for the well-being of animals raised in such farming systems was to develop. The publication of Ruth

Harrison's book, *Animal Machines* in 1964, presented significant criticisms of intensive animal agriculture, and was overwhelmingly successful in influencing the public perception about the inadequacies of modern animal agricultural practices (Ewbank 1993). The United Kingdom government responded to the attention raised by Harrison's book, and within a year, a committee was formed to investigate the welfare of intensively reared farm animals. This committee, known as the Brambell Committee, considered scientific studies of animal stress and behaviour, and published an inquiry into farm animal welfare (Brambell 1965). Codes of recommendations, pertaining to the welfare of farmed chickens, turkeys, cattle, pigs, sheep, rabbits and ducks in the UK were subsequently published (Spedding 1993). Since the 1970's, the science of animal welfare has evolved and developed, and proponents of animal welfare have been increasingly more accurate and specific about their criticisms of intensive, animal agriculture systems (Hurnik and Lehman 1988). Over the last 2 decades, legislation in many countries has been enacted to protect the welfare of farmed animals, as well as animals used for scientific research. The focus of welfare inquiry and criticism is now upon one of the newest and rapidly growing forms of intensive livestock farming, aquaculture.

1.3 A Call For Welfare Assessment In Finfish Aquaculture

Beginning in the early 1990's, a number of articles were published which aimed to identify and generate awareness of the potential welfare problems in intensive aquaculture (Bernoth and Wormuth 1990; Peters 1990; Needham and Lehman

1991; Bernoth 1991; Neukirch 1994). Of common concern was the appropriateness of the husbandry conditions and slaughter methods used by the aquaculture industry. While most of the initial articles raised valid questions to be addressed by scientists, farmers, and the public, much of the literature that would follow was largely based on anecdotal or anthropomorphic reasoning (e.g. Needham 1990; Lymbery 1992). A negative perception of aquacultural practices was growing in some sectors of society, not only from a pre-existing environmental protection stance, but from a welfare position as well. This can be evidenced by the prevalence of anti-aquaculture campaigns and news reports (Lymbery 1992; Morgan 1998; Angus 1998; Hatt 1998), as well as the many legal restrictions placed on the further development of aquaculture in some countries. Therefore, although there has been a need for an assessment of the welfare status of intensively cultured fish, the underlying basis of exactly how the concept of animal welfare relates to fish has remained unclear and largely unexamined in the scientific literature. This is surprising, considering that there have been at least two popular scientific volumes published over the last two decades (Pickering 1981; Iwama, Pickering, Sumpter and Schreck 1997), and a number of reviews which specifically address the biology of the stress responses and the health of fish in aquacultural environments. The notion that "stress" is an important consideration in the successful husbandry of finfish is now commonplace among aquaculturists (Barton 1997); the notion that animal welfare is equally important, both in terms of the well-being of the fish and for

society's acceptance of aquaculture as an ethically justifiable farming practice is not.

The first document which attempted to comprehensively review and examine the rearing methods of farmed fish from a welfare perspective was published by the Farm Animal Welfare Council in 1996 (FAWC 1996). The FAWC is an independent advisory board, which is responsible for defining welfare standards and advising the United Kingdom government of appropriate research and legislative actions (Spedding 1993). The *Report on the Welfare of Farmed Fish* (FAWC 1996) covered many topics, ranging from handling protocols to genetic manipulation of brood-stock, and pointed out many areas of future research that could better define the 'needs' of fish. Since the emergence of the FAWC fish welfare report, a number of government departments, research organizations and aquaculture associations have devoted some resources to fish welfare studies, or have at least begun to voice these welfare issues. A workshop/conference, entitled the "Welfare of Fish at Slaughter" took place in 1997 (Robb 1997), which gave scientists and aquaculture industry representatives a chance to discuss the welfare requirements of fish, and to arrive at commercially appropriate solutions. The United State Department of Agriculture has produced a publication, which was created so government extension personnel could comment upon fish welfare inquiries (Schwedler and Johnson 1997). Some research has examined the consequences of widely used vaccination procedures to the welfare of fish (Midtlyng 1997). The importance of

fish welfare to the future of the aquaculture industry has also been addressed in recent conferences attended by researchers, farmers, government representatives and politicians (Bromage 1999). Currently, there are at least two aquacultural research programs (University of Guelph, and the Norwegian Institute of Fisheries and Aquaculture) which have released preliminary findings and popular press articles concerning fish welfare (Tye 1997; Gregersen 1999; Chandroo 1999; Chandroo, Moccia and McKinley 2000). What still has not been suitably addressed, however, is the *welfare status* of farmed fish. In other words, how exactly does the concept of animal welfare apply to fish, if it does apply all? Such questions must be answered if the welfare of intensively farmed fish is to be evaluated in any meaningful way.

1.4 Why Should Farmed Fish Receive Welfare Consideration?

To answer the question "*why should fish receive welfare consideration?*", an understanding of the animal welfare concept is necessary. The welfare of an animal refers to its "quality of life" (Duncan and Fraser 1997), a concept that is open to much debate among scientists, influenced by value-based subjectivity among different cultures, and cannot necessarily be defined in precise scientific terms. Nevertheless, a number of research approaches to the study of animal welfare have attempted to clarify, elevate or discount the individual elements which are thought to make up the "quality of life" of an animal. Three main approaches have emerged: a "feelings-based" concept that takes into account the subjective experience of the animals (e.g. Duncan and Petherick 1991), a

“function-based” concept which places emphasis on the state of biological functioning of animals (e.g. Broom and Johnson 1993; McGlone 1993), and a “nature-based” concept which suggests that animals should be reared in a manner that suits their “nature” (e.g. Rollin 1993). These three main concepts of animal welfare have been reviewed in Duncan and Fraser (1997). Since these main approaches sometimes lead to similar conclusions when assessing welfare, an “integrative” approach has recently been put forth (Fraser, Weary, Pajor and Milligan 1997). It is not the purpose of this thesis to debate which conceptual approach to animal welfare is most relevant to an animals well-being or “quality of life”. In fact, a case for fish welfare could be reasonably argued within the scope of any one of the three animal welfare concepts. However, it is the approach put forward by Duncan (1993) that provides the means and conceptual framework to answer the question “*why should farmed fish receive welfare consideration or a welfare status?*”. Therefore, for the immediate discussion, it is Duncan’s “feelings-based” concept that will be adopted. In this approach, it is not necessarily the state of health or amount of stress that an animal may have that matters to its welfare, but the possession and state of a number of cognitive capacities (Curtis and Stricklin 1991; Duncan and Petherick 1991; Duncan 1996). The cognitive capabilities in question are those which form the basis of sentience. Hence, sentience is a prerequisite to having a welfare status (Wood-Gush, Dawkins and Ewbank 1981; Duncan 1993; Duncan 1996). It follows that if fish are to be given welfare consideration, they must reasonably demonstrate the cognitive characteristics of a sentient being.

1.5 Sentience In Animals And Cognitive Ability

1.5.1 “Basic” Cognitive Abilities

The simplest of the cognitive processes which may characterize sentience involve “feeling” and “perceiving”, while being in a state of “awareness” (Duncan and Petherick 1991). A “feeling” is a specific internal sensory stimulus which an animal is “aware of”, and “perceiving” is the detection and interpretation of signals that normally originate in external events (Duncan 1993). Therefore, according to Duncan (1991), a sentient animal is said to be “aware of” a stimulus if it “feels” it (for internal events) or “perceives” it (for external events). It follows that for fish to have a welfare status, they should, in some way, be aware of their internal and external states.

1.5.2 “Flexible” Cognitive Abilities

In animals that have a plastic, flexible neural system, the capacity for “memory” and “learning” can be associated with cognitive functioning (Duncan and Petherick 1991). “Memory” can be defined as an acquired set of information, retained by an animal and potentially available for further use (Hurnik, Webster and Siegel 1995). “Learning” can be defined as the acquisition of new neural functions within a plastic neural system (Duncan and Petherick 1991), which is largely the result of, or influenced by experience (Hurnik et al. 1995). Hence, “memory” and “learning” in the sentient organism is distinct from the fixed, “hard-wired” neural systems of non-sentient organisms. Hard-wired neuronal systems can in many circumstances negate the need for cognition, and is thought to be

largely genetically controlled and unaffected by experience (Bunge and Ardila 1987). If it can be shown that the species of farmed fish in question possess “memory” and “learning” capabilities, then they may be subject to additional welfare consideration.

A consequence of “memory” and “learning” in sentient animals is the capacity for “expectation”, “anticipation” and “recognition” (Duncan and Petherick 1991). According to Duncan and Petherick (1991), if an animal can learn (e.g. push a lever to obtain food), then it can reasonably be hypothesized that it has expectations. Animals that have expectations will, therefore, regulate the amount of effort they put into doing something (Duncan and Petherick 1991). If an animal is capable of “individual recognition”, then it may anticipate the actions or have expectations of conspecifics. “Individual recognition” is the process by which an item is identified in the environment, by some form of unique label, and is usually used but not limited to conspecifics (Duncan and Petherick 1991). If it can be shown that the species of farmed fish in question has expectations, or can anticipate based on expectations, then the range of welfare considerations must be further expanded.

1.5.3 Subjective Experience And "Affective" States

The subjective experiences of an animal are not directly accessible to scientific investigation (Duncan 1993), but many scientists have come to the conclusion that the existence of subjective states, in animals with a flexible neural system

(i.e. higher brain functions), are likely to exist (Bunge and Ardila 1987). The term “affect” is used to denote a large variety of conscious experiences, including drives (hunger and sex) and emotions (pleasure and fear). Affective states are thought to be adaptive products of natural selection, which play an important role in the causation and motivation of some types of behaviour (Fraser and Duncan 1998). Positive and negative subjective experiences, therefore, may be elucidated and measured indirectly by assessing how motivated an animal is (i.e. how hard it will work) to avoid or obtain various aspects of its environment (Duncan 1993). It follows that if evidence suggests that fish are capable of subjective experiences, then the degree of welfare consideration for farmed fish must be again adjusted.

1.5.4 Fish Welfare Implications

In general, it is thought that somewhere along the phylogenetic scale, there is a level of cognitive development present which makes probable the existence of sentience (Duncan 1993). From an anatomical point of view, the teleost fish (e.g. Salmonidae), which are intensively farmed, are considered to have simpler, yet contemporary and functionally complex brain organization when compared with other vertebrates (Savage 1980; Finger 1983; Guthrie 1983). There is a substantial amount of work within the scientific literature that suggests overwhelmingly that cultured species of fish possess both “basic” and “flexible” cognitive abilities (e.g. Davis and Kassel 1983; Demski 1983; Overmier and Hollis 1983; Scherer 1992; Pitcher 1993). Therefore, it is entirely reasonable to

suggest that farmed fish are sentient animals and it is appropriate to consider their welfare.

1.6 The Cognitive Ability Of Fish And Its Relation To Welfare

There is extensive literature that deals with the general behaviour and learning processes of fish, and a growing body of knowledge that considers the brain-behaviour linkage of fish with respect to sensory processes, defensive reactions and various aspects of social behaviour. Although much of the work is relevant to the fish welfare argument, the primary focus of this thesis is to consider the welfare of farmed species (with an emphasis on the Salmonidae), and much of the literature unfortunately falls outside of this scope. Therefore, the discussion will be centred around a selection of key examples from the literature that will illustrate the “basic” and “flexible” cognitive abilities of fish along with other “sentient-characteristics” in an integrative fashion.

1.6.1 Social Behaviour And Awareness

Rainbow trout are aggressive animals that fight each other to form and maintain dominance hierarchies or territories (Abbott and Dill 1985). Recent studies have shown that individual trout possess the cognitive ability to recognize previous opponents, and to modify their own fighting behaviour accordingly (Johnsson 1997). Other experiments have shown that in addition, rainbow trout can preview and assess the individual-specific fighting ability of future opponents. Johnsson and Åkerman (1998) demonstrated that an individual rainbow trout watching another pair of trout involved in a fighting contest will subsequently modify it's

own fighting tactics upon contests (size matched) with previewed individuals. Aggressive species of farmed fish may also communicate with each other during contests. In fighting contests between Atlantic salmon (*Salmo salar*), a fish can suddenly change its body colour (independent of “stress-related” body colour alterations) to indicate submission to an opponent (O'Connor, Metcalfe and Taylor 1999). Darkening of body colour usually causes the winner of the bout to reduce its aggression towards the darkened fish (O'Connor et al. 1999). Subordinate Arctic charr (*Salvelinus alpinus*) also use skin darkening as a social signal (Höglund, Balm and Winberg 2000).

In the preceding examples, both “basic” and “flexible” cognitive abilities were demonstrated by fish. A fish could “perceive” and recognize other fish in its environment, and through experience, interpret the fighting skills of future opponents as either superior or inferior to its own. These examples also suggest that some degree of self-awareness may occur in fish. Self-awareness has been defined by animal welfare scientists as an animal's ability to abstract and to form a conceptual framework of its environment so that it can perceive itself and its actions in relation to the environment (Wood-Gush et al. 1981). Self-awareness, like consciousness, is probably not an “all or none” phenomenon, but rather is graded (Bunge and Ardila 1987), and it has been postulated that animals with complex social behaviour are more likely to have some sense of “self” (Humphrey 1981). The fact that an Atlantic salmon could signal submission to modify the behaviour of its opponent's and that a rainbow

trout would alter its own aggressive behaviour based on assessments of other, fighting fish, suggest that those fish could perceive themselves (or at least their actions) in addition to their environment.

1.6.2. Pain and Fear

The information pertaining to pain and fear in fish is fragmentary and scarce. This is unfortunate, as it fuels the misconception that pain, fear and perception of those states in fish is unreal or unimportant, and hence any discussion on the subjective nature of pain or fear in fish is immediately futile. For example, Brown (1985) has stated that "the question of whether fish feel pain is a moot point, since it is impossible for workers to distinguish between the response of fish removing themselves from adverse stimuli from the actual measurement of pain...". That suggestion and others made in Brown's report seem to be based on the fact that responses of fish to severe wounds may appear meager or absent, while the reflexive actions to acute or potentially painful stimuli are thought to be "hard-wired" and not subject to cognitive processes. Stoskopf (1994) argues that it would be an unjustified error to assume that fish do not perceive pain in these situations, only because their responses do not correspond with those traditionally observed in other vertebrates. Instead, fish may simply be reacting in unfamiliar ways (subtle alterations in posture and water column utilization), or may have other unidentified coping mechanisms. After reviewing the neurological, pharmacological and behavioural evidence of pain perception in fish, Gregory (1999) comes to the conclusion that "the

appropriate question appears not to be *do fish feel pain?*, but rather, *what types of pain do fish experience?*". Even though there are very few studies to date that have examined pain perception and fear in fish, the available information suggests that both pain and fear are experienced in ways similar to higher vertebrates.

The receptors, neuronal pathways and specific transmitter molecules that convey information about pain in vertebrates is collectively termed the nociceptive system (Broom and Johnson 1993). Activation of nociceptor nerve fibers is associated with a number of painful sensations in humans and aversive behaviour in many animals (Kitchell and Johnson 1985). Anatomical studies of primitive fish (Lampreys, *Petromyzon marinus* and Hagfish, *Ichthyomzon unicuspis*) have confirmed the existence of functional nociceptor nerve fibers. In these studies, destructive stimuli (pinching, puncturing, burning) of the skin resulted in electrophysiological activity of ganglia innervating the fishes head and of dorsal cells in the fishes brain (Matthews and Wickelgren 1978; Rovainen and Yan 1985). In higher vertebrates, it is generally accepted that the perception of pain involves the cerebral cortex (neocortex) activity (Rose and Adams 1989). There are anatomical differences between vertebrate species in the cerebral cortex, and the equivalent structure in fish is the telencephalon (Echteler and Saidel 1981). The telencephalon of fish regulates avoidance learning, and plays a role in the behavioural responses of fish to noxious stimuli (Overmier and Papini 1986).

The preliminary biochemical evidence for pain perception in fish suggests that pain pathways and the biochemical mediation of pain are in some ways similar to mammals and other vertebrates. In mammals, adrenocorticotropin may be released from the pituitary gland when noxious stimuli are encountered (Chapman and Nakamura 1999; Van de Kar and Blair 1999). Endogenous opioids (morphine-like substances) are also produced in higher vertebrates, which can suppress the intensity of pain experienced (Broom and Johnson 1993). A number of studies have shown that the nervous systems of teleost fish also produce chemical compounds related to adrenocorticotropin hormone and pain mediating opiates (Hon and Ng 1986; Ng and Chan 1990). Jansen and Green (1970) and Ehrensing, Michell, and Kastin (1982) demonstrated that analgesia could be achieved in goldfish (*Carassius auratus*) subjected to electric shocks by adding morphine to the tank water. Similarly, Chervova (1996) found that it was possible to modulate "pain-induced" behaviour of rainbow trout using opiate and non-opiate analgesics. Using opiate antagonists, Ehrensing et. al. (1982) found that morphine analgesia in goldfish could be blocked through similar modes of action as in higher vertebrates. In addition, it has been reported that at least six different protein binding sites, which may act as receptors for pain modulating molecules, are present in teleost fish (Stoskopf 1994; Gregory 1999).

Subjective experience is an intrinsic component of pain in humans and higher vertebrates (Rose and Adams 1989; Chapman and Nakamura 1999). There is

evidence to suggest that pain in fish is not limited to a sensory message of tissue trauma, but is also “perceived” and is responsible for a negative affective state as in higher vertebrates. The behavioural reflexes and reactions of all animals to noxious stimuli may be categorized as “perception linked” or “non-perception linked” (Kitchell and Guinan 1990). The probability that an animal can perceive pain is dependent upon the cognitive ability and functioning of that animal (Kitchell and Guinan 1990). The relationship between cognitive function and pain perception is commonly elucidated through the learning abilities of the animal (with respect to pain), and through brain ablation experiments (Kitchell and Guinan 1990; Bateson 1991).

In general, teleost fish show learning capabilities that approximate those of other vertebrates that are expected to experience pain (Overmier and Hollis 1983). Briefly, learning in teleost fish can encompass non-associative processes (i.e. habituation, sensitization, pseudoconditioning), associative processes (classical conditioning, instrumental training, avoidance learning) and integrative processes (transfer of control, successive reversal learning) (reviewed in Overmier and Hollis 1983; Savage 1980). Most of those learning processes can be facilitated by pain-producing stimuli, such as electric shocks, and are subject to modulation upon telencephalon (the teleost forebrain) ablation. With experience, fish can learn to avoid situations or locations where they have experienced noxious stimuli which are expected to produce pain (Overmier and Hollis 1983). Rainbow trout may, through experience, associate an approaching conspecific with the

physical consequences of receiving the nip that normally follows such an approach, and learn to retreat (Pitcher 1993). Fish also may actively avoid environments that were once rewarding (food liberating) if a noxious stimulus is introduced there (Overmier and Hollis 1983). Fish may interpret physical sensations as positive or negative; ectoparasites that irritate the skin and gills of atlantic salmon cause them to initiate a leaping behaviour in an attempt to rid themselves of the infestation (Furevik, Bjordal, Huse and Ferno 1993). Fish also find the physical removal of such parasites by wrasses ("cleaner fish" that remove parasites from the bodies of other fish) as a positive sensation (Losey Jr and Margules 1974). Although much remains to be learned about the mechanisms of pain perception in fish, it is reasonable to suggest in light of the available information that fish are conscious of pain, and judged by their behavioural reactions, find it negative.

Fear may be defined as a psychophysiological response to perceived danger (Jones 1997), and is a phenomenon which acts as a powerful motivator to evade perceived threats (Webster 1995). Fear in fish has been characterized through branchial responses and adverse behavioral reactions. Pavlovian fear conditioning in fish, as elucidated through branchial responses, is dependant upon cognitive mechanisms (Davis and Klinger 1994). For example, in goldfish, fear responses to an imminent electric shock could be blocked by administering a brain N-methyl-D-aspartic acid receptor antagonist (an anterograde memory blocker) (Davis and Klinger 1994). "Fearful" responses of fish to noxious or

startling stimuli which are manifested as vigorous, rapid escape maneuvers are collectively defined as "fast-start" responses. The "fast-start" response is characterized by a high-energy swimming burst, either starting from rest or imposed upon periods of steady swimming (Domenici and Blake 1997). The actual response itself may be classified as a fixed action pattern (Eaton and Nissanov 1985), which is triggered by the firing of a pair of large neurons located in the brain stem (Eaton, Lavender and Wieland 1981). These neurons receive a rich supply of connections from sensory and integrative centers in the brain, and not surprisingly, "fast-starts" are initially dependent on sensory information which allows the fish to coordinate the trajectory of its response in relation to the stimulus (Bisazza, Rogers and Vallortigara 1998). The "hard-wired" nature of the escape responses in fish has fostered the attitude that, much like removing a finger placed inadvertently on a hot surface, initial awareness of the offending stimuli and the motivational or affective state of the animal are irrelevant for such responses to occur (i.e. they are not linked to perception). This interpretation of the escape response is overly simplistic however; while the "fast-start" response itself is a fixed action pattern, the context in which it is elicited by a fish varies. "Fast-starts" are used by fish in social interactions, during feeding behaviour, to escape predators, and for predatory strikes (Domenici and Blake 1997). Therefore, an awareness of a situation is necessary for proper use of a "fast-start" response; a fish would "want" to speed towards prey and away from a predator. This opens the possibility that under certain conditions, there is likely to be a motivational basis for performing "fast-starts". For example, Canfield and

Rose (1993) demonstrated that feeding maneuvers (capturing of a cricket) in goldfish incorporated "fast-starts". Since the motivation for feeding is necessarily different than for predator escaping, the authors suggested that higher order neural processes may modulate sensory activation of the neurons which invoke the "fast-starts". Canfield and Rose (1993) also suggested that there may be a voluntary component for the "fast-start" activation within a feeding context. Those suggestions are supported by the finding that alternative neural pathways that invoke "fast-starts" do exist (Eaton, Lavender and Wieland 1982). Other studies have suggested that motivation may play a role in determining the "fast-start" response of fish when escaping predators. Webb (1986) found that the prey speed for fish that were chased by predators was 3 to 15% faster than the speed when predators attacked but did not give chase.

Further evidence for the existence of a motivational drive in "fast-start" reactions, with particular reference to fear, were demonstrated in experiments performed by Cantalupo, Bisazza and Vallortigara (1995). Using a species of teleost fish (mosquitofish, *Girardinus falcatus*), the investigators measured the direction of turning during an escape response evoked by a simulated predator. The test apparatus consisted of a rectangular tank in which a "predator shape" could be rapidly introduced and viewed by the fish through a transparent side of the tank. The simulated predator was presented only when the fish swam across the median portion of the tank, in a central position where the fishes central axis was perpendicular to the predator. Upon presentation of the predator, the fish would

perform an escape response, either turning left or right, away from the predator. There are two findings of this study which bear relevance to motivation and fear in fish. First, it was noticed by the investigators that with repeat testing of the same animal, the fish tended to avoid the centre of the tank where the stimulus presentation would occur. If the fish happened to swim in the tank centre, it tended to zig-zag rapidly (Bisazza et al. 1998). It therefore appears that the fish developed an active strategy to avoid an encounter with the stimulus. Since the zig-zag swimming and the escape response were used by the fish to prevent presentations or to remove itself from a stimulus previously perceived negative, it is plausible that the fish could anticipate the "frightful" stimuli, and the escape response was in part motivated by an affective state of fear. The second point of interest in this study relates to the direction (left or right) of the escape response shown by the test fish. The fish tested in Cantalupo et al. (1995) showed a tendency to escape to the left, and after repeated predator occurrences, were biased to escape to the right. The authors speculated that the change in escape direction from left to right was related to the way that the fish categorized the predator stimulus. In animals with laterally placed eyes, as in most fish, the visual scenes on the right and left sides may evoke different types of behaviour, as a result of differing modes of analysis of perceptual information carried out by the left and right sides of the brain (Bisazza, Pignatti and Vallortigara 1997; Bisazza, De Santi and Vallortigara 1999; Vallortigara, Rogers and Bisazza 1999). Cantalupo et al. (1995) reasoned that an initial escape to the right would keep the fishes left eye in contact with the predator stimulus, possibly because of

the involvement of the right-sided structures of the brain in response to fright. The right side of the brain is subject to affective responses in many vertebrates. Since presentation of the model to the fish was not followed by attacks and injuries, the fish may have categorized subsequent presentations of the stimulus as innocuous (but still to be avoided), with a shift towards control by the left-sided regions of the brain. A similar phenomenon has been shown to occur in the domesticated chick (Andrew and Brennan 1983). Jones (1997) notes that "fear responses" of animals may be altered and integrated according to changes in the perceived potency of the threatening stimulus, and consequently, the intensity of the internal fear state. The cognitive processing and categorization of startling stimuli as initially threatening, but later innocuous, argues that fish can experience fear in similar ways to other vertebrate animals.

In summary, no single criterion can provide an all-or-none test for the existence of a subjective sense of pain or fear in animals. Instead, the evidence needs to be considered as a whole in order to assess the abilities of an animal. It is clear from the above discussion that there are strong grounds for supposing that fish can perceive and are conscious of pain and fear.

1.6.3 Psychological Aspects Of Stress

In general, the concept of stress infers some type of coping or preparatory response (the "stress response") of an organism at some level of biological organization in response to some stimulus (the "stressor"). In fish, there is an adrenergic stress response which results in a liberation of adrenaline and noradrenaline to the blood stream, and a hypothalamo-pituitary-interrenal (HPI) stress response, which ultimately results in an increased blood cortisol concentration (Sumpter 1997). A number of investigators, however, have also adopted a more encompassing view of stress in fish, by realizing that the stress phenomenon is an integrative process that takes into account both the "perception" of the stressor by fish and the memory of prior experiences in the process that organizes the stress response (Barton 1997). This has prompted a few authors to discuss stress in fish not just from a strict physiological stance, but from a psychological one as well. The psychological aspect of stress in fish has been viewed and discussed in the context of being caused by biotic or abiotic factors.

Schreck (1981) was the first to suggest that the "psychological" component of stress was important in determining the severity of a stress response in fish (in an abiotic context). Schreck proposed that the psychological well-being of a fish was a factual, tangible phenomenon, that could be elucidated by "awareness" or positive conditioning experiments. Although very little research has addressed the issue of "psychological well-being" in fish, the results of such studies clearly

demonstrate the "psychological" component of stress. In one such experiment, Schreck, Jonsson, Feist and Reno (1995) were able to positively condition chinook salmon (*Oncorhynchus tshawytscha*) to a stressor (emptying the fish tank until the water just covered the fishes bodies). After conditioning, the fish's physiological stress response (cortisol) to subsequent stressors (transportation) was significantly lowered as compared to controls. In addition, the "psychologically hardened" fish had a higher rate of survival in a number of "challenge tests" after the initial transportation stressor (Schreck et al. 1995).

Since many species of farmed fish form social hierarchies, or defend territories (Wedemeyer 1997), psychological stress in a social context has been more thoroughly studied in fish. In a social context, psychological stress in animals has three components (Zayan 1991). The first consists of a set of negative affective experiences, such as fear. These aspects were discussed in section 1.6.2, and are additionally commented on in the proceeding discussion. The second component consists of a "perceptive" process, by which familiar conspecifics are recognized as damaging, and strange conspecifics are viewed as threatening. The "perceptive" process by which conspecifics can be recognized with respect to aggressive ability has been demonstrated in section 1.6.1. The third component of social stress relates to the cognitive process in which animals can "anticipate" the presence or actions of "stressful" conspecifics.

Fish in stable dominance hierarchies may be classified as dominant, subordinate, or of intermediate status. There is evidence that suggests that not only can the subordinate fish in a hierarchical situation anticipate the action of dominant fish, but it can be affected psychologically as well. A number of studies using rainbow trout and arctic charr (*Salvelinus alpinus*) have demonstrated that the chronic stress experienced by subordinates is more related to the threat imposed by the presence of the dominant fish, rather than actual aggressive encounters (Winberg and Nilsson 1993; Winberg and Lepage 1998; Øverli, Harris and Winberg 1999). This suggests that the experience of a fish being subordinate under captive conditions probably involves an affective state of fear. If an animal is capable of experiencing fear, then the motivational state of that animal could also be expected to be altered. The central serotonergic system of fish seems to have an influence on the motivational state, and consequently, on the behaviour of the fish. The subordinate experience in many vertebrate species, including fish, is associated with an increase in brain serotonergic activity (Winberg and Nilsson 1993). Socially subordinate fish with an increase in brain serotonergic activity are behaviourally inhibited (i.e. less motivated to fight), and show reduced locomotor activity (Winberg and Nilsson 1993; Øverli et al. 1999). Interestingly, those findings are in line with the observations and adaptive functions of "low-mood" as described in higher animals (Nesse 2000).

The preceding examples have shown that the stress responses of fish are not only manifested physiologically, but psychologically as well. A fish can

“perceive” biotic and abiotic factors from its environment and interpret them as either positive or negative. In some cases, the interpretation may lead to an affective state of fear. Stressors that psychologically affect fish should be accounted for in the range of welfare considerations for farmed fish.

1.7 Assessment of Welfare in Aquaculture

The foregoing review of the pertinent capabilities of fish strongly suggests that the concept of animal welfare can be applied legitimately to farmed fish. Animal welfare is discussed and pursued because of an ethical concern by society over the quality of life of animals. Inherent to the concept of animal welfare is that animals have the capacity to suffer, and therefore the “quality of life” of an animal is greatly determined by how much it may suffer under captive conditions (Duncan and Fraser 1997). The previous discussion has illustrated that states of pain, fear and psychological stress are likely to be experienced by fish. The affective states normally associated with pain, fear and psychological stress imply that fish can indeed suffer, and that welfare consideration for farmed fish should take into these states into account. Therefore, the welfare of farmed fish should include, but range beyond, basic life-support (suitable water conditions and adequate feeding), and should be extended in a manner which corresponds to any of the cognitive abilities they may possess. Of particular importance are those cognitive abilities that relate to the ability of fish to experience pain and fear.

1.7.1 Biological Indicators And Welfare

There has been extensive research to develop physiological indicators to monitor and assess the responses of fish to environmental stressors. Much of this research has used the hormone cortisol and the related metabolic responses to indicate "stress" in fish (Barton 1997). As a result, the behavioural, physiological and psychological reactions of fish are almost always interpreted within the concept of this physiological "stress". Consequentially, the well-being of fish (most often understood as solely pertaining to health or growth) has predominantly been equated to an endocrine or metabolic indicator. However, the reverse of this relationship is not always true, especially when the well-being of a fish takes into account experiences of pain and fear. Since the physiological "stress" response of animal is not necessarily indicative of how that animal may 'perceive' any given situation, the integration of a number of scientific methods to assess welfare has resulted (Mench 1993). By no means is this a criticism of stress physiology, since "stress" and the indicators of stress have significant importance to a number of animal management and husbandry issues in aquaculture. Furthermore, in the correct context, endocrine or other metabolic indicators may be used in welfare assessments when there is a demonstrable relationship between cognitive factors and physiological responses (Mench 1993). Since physiological responses can depend upon the animals perception of a stressor, indicators such as plasma cortisol may give insight into how an animal experiences and interprets a situation (Mendl 1999). Research of this

type has already been initiated using farmed species of fish (Schreck et al. 1995).

Any method that is devised to assess the welfare of farmed fish should take into account both the behavioural and physiological responses of fish within the context of the cognitive states of the fish. Behaviour, in particular, can provide excellent cues about the preferences, needs, and internal states of animals (Mench and Mason 1997). Suppression of normal behaviours and behavioural changes of animals can be indicative of environmental disturbances, pain and distress (Mench and Mason 1997). Studying behaviour may provide insight into the affective states of animals. For example, if affective states of suffering, such as fear and pain in animals can be defined operationally, then behaviour can be used to determine how negatively the animal finds those experiences, for example, by observing how hard it will work by avoiding those experiences (Duncan 1996).

The value of using behaviour for evaluating welfare in farmed fish should be significant, as overt behaviour encompasses many of the observable characteristics which have been used in successful welfare assessments in other farmed animals (behavioural suppression, avoidance etc.). Fish behaviour has been shown to be a sensitive indicator to problematic rearing techniques used in the aquaculture industry (Schreck et al. 1997), as well as in itself being a source of significant stress (Wedemeyer 1997). With all these positive attributes for

using behaviour as a welfare indicator, it might be expected that such methods are widely used by the aquaculture industry (albeit primarily as a “stress” indicator). This is not the case however. Part of the reason that behaviour has been widely used for welfare assessment for terrestrial animals is that the behaviour of interest is easily observable. In aquaculture, major observational restrictions are imposed by the rearing environment itself (light intensity, turbidity, depth, distance), and consequentially, most conventional observational techniques have limited use (Baras and Lagardère 1995). Furthermore, behavioural assays performed in the laboratory may give misleading conclusions due to the discrepancies between experimental and aquacultural conditions (Baras and Lagardère 1995). Clearly, it would be of great benefit if techniques were devised that could monitor and measure the behaviour response of fish *in situ*, under intensive rearing conditions. With the recent developments of underwater biotelemetry technology, the opportunity now exists to remotely monitor and evaluate the activity and behaviour of fish.

1.7.2 Biotelemetry As A Behavioural Indicator

With the recent application of physiological telemetry systems to fisheries science, quantitative information describing the locomotory activity of free - swimming fish *in situ* can be obtained and recorded (Kaseloo, Weatherley, Lotimer and Farina 1992). An implantable, wireless device, capable of detecting and transmitting estimates of electromyogram (EMG) activity has allowed researchers to quantify swimming activity of wild fish (McKinley and Power 1992;

Demers, McKinley, Weatherley and McQueen 1996; Hinch, Diewert, Lissimore, Prince, Healey and Henderson 1996; Weatherley, Kaseloo, Gare, Gunn and Lipicnik 1996). Since changes in locomotory activity appear to be sensitive indicators of the fishes behavioural response to environmental conditions and social stressors (Scherer 1992), it is possible that biotelemetry may be used as a behavioural indicator of value to welfare assessments. Furthermore, with the use of controlled water-velocity swim chambers, and respirometers, telemetered locomotory activity has also been correlated to the actual swimming speed and oxygen consumption of fish in the laboratory, thereby permitting estimates of those variables for free ranging fish in natural or captive environments (Briggs and Post 1997a,b; Cooke, Chandroo, Beddow, Moccia and McKinley 2000). This information could theoretically provide physiological or animal performance correlates pertinent to fish welfare.

1.8 Purpose And Objectives

The purpose of this thesis research was to develop and exploit EMT technology as a quantitative and objective behavioural indicator in order to aid in the assessment of the welfare status of fish reared in intensive aquacultural conditions. Towards this end, a number of experimental investigations and objectives were pursued. The first study was designed to validate the use of the EMT as a behavioural indicator in rainbow trout by correlating radio-transmitted EMT signals of the flank muscle contractions with video-recorded swimming behaviour. To date, EMT has not been used extensively to identify specific

locomotory behaviour per se, therefore the characteristics and limitations of EMT signals as a proxy for behaviour must be identified. The second and third experiments were designed to investigate and evaluate, using EMT, the behavioural response of rainbow trout to a sample of common aquacultural rearing activities and conditions. Specifically, the fishes response to a transportation episode, and two types of artificial lighting systems were examined. The final objective of this thesis was to initiate the development of a “welfare indexing system”, in which the effect of intensive rearing practices on the welfare of farmed fish can be assessed and evaluated as acceptable or unacceptable from a welfare perspective.

Chapter 2: General Methodology

The following sections describe the experimental animals, materials and procedures which have been common to the investigations presented in the subsequent chapters of this thesis. Information pertaining to the experimental design, environmental conditions, statistical methods and animal characteristics for individual experiments are described in the methodology section of the appropriate chapter. All experimental techniques and procedures were conducted under the approval of the Animal Care Committee (Animal Utilization Protocol 98RO97) of the University of Guelph, under the guidance of the Canadian Council on Animal Care.

2.1 Experimental Animals

Hatchery reared, domesticated rainbow trout (*Oncorhynchus mykiss*), raised at the Alma Aquaculture Research Station (University of Guelph, Alma, Ontario) were used for all the experiments reported here. The experimental trials were conducted using large, market size fish (weight range approximately 700 to 1500 g).

2.2 The Electromyogram Telemetry System

The telemetry system consisted of EMG sensing radio-transmitters, which are surgically implanted into the fish; a programmable radio receiver which detects and records transmitter signals; and computer software required to download the recorded transmitter signals. The commercially available EMG sensing

transmitters, telemetry radio receiver (SRX_400) and software used in this study were manufactured by Lotek Engineering Inc., Newmarket, Ontario.

The EMT transmitters were cylindrical, 50 mm in length, 16 mm in diameter and weighed 18 g in air. In addition to a single radio antenna, each transmitter included a pair of Teflon covered electrodes, which are twisted together except near the distal end where they are inserted into the fishes musculature (Figure 2.1). Cylindrical gold tips (9 carat, 5 x1 mm) were affixed to the end of each electrode. When the gold tips are anchored into the red, lateral muscle band of the fish, the electrodes can detect the waves of electrical activity (EMG's) in this muscle type that contribute to a muscular contraction. These electropotential events are processed (integrated and amplified) and the transmitter uses this electrical activity to charge a capacitor. When a factory preset capacitance has been reached, a pulse radio signal is emitted from the transmitter. These signals are detected by the SRX_400 radio receiver, and the interval of time between successive pulse signals is calculated by the receiver and recorded as a pulse interval (EMG_i). This is a numeric value, given in units of milliseconds (ms), and is inversely related to muscle activity. In other words, as the muscular activity of a fish increases, the transmitter capacitor charges more rapidly, thereby decreasing the pulse interval value calculated and recorded by the receiver. Each transmitter broadcasts at a different radio frequency, so the EMT information, collected sequentially on a multitude of fish with operating tags, can

be kept distinct for individual fish. The radio receiver stores the EMT information, until being periodically downloaded to a computer.

2.2.1 Surgical Techniques

The surgical procedures were conducted under the approval of the Animal Care Committee of the University of Guelph (Animal Utilization Protocol 98R097). Two days before surgery, fish were taken off feed. Transmitters were implanted using procedures similar to those reported by Beddow and McKinley (1998) and Cooke et al. (2000). All surgical instruments were dipped in alcohol, rinsed and flamed immediately before use. Transmitter electrodes and gold tips were cleaned with alcohol, and rinsed with autoclaved water. Rainbow trout were netted from their holding tank and individually anaesthetized in an aerated, temperature controlled solution of tricaine methanesulfonate (MS - 222) at a concentration of 70 mg/l. The fish were then placed ventral side up on foam V-shaped surgical table, and their gills were irrigated continuously with fresh, aerated anesthetic solution. Using a scalpel, a 3 cm long incision was made in the mid-ventral abdominal wall, just anterior to the paired pelvic fins. A blunt probe was used to open the peritoneal cavity, to reduce the risk of damaging internal organs. To anchor the gold tips into the lateral red muscle, a custom tool was devised, which was constructed of the sharpened, stainless steel portions of two 16 gauge intravenous catheter needles (Surflo[®], Terumo Medical Corp.), affixed 10 mm apart. The individual gold tips from each pair of transmitter electrodes were inserted into the barrel at the sharpened end of each catheter needle, and held

in that position by gently applying tension to the Teflon coated electrodes. The device was then inserted through the incision into the peritoneum (shielded by a blunt probe), and positioned into the red axial muscle, to a depth just below the integument. A stylette was then inserted into the unsharpened distal ends of the catheter needles, and was used to push the gold tips from the catheter barrels into the musculature, thus securing the electrodes. The placement of electrodes, lengthwise (anterior to posterior) along the lateral red muscle band, was standardized in each fish by aligning the paired electrodes with the anterior portion of the dorsal fin (Figure 2.1) (Beddow and McKinley 1999). The transmitter was then gently inserted into the peritoneal cavity through the abdominal incision. The transmitter antenna was fed back through a 16 gauge hypodermic needle, inserted just posterior to the incision, and was allowed to protrude from the body wall. The abdominal incision was then closed using 3 to 4 independent sutures (3-0 silk, Ethicon). An external, 10 cm anchor tag (Floy Tag & MFG., INC.), was then attached in strategic positions around the dorsal fin of each fish. The coloured anchor tags allowed for easy, visual identification of individual fish throughout the experiment. The surgical procedure took a maximum of 10 minutes to complete, at which time the fish were placed in a 2x2 meter semi-square tank with fresh, well-aerated, temperature controlled water to recover.

2.2.2 EMT Data Standardization

It has been previously shown that the EMT output recorded from a number of tagged individuals can vary even when the fish demonstrate identical locomotory activity, such as when they are resting (stationary), or swimming at the same speed (Weatherley et al. 1996; Økland, Finstad, McKinley, Thorstad and Booth 1997; Beddow and McKinley 1998; Beddow and McKinley 1999). In each instance when this has been observed, a clear, linear relationship between swimming speed and EMT pulse intervals for each individual fish still exists, except that the relationship for each individual occurs over a different range of EMT values (e.g. between 2100 to 1300 ms for one fish and 1700 to 800 ms for another). These differences can also be observed during surgical procedures, where the baseline EMT output from an anaesthetized, immobilized fish can differ by as much as 500 ms (unpublished observations, this study). Most of this variation is usually associated with the electronic variability inherent to the transmitters, rather than to the muscle-associated differences between fish, assuming that electrode placement is standardized (Beddow and McKinley 1999). Økland et al. (1997) found that when adjustments were made for the differences in resting EMT levels among individuals, by dividing the EMT values during various states of activity by the EMT average obtained at rest, a higher correlation between swimming speed and EMT pulse intervals for the entire group of fish was obtained. Weatherley et al. (1996) have also used this correction method, to compare muscular efforts among different fish. Those studies have assumed that this correction can standardize activity readings

among individuals, ensuring that different levels of EMT indicated activity measured among individuals are based on changes in muscle activity, rather than a difference in non-biological transmitter range.

In this study, EMT signals were standardized for each fish by measuring EMT values under pre-defined conditions or behavioural states (i.e. "resting") and using these as basal measurements. All other recorded activity from that fish throughout the experiment (experimental EMT values) was then calculated for each fish as the percent increase or decrease from its basal EMT value, and is defined here as the "activity index". The activity index was calculated as in (Cooke et al. 2000) according to the following equation:

$$\text{Activity Index} = [1 - (\text{experimental EMT values} / \text{basal EMT})] \times 100$$

Activity index data increases reciprocally with fish activity, rather than inversely as when 'raw' EMT pulse intervals are related to activity. The basal values were, at minimum, the mean of 200 to 500 EMT signals for each fish. The environmental conditions or behavioural state when basal EMT values were recorded from fish are further defined and explained where warranted in each thesis chapter.

2.3 Swim Tube Protocols

In both preliminary work relating to this thesis (Cooke et al. 2000) and in experimental work presented in subsequent chapters, a series of trials were carried out in which fish implanted with EMT transmitters were made to swim at controlled speeds. During this procedure, EMT values were calibrated with the swimming speed and oxygen consumption (V_{O_2}) of the fish. In addition, the swimming endurance and fatigue point of the tagged fish was determined. The swimming tests, EMT and oxygen measurements were done in a 120 litre Blazka-type swim speed chamber/respirometer, which is pictured in Thorstad, Finstad, Økland, McKinley and Booth (1997).

The Blazka-type swim chamber/respirometer is characterized by a tube within a tube (coaxial) design. A propeller pulls water past the fish, located in the inner tube, at a controlled, constant velocity, forcing the fish to swim against the current. Water exiting the proximal end of the inner tube is then deflected by a domed end-cap into the outer tube, and is subsequently returned to the distal end of the inner tube. The cross-sectional diameter of the inner and outer tubes were 24 and 44 cm, respectively. In a swimming tube of this design, errors in estimating the swimming speed of fish may be produced due to hydrodynamic “edge effects” and “blocking effects”. The decrease of water velocity near the inner tube wall (the edge effect) was minimal, and was previously calculated to be less than 4 cm/s at any speed. Since the fish tested did not occupy more than 10% of the inner tube cross-sectional area, we did not have to correct for solid

blocking effects (Beamish 1979). Oxygen levels in the swim chamber were monitored continuously by re-circulating a small fraction of the chamber water over a digital oxygen probe (Orion, Inc.), using a small peristaltic pump fitted with gas-tight tubing. The oxygen probe was calibrated with an air saturated water sample before use. During the tests, the oxygen concentration of water in the swim chamber never fell below 80% air saturation.

All calibrations and swimming performance tests were performed simultaneously. Fish were netted into the chamber, and allowed to acclimate to the chamber at a constant water velocity of 0.1 m/s for a 15 minute period. The water velocity was then increased every 15 minutes by 0.17 m/s intervals until the fish fatigued. During each water velocity increment, EMT values were recorded when the fish maintained a constant position in the tube and was actively swimming. Oxygen readings were also taken at the beginning and end of each successive 15 minute interval. Fatigue was considered to have occurred when the fish could no longer maintain position in the chamber and was impinged against the blocking screen. The time of fatigue was recorded and used to calculate the critical swimming speed (U_{crit}). The critical swimming speed is a measure of prolonged swimming ability, and was calculated according to Brett (1964).

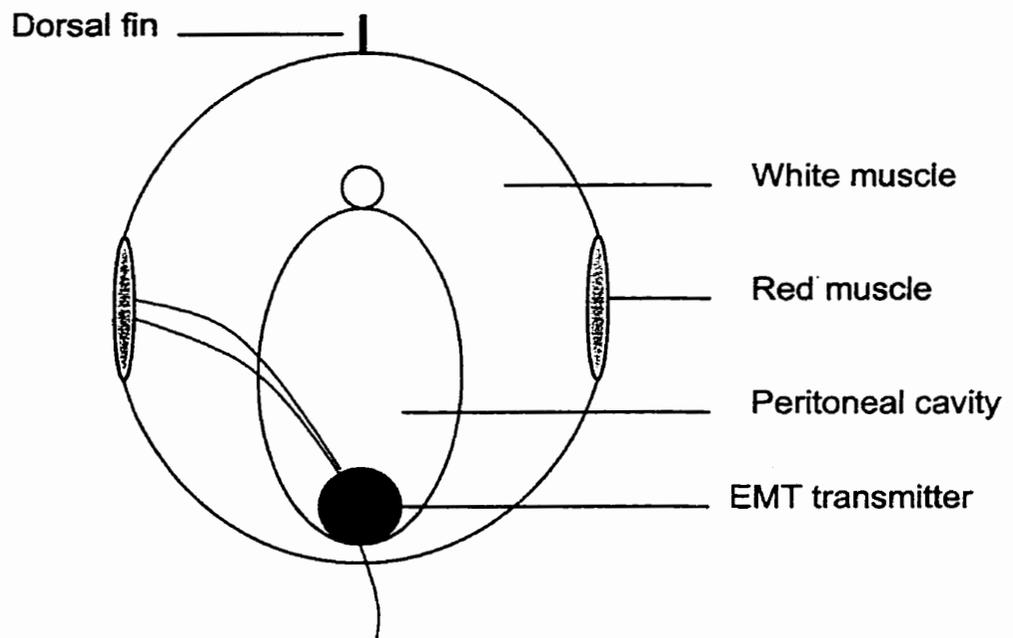
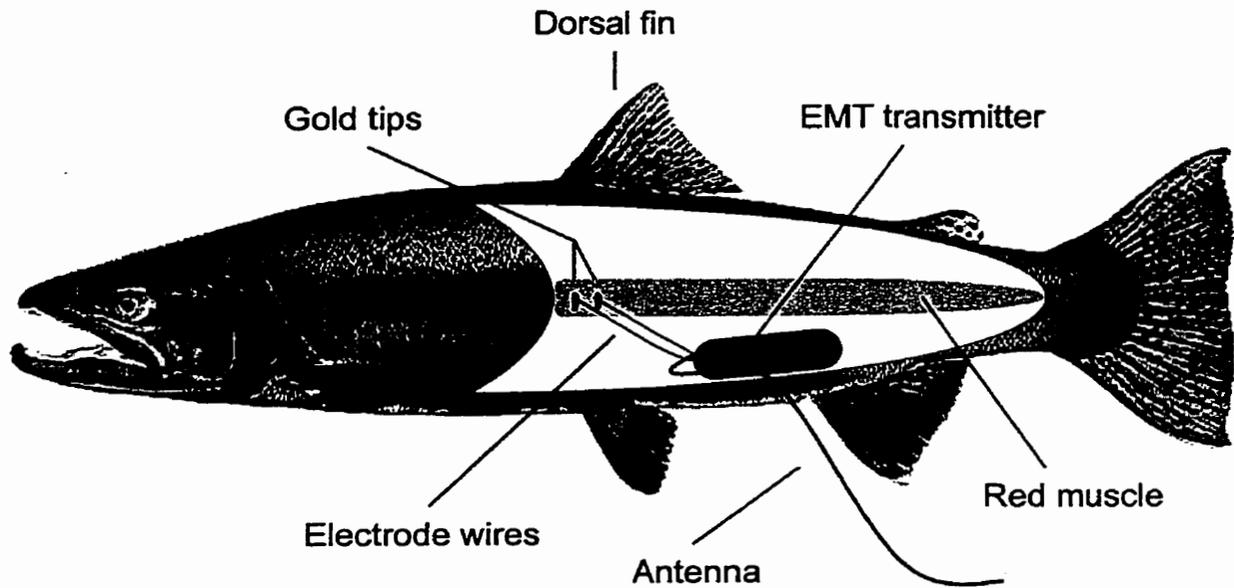


Figure 2.1 EMT transmitter, electrode and antenna position in a surgically implanted rainbow trout.

Chapter 3: Utilization Of EMG Telemetry As A Behavioural Indicator: The “Light-Shock” Response Of Rainbow Trout

3.1 Abstract

The objectives of this experiment were to, 1) characterize the swimming behaviour involved in the “light-shock” reaction of rainbow trout, and, 2) validate the use of EMT as a behavioural indicator, by correlating EMT signals with video-recorded swimming activity. Video recorded observations of the “light-shock” reaction of rainbow trout, induced by “instant-on” lighting, were analyzed and could be correlated with EMT signals, suggesting that telemetry data were an accurate measure or proxy of swimming behaviour. EMT may therefore allow for the objective quantification of both fish activity and behavioural responses to rearing methods used in aquaculture, and may thus prove to be a valuable tool to evaluate aquacultural husbandry protocols from a welfare perspective.

3.2 Introduction

Aquacultural production systems and research facilities are usually equipped with artificial light fixtures. Timing and dimming control devices are commonly used to adjust the latency and duration of the photoperiod to suit a number of needs relating to the growth, smoltification and reproduction of fish (Heinen 1998; Boeuf and Yves Le Bail 1999; Duncan, Mitchell and Bromage 1999). Although there is a wealth of information prescribing the optimum photoperiod length for culturing various species of fish, few investigations have addressed the

optimum, or minimum acceptable rate at which the light intensity changes during twilight (the dawn and dusk periods). Often, artificial lighting systems have an abrupt on-off control, subjecting the fish to instantaneous activation or deactivation of the light source at maximum intensity.

Light sensitive fish subjected to a sudden change from darkness to brightness demonstrate a number of behavioural reactions, collectively termed as the “light-shock” reactions (McHugh 1978; Heinen 1998). This behaviour includes species-specific changes in locomotory activity, which may occur along with other physiological responses. Detailed information describing the behaviour is sparse, consisting mainly of qualitative observations. Notwithstanding, the “light-shock” response has been interpreted as a stressful event, with negative implications for adult fish (Lahaye et al. 1978; Mork and Gulbrandsen 1994), and has been associated with elevated mortality and other “stress signals” in larvae and fry (Marliave 1977; McHugh 1978; Jørgensen 1983). The fact that little detailed information is available on the locomotory response of fish to this aspect of lighting systems in fish culture facilities may simply be a function of the difficulty associated with measuring such activity, behaviour or other biological indicators under normal aquacultural conditions (Scherer 1992; Baras and Lagardère 1995).

With the recent introduction of physiological telemetry systems to fisheries science, quantitative information describing the locomotory activity of free-

swimming fish *in situ* can be obtained and recorded (Kaseloo et al. 1992). Since previous studies observing the “light-shock” reactions in cultured species of salmonids have suggested that fish show a locomotory response to abrupt, “instant-on” light activation (Mork and Gulbrandsen 1994), the opportunity exists to record, quantify and objectively assess the activity of fish to “instant-on” lighting systems using EMT.

Therefore, the objectives of this experiment were to, 1) characterize the “light-shock” reaction of rainbow trout, and 2) to validate the use of EMT as a behavioural indicator by correlating telemetered EMT signals with video-recorded swimming activity. To date, EMT has not been extensively used as an indirect measure, or proxy, of fish behaviour per se, but instead has served primarily as an indicator of overall swimming activity. To examine the potential of EMT as a behavioural indicator, it is necessary to study visual observations of the locomotory activity of fish, in concert with EMT signals. Therefore, the behavioural response of rainbow trout to “instant-on” light activation will be characterized using conventional video recording methods. This study demonstrates the effectiveness of EMT as a behavioural indicator of fish, and reports a precise description of the “light-shock” reaction of rainbow trout.

3.3 Methodology

3.3.1 Experimental Animals & Animal Care

Eighty rainbow trout were randomly selected from a stock population and had a mean weight and fork length of 1145 ± 385 g and 422 ± 28 mm respectively at the beginning of the study. Fish that were implanted with EMT transmitters (n=9) were taken from this group (see Appendix A). Coloured anchor tags were also attached to the tagged fish, and allowed for easy, visual identification of individual fish. After the surgical procedure was completed (described in Chapter 2) the fish were placed in a 2x2 m, semi-square tank (i.e. a square tank with curved corners) supplied with fresh, well-aerated water to recover. All experimental protocols were conducted under the approval of the Animal Care Committee of the University of Guelph (Animal Utilization Protocol 98R097).

3.3.2 Video And Telemetry Equipment

The fish were video-recorded using a colour surveillance camera (Panasonic WV-CD110A) with a 3.7 mm auto-iris lens (Cosmicar/Pentax HX-2 series). The camera was mounted on a standard metal tripod and extended to a length which allowed for a view of the entire tank surface area (3.89 m^2), and throughout the water column (0.32 m). The camera was powered by a colour monitor (Panasonic WV-CM110A), which was in turn connected to a VCR (Panasonic AG-6040). The VCR was programmable, so the date and exact time (HH:MM:SS) were displayed and recorded along with all observations. The telemetry receiver (SRX_400) also records the exact ('real') time (HH:MM:SS)

and date at which each EMT pulse interval is recorded. The radio receiver stored the EMT information which was periodically downloaded to a computer.

3.3.3 Environmental Details

Following surgery, the bio-density of each tank was adjusted to between 24 and 27 kg/m³ by the addition of non-tagged individuals, and adjustments to the tank water depth. In total, there were 23 to 26 individual fish in each of the 3 experimental tanks used throughout the study. The tanks were left uncovered, and the fish were hand-fed once daily to satiation (6 Pt., Shur-Gain). Experimental tanks were located in a windowless room, which featured light-proof, double doorways. The recording consoles used during the experiment were kept and controlled in a separate room. The programmable, automated lighting system used to illuminate the room that contained the experimental tanks consisted of four, ceiling mounted 150 watt frosted, medium base tungsten bulbs (Sylvania - A2348-0017650B). Using a digital light meter (RCC 340 FC/Lux), the light intensity at the tank-water surface was measured, and ranged between 310 to 350 lumens. There were no sources of light during night (i.e. complete darkness) periods.

Two months prior to the experimental trials, and also during surgical procedures, the experimental fish were held indoors, under artificial lighting. During twilight, the lights were phased from 0 to 100 % intensity on or off over a 30 to 35 minute time period, mimicking natural conditions for southern Ontario at this time

(Elmira, Ontario, June 1998 - April 1999). Water supplied to the fish had a temperature of $8.5 \pm 0.1^\circ\text{C}$, a dissolved oxygen concentration of 10.6 mg/l (oxygen saturation of 100.8%) and a pH of 7.6 ± 0.1 . Water was delivered to the tank at 22 l/min, which generated a mild circular flow within the tank, and a theoretical turnover rate of 1.03 turnovers per hour.

3.3.4 Experimental Protocol

There were 3 independent recording events within each experimental trial, and 3 trials were performed. During each recording event, the fish's reaction to "instant-on" light activation was documented by time-synchronized video and telemetry recordings. Each recording event took place once daily, at dawn, over 3 to 4 consecutive days. The time of "instant-on" light activation during the experimental trials was the same as under the fishes previous rearing photoperiod. Ten minutes prior to "instant-on" light activation, time-synchronized EMT and video recordings filming the entire tank began (in darkness), and continued up to 10 minutes after lights-on. During the second twilight period of the day (i.e. dusk), the room lighting instantaneously shut off at the same time at which the phased lighting system would have slowly begun to reduce the light intensity. No recordings were taken from this period. From each recording event (n=9), there were 20 minutes of continuous EMT sampling and 10 minutes of visible fish activity filmed by the video camera. To avoid bias, the EMT data analysis and video transcription were done separately.

3.3.5 Behavioural Categories Derived From Video recordings

A combination of fast swimming speeds and a relatively large number of fish in each tank made it impractical to score activity on the individual from which telemetric data was being collected. Hence, the fish activity, recorded by video, was analyzed on a per tank basis. Analysis of the videotapes for distinct swimming behaviour revealed three main behavioural locomotory categories as follows;

Category "A": Characterized by burst and glide swimming, "fast-start" responses (Domenici and Blake 1997), fast undulatory swimming (Webb 1976) and "snout swimming" (Mork and Gulbrandsen 1994). The fish were swimming with no discernible pattern or positioning with respect to each other.

Category "B": Fish were designated to this category when at-least $\frac{3}{4}$ of the tank population arranged themselves within close proximity to each other, swimming against the water current. The predominant, conspicuous swimming activity was observed to be restricted to tail beating (lateral movements of the caudal fin) (Webb 1971) or slow, uniform undulations which allowed the fish to maintain positional equilibrium against the water current.

Category "C": Fish were designated to this category when they were no longer in close proximity to each other (as observed in category "B") and swam without

discernible pattern or positioning with respect to each other. Undulatory swimming was generally slower than found in category "A".

3.3.6 Telemetry Recordings, Data Standardization And Analysis

For each recording event, an individual fish was selected and EMT signals were recorded from its tag. Since each EMT tag operates at a distinct radio frequency, the selection of an individual for each recording event was predetermined by random assignment of tag frequency to recording event. The EMT signals for each individual were averaged every 30 seconds, beginning at the time of "instant-on" light activation. Therefore the mean \pm 1 standard deviation (SD) of EMT signals for each consecutive 30 second period after "lights-on" was obtained and used for subsequent analysis.

EMT signals were standardized for each fish by obtaining pre lights-on EMT values and using them as basal measurements. These basal recordings were obtained during the 10 minute period prior to light activation. All other activity throughout the experimental recording period (experimental EMT values) were then calculated for each fish as the percent increase or decrease from it's pre-light activation basal EMT value, and is defined here as the "activity index". It was assumed that the pre-light activation, basal EMT measurements for each experimental fish would stay constant over the 3 to 4 consecutive days during which the recording events took place. This assumption was supported by data from a separate, on-going experiment which was designed to examine the

effects of various light regimens on EMT levels over time (see Chapter 5). The standard deviation, resulting from averaging each 30 second recording period was not “standardized”, since that measure appeared to be either independent or less sensitive to differences in transmitter range among individuals.

The mean of the standard deviations (mean SD \pm SEM; n=8) calculated from each 30 second EMT recording interval after “lights-on” for each fish is defined here as the “variance profile”. To illustrate the relationship between the behavioural categories, activity index and variance profile measurements, a purely descriptive analysis of the data was necessary. Additional analysis was performed using the SAS[®] system, Version 6.1. In order to disassociate the activity index mean and variance, a log transformation was performed on the data prior to statistical analysis. A general linear model was used to analyze the variance associated with the log transformed activity index data (Proc GLM, SAS[®]):

$$\Upsilon_{ijk} = \mu + \alpha_i + \beta_j + \Phi_{k(j)} + \varepsilon_{k(j)}$$

where;

Υ_{ijk} = the activity index observed in the i^{th} behavioural category, during the

j^{th} replicate by the k^{th} fish;

μ = the overall mean of the activity index;

α_i = the fixed effect for behavioural category ($i = 1,2,3$);

β_j = the fixed replicate effect ($j = 1,2,3$);

$\Phi_{k(j)}$ = the fixed effect for fish nested within replicate;

$\epsilon_{k(i)}$ = the random error term.

Significant differences in behavioural categories were further elucidated using Tukey's pair-wise comparisons (Steel, Torrie and Dickey 1997).

3.4 Results

EMT signals were successfully recorded from all but one individual. The data from this fish were incomplete due to transmitter malfunction, and were therefore excluded from the analysis. All fish survived the surgical procedure, and there were no significant differences ($p > 0.05$) in the fork length or weight of fish between replicate tanks.

The behavioural response of fish to "instant-on" light activation was distinguishable as three, temporally sequential categories as described in the methodology. The behavioural category "A" followed by "B" followed by "C" was observed in all video recorded events ($n=9$). When the lights were activated, activity described in category "A" occurred for a mean time of 1.61 ± 0.91 min, followed by "B" type activity for a mean time of 4.76 ± 2.06 min ending with "C" type activity which occurred for a mean duration of 3.13 ± 1.60 min. When the mean duration of each behavioural category was expressed as a proportion of the total recording time after light activation (10 min), the fish's activity levels as measured by EMT appeared to be associated with the video recorded behaviour observed (Figure 3.1). Statistical analysis of the log transformed data revealed that both the behavioural categories ($p < .0001$) and replicates ($p = .0463$) were

significant in affecting mean activity index levels. Pair-wise comparisons showed that only category "A" was significantly different from all other behavioural categories (Table 3.1).

3.5 Discussion

Readily describable behaviour often occurs in repeated, recognizable units (Noakes 1992). In this study, three distinct, behavioural units were observed in fish in response to "instant-on" light activation. Descriptively, the EMT derived activity index and variance profiles reasonably characterized and identified the behaviour of the fish in each of the behavioural categories (Figure 3.1). A high level of variation was apparent in the data (i.e. significant replicate effects). The high level of variation may be due to the fact that EMT recordings from individual fish were necessarily compared to a group measure of swimming activity. Nonetheless, the activity index demonstrated a statistically significant relationship with the behavioural categories. Consequently, it is suggested that EMT data can be used as an accurate measure, or proxy, of swimming behaviour. In addition, a comparison of the data with studies reporting swimming activities associated with the "light-shock" reaction (Mork and Gulbrandsen 1994), and startle-responses (Domenici and Blake 1997), has suggested that the "light-shock" reactions of rainbow trout are most likely a type of "fast-start" response followed by a recovery period.

3.5.1 "Fast-starts" And The "Light-shock" Reaction

C-type "fast-starts" are stereotypical patterns of high-energy swimming bursts found when fish are presented with an abrupt or unexpected stimulus (Hale 1999). That behaviour collectively termed as "fast-starts" in the literature has been associated with startle-responses, escape-responses, post-feeding turns, predator strikes, and some social interactions (Domenici and Blake 1997). C-type "fast-starts" have been shown by trout when flood-lights are switched on from darkness (Webb 1975), striking the water surface with an object (Eaton et al. 1981; Eaton et al. 1982; Jayne and Lauder 1993), by touching or thrusting a rod towards the fish (Harper and Blake 1990; Hale 1999; Wakeling and Johnston 1999) or by applying an electric shock stimulus (Webb 1978). While we did not use high-speed cinematography, which would enable the precise identification of the C-type "fast-starts" (Harper and Blake 1989), our results suggest that the "light-shock" reaction incorporates "fast-start" responses for the following reasons. Mainly, the "instant-on" lighting acted as an abrupt stimulus, which has been found to elicit fast-starts in rainbow trout (Webb 1975). In addition, the C-type "fast-starts" have been kinematically described in 3 stages; a preparatory stroke, a propulsive stroke, and a third variable stage which can involve continuous swimming with propulsive acceleration strokes or unpowered glides (Webb 1975). It can be reasonably expected that those swimming movements would appear on normal speed film as "burst and glide" maneuvers, followed by fast, undulatory swimming, as was observed in the video recordings of the present study. Finally, the "fast-start" reactions are stereotypic movements that

occur in a programmed sequence (Eaton and Nissanov 1985). Hence, it is not surprising that the activities observed in each category was similar in duration for each fish recorded.

Studies describing “fast-start” kinematics usually focus on the initial response of the fish, and subsequent behaviour during which the fish returns to ‘normal’ behaviour is often not described. However, two studies describing the “light-shock” reactions of fish coincide with the activity index patterns observed in this study. Heinen (1998) states that the “light-shock” reaction may involve a reduction in colour, sinking or diving to the bottom, relative immobility or hyperactivity but does not mention any sequence shown in those activities. Mork and Gulbrandsen (1994) subjected rainbow trout to sudden light activation and reported an initial bout of “chaotic” swimming where the fish swam near the bottom of the tank, followed by a period of slackening of total fish activity, followed by a slight increase in fish activity where-after the fish began to resume their initial spatial arrangements. The findings of this study are in agreement with these previous observations of rainbow trout “light-shock” behaviour, and further clarify the behaviour by identifying and quantifying the sequence and duration of swimming activity making up the behaviour in the “recovery period”.

In this study, an arbitrarily selected 10 minute recording period was used to record the actual duration of the behavioural categories. While it is likely that the actual duration of behaviour found in categories “A” and “B” were estimated in

this study, it is not known how much longer the behaviour found in category “C” persists. Mork and Gulbrandsen (1994) reported that trout required a maximum of 15 minutes to resume their normal tank positioning after exposure to sudden light activation. Wakeling and Johnston (1999) also used a 15 time period as a recovery interval when eliciting “fast-start” behaviour in the common carp (*Cyprinus carpio*). Based on the results of those studies, the behaviour recorded in category “C”, which occurred over the later time periods of the recording period, may be hypothesized as a return to some type of “normal” behaviour as was noted by the intermediate activity index and variance profiles. In light of the fact that some fish, including rainbow trout, seem to recover from the “light-shock” reaction within a 15 minute time interval (Wakeling and Johnston 1999; Mork and Gulbrandsen 1994), it is suggested that the activity in category “C” is underestimated in duration by at least 5 minutes, and may have further changed after our recording periods had stopped.

3.5.2 EMG Telemetry As A Behavioural Indicator

Radio-telemetry offers practical solutions to obtaining data from wild animals, and is also exempt from problems that have plagued activity measurements of free-living animals to date (Scherer 1992). However a major limitation that remains has been the inability of users to determine what the radio-tagged animals are doing in terms of locomotory behaviour. Two published studies have used EMT transmitters (similar to the tags used in this study) to examine reproductive behaviour of lake trout (*Salvelinus namaycush*) by means of

transmitted EMT signals (Kaseloo, Weatherley, Ihssen, Anstey and Gare 1996; Weatherley et al. 1996). In both studies, behaviour was inferred from overall levels of EMT activity, and it was noted by the authors that care needed to be taken when interpreting such records; e.g. activities that resembled reproductive episodes were possibly other activities, such as sustained predatory feeding. Sporadic bursts of high muscle activity, that did not affect the overall EMT values were also observed by Weatherley et al. (1996), however it appears they could not be further analyzed since they were insufficient to affect overall EMT values. Although the EMT transmitters were designed to highlight changes in swimming activity, we suggest that in order to unequivocally determine specific behavioural responses electromyographically, the following protocols should be heeded. First, it is necessary to describe the behaviour so that definitive, quantifiable categories can be constructed, in order to be correlated to telemetric EMT signals. Second, the EMT data analysis should focus on overall levels of activity combined with a measure of the variance in activity patterns which would indicate spontaneous activity at those overall levels. These protocols should result in more precise interpretations of behaviour or behavioural units from EMT data. In this study, the activity index was sometimes synonymous with the variance profile. Future work should also further examine this relationship, in order to identify more accurately similarities and differences between these variables during swimming behaviour.

3.6 Conclusions

The data presented here highlight the locomotory response of cultured rainbow trout to “instant-on” light activation, and in doing so introduce a novel application of EMT. It is suggested that when EMT records are analyzed in terms of both overall EMG i levels and EMG i signal variation, the swimming behaviour of tagged fish may be distinguishable when visual observations are not possible. The abrupt, “instant-on” artificial lighting used in this study are largely prevalent in many fish culture facilities (Heinen 1998). The findings of the present study show that such abrupt lighting causes a series of “fast-start” responses related to avoidance or escape maneuvers in rainbow trout. Therefore, this lighting system may be considered as an environmental stressor (Adams 1990), and it is recommended that other technologies, such as gradual, phased-in lighting controllers, supplant this type.

Any implantable technology or methodology required to indicate the swimming behaviour of fish should allow for quantitative, and objective measurements to occur in a manner that ideally does not effect the behaviour of the subjects. If the tool is to be used for purposes of behavioural evaluation in rearing situations, then there is also a factor of “field-practicality”. In addition, the data produced should be readily translatable into an understanding of what the animal is doing. Since the EMT tagged fish are free-living, feed and swim normally when tagged (Kaseloo et al. 1992; Adams et al. 1998), EMT may allow for the evaluation of a wide range of rearing conditions with respect to fish physiology and behaviour.

This type of information could aid in improving the environmental conditions pertinent to the welfare of farmed fish by offering scientifically based information on which to base husbandry recommendations.

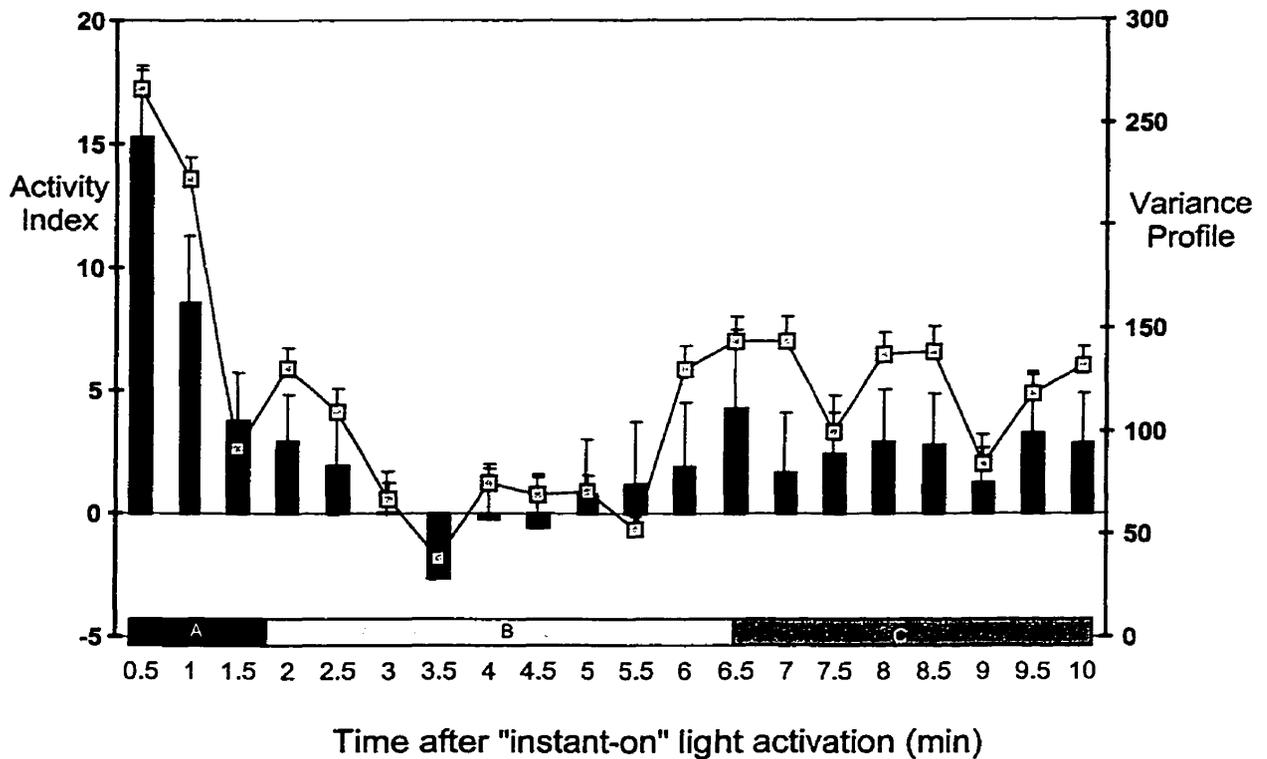


Figure 3.1 Swimming behaviour of rainbow trout in response to "instant-on" light activation as recorded by EMG telemetry and video. Bars (left y-axis) represent the mean activity index of 8 individuals. The plotted line values (right y-axis), located over each bar, is the mean variance for that bar. The partitioned horizontal bar underneath the x-axis represents the behavioural categories denoted as "A", "B", and "C" determined by video analysis from left to right. Each partition represents the mean duration of that category expressed as a proportion of the total recording period (10 min). The error bars are ± 1 SEM.

Table 3.1 Pair-wise comparisons of the behavioural categories

	"A"	"B"	"C"
"A"	x	<.0001*	0.0037*
"B"	<.0001*	x	0.25
"C"	0.0037*	0.25	x

The asterisk * denotes significantly different Tukey-Kramer pair-wise comparisons ($\alpha = 0.05$) of the behavioural category least square means.

Chapter 4: Behavioural And Energetic Responses Of Fish During Transportation

4.1 Abstract

Behavioural and energetic responses of hatchery-reared rainbow trout to a transportation episode were investigated. Fish implanted with EMT transmitters were transported in a shipping tank, for 50 minutes by truck, and then allowed to recuperate for 48 hours in resting tanks. EMT recordings of fish activity indicated that vigorous swimming activity occurred during transportation. Telemetry recordings also indicated that fish locomotory activity had returned to baseline levels within the 48 hour period post-transport. Despite the 48 hour resting period, the swimming performance of transported fish was impaired. Respirometry measurements of fish taken after transportation indicated that oxygen consumption was elevated. The rise in oxygen consumption could be attributed to handling procedures and the intense swimming levels observed during the transportation episode. This study demonstrated that EMT could be used to monitor the behaviour of fish during routine aquacultural procedures. A combination of biological indicators, however, are required to estimate the full impact of these aquacultural procedures on the fishes condition.

4.2 Introduction

The practice of transporting live fish in aquaculture is an ordeal that fish are regularly subjected to, and has been unequivocally identified as a physiological

stressor (Specker and Schreck 1980; Schreck 1982; Carmichael 1984; Davis and Parker 1986; Robertson, Thomas and Arnold 1988; Maule, Schreck, Bradford and Barton 1988; Schreck, Solazzi, Johnson and Nickelson 1989; Barton 2000). Transportation procedures typically subject fish to an initial capture and loading procedure, a period of time spent in the transport containers, and subsequent release to another environment which is accomplished by using a simple dipnet or emptying of the carrier contents via a "fish hose".

In order to determine what procedures the fish are most sensitive to, a number of biological stress indicators have been used to monitor the physiological reactions of fish at various points during the transportation procedure. The indicator most often used has been the concentration of plasma cortisol in fish. Cortisol is a hormone released into the bloodstream of fish following various stressors, including the perception of potentially harmful stimuli (Mazeaud, Mazeaud and Donaldson 1977). Using plasma cortisol as an indicator of stress, it has been found that a stress response occurs in fish regardless of the transportation or handling method employed (Barton and Peter 1982). The only exception may be when the fish are immobilized prior to transport by an anesthetic agent (Robertson et al. 1988).

Since the stress responses of fish to transport currently cannot be avoided, researchers have proposed pre- and post-transport protocols, designed to increase the overall health and survival of transported fish when subsequently

released. Strategies incorporated before transportation include the feeding of immunostimulatory feeds (Jeney, Galeotti, Volpatti, Jeney and Anderson 1997), and by positively conditioning the fish to stressful experiences associated with transport (Schreck et al. 1995). Post-transportation strategies have examined the incorporation of fish-resting periods immediately after transport (Schreck et al. 1989; Johnson et al. 1990; Tipping 1998). Presumably, the rest period would allow the fish to recover enough to optimally cope with the challenges of new environments. However, no studies have adequately identified the characteristics of a recovered state in fish after transportation. Since plasma cortisol levels are elevated after transportation in many fish, it has been suggested that fish may be in a better condition, and thus suitably rested, when cortisol values drop to pre-transported baseline levels (Specker and Schreck 1980; Schreck et al. 1989). However, a resting time recommended on cortisol values alone (or any sole indicator for that matter; see Adams 1990), may not accurately reflect the overall recovery state in transported fish (Barton 2000). Apparently, some behaviour that may signal recovery in stressed fish returns to normal prior to cortisol returning to baseline levels (e.g. Olla, Davis and Schreck 1992), while other behaviour or performance measures remain adversely affected even after plasma cortisol concentrations have reached pre-transportation baseline levels (e.g. Maule et al. 1988). Hence, the return of one physiological stress parameter to a baseline level may not have bearing on other physiological pathways which may be affected, and often go unmeasured. In addition, fish species can vary in their physiological responses and susceptibility

to the effects of handling, transportation, and to the environments that they may find themselves released into (Korovin 1982; Davis and Parker 1986; McDonald, Goldstein and Mitton 1993; McDonald and Robinson 1993; Olla, Davis and Schreck 1995; Barton 2000). Therefore, it follows that a variety of biological indicators should be used to monitor the recovery status of fish, carefully selected with the species, life stage and its future environment in mind.

Many post-transport release environments demand the unimpaired ability to swim and an appropriate behavioural repertoire. Therefore, the incorporation of whole-organism responses, such as swimming performance, or other behaviour relating to survival, may improve the accuracy of indices that are used to determine the recovery status of fish (Adams 1990; Scherer 1992; Olla et al. 1995). Behavioural indicators may be able to show that fish are responding to a stressor even when clinical physiological indicators such, as plasma cortisol, disappear (Schreck 1990). The behavioural responses of fish may also reflect a variety of physiological and metabolic alterations (Mesa and Schreck 1989; Schreck 1990; Winberg and Lepage 1998). Swimming behaviour of fish in response to “stressful” rearing stimuli has been observed when adding mild salt solutions to transportation containers (Barton and Peter 1982), to artificial, abrupt lighting systems (Mork and Gulbrandsen 1994; Chandroo et al. 2000), to wave conditions in fish cages (Srivastava, Brown and Allen 1991), to high intensity infrasound (Knudsen et al. 1997) and to conspecific social stress (Øverli et al. 1999). Due to the practical difficulties inherent to field observations, swimming

activity is often reported in a non-quantitative manner, which can preclude the use of such data for behavioural tests or assays. For example, Barton and Peter (1982) noticed hyperactivity and erratic swimming of rainbow trout in transport tanks at time of stocking when the tank water contained 0.5% salt, although the behaviour was not quantified and no observations of this behaviour during transportation were reported. Since the locomotory activity of free-swimming fish may be objectively characterized and quantified using EMT, the opportunity exists to gain a clearer understanding into the swimming behaviour of fish during transportation protocols.

The purpose of this study was to examine the swimming activity and behavioural responses of rainbow trout during transportation using EMT. In addition, a 48 hour post-transport resting protocol was evaluated by comparing the muscular activity, oxygen consumption and swimming performance of fish given the resting period with fish that were not. This use of EMT will provide unique insight into the behavioural and physiological impacts of transportation procedures to fish, and will further elucidate the value of post-transport resting periods.

4.3 Methodology

4.3.1 Experimental Animals & Animal Care

Hatchery reared, domestic rainbow trout, raised at the Alma Aquaculture Research Station (University of Guelph, Alma, Ontario) were used in this study. Experimental fish (n=12) to which telemetry devices were surgically inserted

were randomly chosen from stock tanks (mean weight \pm SEM = 1259 ± 370 g; mean fork length \pm SEM = 440 ± 45 mm) (see Appendix B). All experimental and surgical protocols were conducted under the approval of the Animal Care Committee (Animal Utilization Protocol 98RO97).

4.3.2 Experimental Protocol

Following surgery (described in Chapter 2), additional rainbow trout were netted from stock tanks and added to the tank containing the 12 tagged fish, and the bio-density was adjusted to 30kg/m^3 . The fish were given a 48 hour rest period, during which EMT signals were monitored to ensure that the tags were functioning properly. These EMT signals were not used for subsequent analysis.

4.3.2.1 Pre-Transport period and Data Standardization

Over the next 4 days, EMT signals were recorded from each tagged fish. The receiver was programmed to scan for each tag frequency and to record 30 sequential EMT values from each tag. This was done continuously over the 4 day period, except during times that the system was off-line in order to download data. The data collected during this period were used to establish pre-transport basal levels of swimming activity.

4.3.2.2 Transport by Truck

Tagged fish were transported by truck from the Alma Aquaculture Research Station to the University of Waterloo's wet lab (Waterloo, Ontario). Fish were transported for 50 minutes along a paved, secondary highway with light to moderate traffic density. Four EMT tagged fish were transported during each trip, and there were 3 trips in total. At the Alma Aquaculture Research Station, the fish were netted out of the 2x2 m tank into a 20 litre plastic container, and then transferred to the truck-mounted fiberglass tank. Additional fish were added to the 2x2 meter tank from which the tagged fish were taken, to maintain a constant bio-density. The transport tank's dimensions were (1.47x0.97x0.60 m; LxWxH), and was filled with fresh, aerated water just prior to the fish being added. The density of fish in the transport tank was approximately 5.9 kg/m³, so aeration was not supplied. The colour of the transport tank was white, and it featured a locking cover that blocked out most of the daylight. The SRX_400 radio receiver was mounted inside a weather resistant container, and was powered by a DC adapter hooked up to the truck's cabin power source. The receiver was programmed to continuously record 15 sequential EMT signals from each tagged fish during the entire transportation event. Upon arrival at the facility at Waterloo, the fish were netted out into 50 l plastic containers, and transferred to a 2x2 m tank, identical to the tank used at the Alma Aquaculture Research Station.

4.3.2.3 Post - transport period

Within 1.5 hours of arrival, two of the four fish (defined here as non-rested fish) were subjected to a swim tube protocol (test described below), and two fish were allowed to rest for 48 hours (defined here as rested fish). The SRX_400 receiver collected 30 sequential EMT values from each resting fish, and did so continuously over the 48 hour period. The data collected during this period were used to determine post-transport levels of activity. The “resting tank” contained other non-tagged rainbow trout, and was previously adjusted so a bio-density of 30kg/m³ was maintained. At the end of the 48 hour rest period, the rested fish were also subjected to the swim tube protocol.

4.3.3 Environmental conditions

Water used for all procedures at the Alma Aquaculture Research Station, and during transportation had a temperature of $8.5 \pm 0.1^{\circ}\text{C}$, a dissolved oxygen concentration of 10.6 mg/l (oxygen saturation of 100.8%), and a pH of 7.6 ± 0.1 . All transport containers were filled with fresh, aerated water immediately before use. The fresh, aerated well-water used at the University of Waterloo was approximately 9°C , and was similar in all other water quality characteristics. At both facilities, the tanks used to hold experimental fish were covered with an translucent plastic sheeting, and artificial lighting systems were used to provide a photoperiod which mimicked natural conditions for southern Ontario at that time (January to February 1999). Water was delivered to holding tanks at both

facilities via a PVC spout at a rate between 22 and 24 l/min, which created a circular flow within the tanks.

4.3.4 Swim Tube Protocols

At the appropriate time, each rested and non-rested fish was forced (individually) to swim against water supplied at controlled velocities (described in Chapter 2). During this procedure, EMT values were recorded, oxygen consumption (V_{O_2}) measurements were taken and the critical swimming speed (U_{crit}) was determined.

In a similar manner, the fatigue time and V_{O_2} of 12, non-transported, EMT tagged rainbow trout were tested at the Alma Aquaculture Research Station in a published, parallel study (Cooke et al. 2000). Since constraints in the present study did not allow for pre-transportation respirometer assays, fatigue time and V_{O_2} data obtained by Cooke et al. (2000) may serve as substitute controls. The fatigue time data obtained by Cooke et al. (2000) were used to calculate the U_{crit} of fish tested in their study, and are reported here for the first time. The EMT tagged fish (mean weight and fork length = 1017 ± 123 g and 414 ± 26 mm) tested by Cook et al. (2000) were of the same source and stock as used in this study, and were reared under identical conditions at the Alma Aquaculture Research Station. All of the equipment, including the swim chamber/respirometer and the oxygen detecting apparatus, were identical to those used in the present study. The swim test protocols in this parallel experiment were slightly different in

a number of aspects. A shorter acclimation interval (the time a fish spends in the swim chamber before the stepwise increases in water velocity occur) was used for transported fish, in order to complete the testing procedure in the shortest practical time to ensure the fish were truly “not-rested”. Fish tested in Cooke et al. (2000) were given a 30 minute acclimation interval, instead of the 15 minute acclimation interval used in the present study. The duration of the acclimation interval does not affect U_{crit} in rainbow trout (Peake, Barth and McKinley 1997). The water velocity intervals used in both studies after the acclimation period were identical. Another difference in testing protocols was that the swim chamber used in Cooke et al. (2000) was flushed with freshwater periodically throughout the procedure, while in the present study the chamber was flushed with fresh, aerated water only during the 15 minute acclimation period. Since the oxygen concentration did not fall below 80% air saturation during any of the tests in either study, it was assumed that this difference in protocol was negligible.

4.3.5 Statistical Analysis

All statistical analyses were performed with the SAS[®] system, Version 6.1. Prior to combining data among any of the three replicates, tests for differences in weight, fork length and activity levels were conducted. Differences in fish activity index levels during the pre-transport and post-transport 48 hour period (days 1 and 2) were tested using ANOVA. A t-test for unequal variances was used when testing pooled pre- and post-transported fish activity index levels with activity levels during transport. The relationship between the muscle activity index and

swimming speed, for rested and non-rested fish during forced swim trials were generated using linear regression. Differences in V_{O_2} at each swimming speed within and among rested and non-rested groups of fish were tested with the SAS® Proc Mixed procedure. The critical swimming speeds for rested and non-rested fish were tested for differences using a t-test. Differences between U_{crit} , weights, fork-lengths among non-transported fish (Cooke et al. 2000) and transported fish (this study) were also done with a t-test. All tests were performed at an alpha level of 0.05.

4.4 Results

All fish survived the surgery, the transportation episode and the swim chamber protocols with the exception of two fish, which succumbed to abnormal water conditions (chlorine) present in the “resting tank” after transportation during one trial. During the third transportation event, one transmitter failed (battery depletion), so EMT data for that individual could not be obtained. There were no significant differences ($p > 0.05$) between the weights or fork lengths of fish among experiment replicates within this study, or among those fish used in Cooke et al. (2000). ANOVA revealed no significant differences in activity among replicates during transport or resting events, so replicate data were pooled.

4.4.1 Fish Activity During Transportation And Resting Events

In general, the activity levels of fish during transportation (Figure 4.1) was significantly different from the activity levels of fish recorded during pre- and

post-transportation rest periods ($p < 0.01$). There were no differences in the activity levels of fish between pre- and post-transportation resting events ($p > 0.05$). Activity index levels from all fish that were allowed to rest after transportation returned to the levels of activity observed pre-transport, within the first day of arrival and stayed at those levels throughout the 48 hour resting period. During transportation, two levels of fish activity were distinguishable from EMT data collected during transportation (Figures 4.1 and 4.2). The majority of fish ($n = 8$) demonstrated an elevated activity index level, while the remainder ($n = 4$) showed a significant decrease in activity during the transportation period as compared to their pre-transport resting levels.

Since EMT signals were calibrated with swimming speed, the average swimming speed of fish during transport could be estimated. Regardless of activity mode, all fish, except one, reached swimming levels of at least 70 % U_{crit} during transportation (Figure 4.2).

4.4.2 Swim Tube Measurements

Regression analyses indicated that the muscle activity index was positively correlated to swimming speed (Figure 4.3) for both non-rested fish ($F=27.8$, $df=15$, $r^2=0.67$, $p<0.001$) and 'rested' fish ($F=30.5$, $df=20$, $r^2=0.62$, $p<0.001$). A non-significant trend was noted with respect to the calculated slope of the activity index vs. swimming speed for non-rested and rested fish; the slope of the regression line for rested fish was greater than that of non-rested fish.

Swimming speed did not significantly affect the rate of oxygen consumption measured during forced swim trials (Figure 4.4), for both rested and non-rested transported fish ($F_{3,15.4}=0.76$, $p=0.53$). This was clearly different from the positive correlation of those variables observed when testing non-transported fish (see Cooke et al. 2000; Figure 4.5). The mean V_{O_2} for non-rested fish was higher than that of rested fish at each swimming speed tested, but these differences were not significantly different ($F_{3,15.4}=0.01$, $p=0.62$). The average V_{O_2} during forced swim trials for all transported fish was 148 ± 4 mg/kg/hr (mean \pm SEM). At low to moderate swimming speeds (between 0.25 and 0.63 m/s), transported trout, on average, had a higher rate of V_{O_2} than non-transported fish (148 and 108 mg/kg/hr, respectively) (Figure 4.5).

The critical swimming speeds of non-rested and rested trout were not significantly different ($p > 0.05$). The data from both groups were pooled to give a mean U_{crit} of 0.64 ± 0.04 m/s (\pm SEM). In comparison, the mean U_{crit} of non-transported, rested, EMT tagged trout tested in Cooke et al. (2000) was 0.90 ± 0.05 m/s (\pm SEM). A t-test revealed that the mean U_{crit} for transported and non-transported fish were significantly different ($p < 0.05$).

4.5 Discussion

4.5.1 Respirometry Estimate Variability

A comparison of the V_{O_2} estimates determined in the present study and reported in Cooke (2000) with tunnel respirometer derived estimates obtained from the literature (Table 4.1) reveals that maximal V_{O_2} expression of rainbow trout tested according to our protocols are at the lower end of the published values. There are two possible explanations for the lower V_{O_2} values reported in Cooke et al. (2000) and this study. The V_{O_2} of a fish, relative to a large volume respiration chamber, will result in a slow decline in the time-course change in oxygen concentration in the water (Kaufmann, Forstner and Wieser 1989; Steffensen 1989). Therefore, the 10 to 15 minute sampling intervals used in our studies may not have been sufficient to allow for optimum sensitivity for detecting changes in V_{O_2} . Geist, Abernethy, & Blanton (2000) also report variable V_{O_2} determinations of EMT tagged chinook salmon (*Oncorhynchus tshawytscha*) due in part to the large sized respirometer utilized in their study. The lowered V_{O_2} observed in fish may also be expected since the temperature of the water (8.5 to 9°C) used during our determinations are of the lowest in comparison to other studies (Table 4.1). The possible ramifications of lowered oxygen detecting sensitivity in our results is discussed further in the text where warranted

4.5.2 Estimates Of Fish Activity During Transportation

As indicated by EMT signals, fish showed periodic, vigorous swimming activity during each transportation event. On average, these bouts of swimming could

reach 70% of U_{crit} (Figure 4.2). Swimming at this intensity requires the support of both red (aerobic), and white (anaerobic) musculature (Jayne and Lauder 1994). Swimming utilizing the white musculature in fish typically has been associated with vigorous movements, such as burst and glide or fast-start swimming (Domenici and Blake 1997). The erratic pattern of EMT signals recorded during transportation, in combination with high activity index values, suggests that the behaviour of fish during transportation encompassed "burst-glide" type swimming in combination with body turns. Swimming movements of fish were probably performed in reaction to the waves and other water movements generated within the transport tank. Four fish during transportation maintained levels of activity below resting with only occasional bursts of speed above 70% U_{crit} . Although lowered in magnitude, the erratic pattern of EMT signals observed from those fish could suggest a greater degree of swimming complexity, i.e. spontaneous movements, perhaps to maintain a constant position within the tank during transport. Spontaneous swimming in fish is energetically expensive relative to steady, undulatory swimming (Boisclair and Tang 1993). Overall, our results support previous suggestions which imply that reduced survival of fish released to the wild immediately after transportation is in part caused by a transport invoked energy drain in fish (Schreck et al. 1989). The rate of energy utilization in fish is considerable when they swim at intensities approaching their U_{crit} , or which involve fast-starts and other erratic movements (Driedzic and Hochachka 1978; Wieser, Platzer and Hinterleitner 1985; Dobson and Hochachka 1987;). Furthermore, those types of swimming activities can promote the accumulation

of the end-products of anaerobic metabolism, such as lactate, within various organs or tissues of fish (Burgetz, Rojas-Vargas, Hinch, and Randall 1998). Since lactate accumulation in fish has been measured immediately after transportation (Nikinmaa, Soivio, Nakari, and Lindgren 1983; Iversen, Finstad, and Nilssen 1998), but also occurs as a result of routine handling stressors (Vijayan and Moon 1992; Davis and Schreck 1997), it would have been difficult in the past to attribute such findings to swimming behaviour during transport. However, the results of this study demonstrate precisely how fish behaviour during the transport period could affect and be responsible for a portion of the lactate measured in fish after transport. Similarly, other stress variables that are sensitive to moderate or vigorous swimming may also be affected in this manner.

It is not certain as to how the bio-density of fish or the water quality in the transport carrier influenced fish behaviour observed during our transportation trials. Due to the low bio-density and post-absorptive status of fish in the transport tank, the build up of metabolic waste products over the 50 minute transport period was considered negligible. Since a build up of respiratory gasses, such as CO₂, and the use of water additives, such as salt, have sometimes been observed to cause erratic swimming behaviour in rainbow trout (e.g. Barton & Peter 1982), it is possible that water quality alterations common to transportation practices may cause additional behavioural reactions not observed during this study. In a pilot study, Chandroo et al. (2000) monitored the EMT signals from two fish that were transported along the same route as this

study. The magnitude of swimming activity in that transportation episode occasionally reached levels approximately 1.5 times greater than those reported in the present study.

4.5.3 Metabolic Responses Of Transported And Non-transported Fish

Although constraints in this particular study did not allow for pre-transport control respirometry measurements, V_{O_2} and U_{crit} data from the parallel study (Cooke et al. 2000) will be used for comparison. In general, this comparison reveals that at low to moderate swimming speeds (between 0.25 and 0.63 m/s), transported rainbow trout, on average, had a higher rate of V_{O_2} than non-transported fish (Figure 4.5). Transported fish were subjected to a number of physical stressors that were likely to affect their metabolic processes. The fish were first netted (which involved a minor amount of chasing in the tank), subjected to a brief air exposure of less than 10 seconds (via transfer by dip-net), and then placed in the transport carrier. After transportation, fish were again netted, subjected to another brief air exposure, and then either placed in the resting tank, or netted into the swim chamber/respirometer. Physical disturbances, such as capture and handling, can cause fish to struggle, and this has been shown to double the V_{O_2} of rainbow trout and coho salmon (*Oncorhynchus kisutch*) (Barton & Schreck 1987; Davis & Schreck 1997). Large increases in V_{O_2} also occur during erratic, forced exhaustive exercise (Goolish 1989; Scarabello, Heigenhauser, & Wood 1991). Dickson & Kramer (1971) found that physical handling of rainbow followed by forced swimming of those fish at high speeds were sufficient to elicit

a full expression of V_{O_2} (i.e. the active metabolic rate). Another factor that may have influenced the rates of V_{O_2} of fish tested in this study were the brief instances of air exposure experienced by the fish. Air exposure, which occurred when transferring fish by dip net before and after transportation, may exacerbate metabolic imbalances in fish that have been previously exercised. Ferguson & Tufts (1992) found that 30 seconds of air exposure after bouts of burst swimming in rainbow trout caused a greater degree of anaerobic end-product accumulation within the white musculature, as well as increased mortality. Davis & Schreck (1997) observed that among a variety of experimental handling stressors, treatments which involved the exposure of coho salmon (*O. kisutch*) to air caused the greatest elevations in V_{O_2} . Therefore, it is expected that the culmination of transportation disturbances experienced by fish in this study caused an elevated level of V_{O_2} , above that of non-transported fish.

The V_{O_2} for transported, non-rested fish was higher at all swim speeds than that of transported, rested fish (Figure 4.4). Since the fish in the present study were subjected to handling and were active at speeds that have been correlated with the build up of anaerobic end-products (i.e. lactate), it is suggested that the observed differences in V_{O_2} between rested and non-rested fish was due to the subsequent repayment of an acquired oxygen debt. Similarly, Barton & Schreck (1987) observed an anaerobic response in rainbow trout that were made to struggle (sub-maximally), and suggested that a portion of the increased V_{O_2} , post-struggling, while swimming at low speeds, may have been due 'pay-back' of

an oxygen debt. The oxygen debt hypothesis states that after a period of intense exercise, there is an elevated, post-exercise oxygen consumption, i.e. the oxygen debt, that is indicative of an adjustment and recovery from physiological alterations due to strenuous white muscle activation (Scarabello, Wood, & Heigenhauser 1991). Two predominant components of the oxygen debt have been associated with rises in V_{O_2} post-exercise. A fast component (half life of 0.23 hours), in which restoration of the intracellular concentrations of ATP and creatinine phosphate (Schulte, Moyes, & Hochachka 1992) can occur, and a slow component (half life of 2.1 hours), than has been associated with lactate recovery. Lactate accumulation occurs in rainbow trout musculature under circumstances of "stressful" swimming and, at high, sustainable cruising speeds (Wokoma & Johnston 1981; Jones 1982). Davis & Schreck (1997) suggest that increases in V_{O_2} , immediately following handling, can be attributed to the fast component of oxygen debt. Activity index records of rested fish showed that they had returned to pre-transport rates of swimming activity, and maintained that level of activity throughout the 48 hour period prior to testing in the respirometer. Therefore, we speculate that fish allowed to rest for 48 hours would have only demonstrated the elevated rates of V_{O_2} predominantly associated with the handling-induced fast component of oxygen debt. Nikinmaa et al. (1983) found that lactate levels in brown trout (*Salmo trutta*) increased significantly while in transport, and returned to baseline levels within a day. Transported Atlantic salmon also showed a trend of declining plasma lactate levels after resting for 48 hours (Iversen et al.1998). The elevated V_{O_2} of non-rested fish may have been

influenced by both fast and slow components of oxygen debt. Farrell, Gamperl, & Birtwell (1998) hypothesized that metabolic recovery and repayment of the lactate-associated component of oxygen debt in fish could occur during low swimming speeds post-exercise, and be experimentally measured as an elevation of V_{O_2} .

The physiological responses to exercise in trout are often reported to include a positive correlation between V_{O_2} and increasing swimming speed (Brett 1964; Beamish 1979; Weatherley, Rogers, Pincock, & Patch 1982). In this study, forced swimming of trout, from very slow to critical velocities, did not cause a step-wise increase in V_{O_2} . This was an unexpected, but reasonable result in light of the immediate histories of the fish prior to testing, and the characteristics of our respirometer. We suggest that the observed rates of V_{O_2} in transported fish did not increase with progressive swimming speeds because of the inability of our respirometer to resolve the relatively smaller change in V_{O_2} specific to swimming. Driedzic & Hochachka (1978) have reported that the red musculature of fish (which power swimming at low to moderate speeds) are in fact only accountable for approximately 50% of the total oxygen usage when the energy required for respiratory adjustments (Steffensen 1985) and cardiac metabolism (Jones & Randall 1978) are taken into account. Therefore, if the magnitude of V_{O_2} specific to swimming at low to moderate speeds was relatively small as compared to the elevation in V_{O_2} caused by the transportation invoked stressors, a situation may have arisen where our respirometry protocol was not sensitive

enough to detect these smaller changes. Further difficulties in resolving the V_{O_2} of stressed fish may be attributable to the small sample sizes used in this study.

4.5.4 Swimming Performance Of Transported And Non-transported fish

The swimming performance of transported fish was observed to be impaired when compared to the performance of non-transported fish tested in Cooke et al. (2000). Furthermore, this comparison shows that the maximum aerobic capacity of transported fish was not attained at U_{crit} . This is not a novel finding, since a number of studies have demonstrated that U_{crit} is not always coupled with maximum V_{O_2} in the Salmonidae (Alsop and Wood 1997; Farrell et al. 1998) and other teleosts (Reidy, Nelson, Tang and Kerr 1995). This finding is interesting, however, as it may be indicative of the physiological alterations that occur in fish when they are transported. The fishes experience during the transportation episode may be viewed as a bout of extended exercise. Physical disturbances and extended exercise have significant physiological impacts affecting the swimming musculature and respiratory structures of trout (Jones and Randall 1978; Milligan and Wood 1982; Gonzalez and McDonald 1992; Butler and Day 1993). Since U_{crit} and V_{O_2} are reflective of the biochemical and functional status of trout musculature and gill processes (Waiwood and Beamish 1978; Schreck 1990), alterations in those measures due to muscular or ionoregulatory disturbances should have been resolvable in the respirometry tests. It is speculated that the reduced U_{crit} and elevated, but non-maximal V_{O_2} in transported fish was caused by an interaction of anaerobic end-product

accumulation within the fishes musculature, and disturbances in ionoregulation (i.e. the osmorepiratory compromise; see Gonzalez and McDonald 1992). The relationship of prolonged swimming and ionoregulatory disturbances to muscle fatigue and V_{O_2} have been examined in rainbow trout (Milligan and Wood 1982) and brown trout (*Salmo trutta*) (Butler and Namba 1992; Butler and Day 1993; Day and Butler 1996). Transportation invoked ionoregulatory disturbances have been observed in various *Salvelinus* species (McDonald, Goldstein and Mitton 1993), although the post-transport swimming ability of those fish was not tested.

4.5.5 The Effect Of Post-transport Resting Periods

In terms of swimming activity, EMT signals recorded after transportation indicated that fish were resting. Based on those results alone, however, we could not arrive to the overall conclusion that fish had achieved a recovered state, and this was evidenced by the reduced swimming capacity of those fish. Similarly, Maule et al. (1988) found that although plasma cortisol titers indicated that chinook salmon were rested after transportation, their swimming performance was still impaired. It is possible that the resting environment could have been perceived as stressful by the fish, as we did not measure for other stress indicators of fish in the resting tank. In most studies, including the present one, in which the fish are rested after transportation, the resting container is designed to be non-stressful (e.g. optimum water quality, darkened, covered tanks). However, it is undoubtedly still an alien environment, for reasons that we may not immediately perceive. McDonald & Robinson (1993) found that transported

trout recovered much faster when returned to their own race-ways, rather than when introduced to a new environment that was designed to be non-stressful. Tipping (1998) reported that allowing rainbow trout to rest for 24 hours post-transport in a novel resting environment did not improve post-release survival of those fish.

Post-transport survival of fish released to natural environments is probably more related to the physiological and behavioural factors involved in acclimation processes (Munakata, Björnsson, Jönsson, Amano, Ikuta, Kitamura, Kurokawa, & Aida 2000), the genetic make-up of the fish (McDonald & Robinson 1993), and the timing of the actual release corresponding to annual cycles of natural fish behaviour (e.g. migration) (Hansen & Jonsson 1989). Therefore, post-transport resting periods may only be of value if they allow for an adequate time for fish to acclimate to novel circumstances in the release environment. Johnson et al. (1990) found that transported coho salmon that were acclimated to the release site for 6 weeks showed higher rates of survival than fish immediately released. Since acclimation to novel post-transport conditions can take many days or weeks (Korovin et al. 1982; Nikinmaa et al. 1983; Johnson et al. 1990) and involves a number of biological pathways, a multitude of biological indicators are necessary to successfully monitor the progression of acclimation. For example, swimming stamina tests and behavioural assays would be particularly useful to identify the later stages of acclimation, and indicators able to monitor the fishes response to acute stressors, such as cortisol and swimming activity

measurements, should preside when testing in the early stages of acclimation. One trend, which may be of interest for future research, were the differences observed between the regression derived slope of muscle activity vs. swimming speed among rested and non-rested fish (Figure 4.3). The greater slope calculated for rested fish may be indicative of muscle recovery. However, that interpretation cannot be substantiated due to the low numbers of experimental animals tested, and the differences in slope may very well be due to variation specific to the EMT transmitters themselves. While short (24 to 48 hour) post-transport resting periods may not be effective in increasing fish health and survival, they may have other applications. For example, resting farmed fish intended for human consumption for short time intervals after transportation may be a suitable practice to allow the white muscle metabolites to return to baseline levels, as not to affect flesh quality (Erikson, Sigholt and Seland 1997; Robb, Kestin and Warriss 2000).

4.6 Conclusions

The findings of this study demonstrate the merit of integrating behavioural measures when assessing the impact of aquacultural procedures to the fish. Studies examining fish transportation protocols generally regard the netting and loading procedures as the major causes of stress, but disregard transportation per se as a stressor (Barton & Peter 1982; Maule et al. 1988; Robertson et al. 1988; Schreck et al. 1989; Iversen et al. 1998). Those conclusions are based on the fact that plasma cortisol levels in fish usually decrease during the transport

period. However, the results of this study illustrate how the behavioural responses of fish during transportation could contribute to the overall magnitude of "stress" experienced by fish. Furthermore, we suggest that combinations of biological indicators are necessary when attempting to determine the recovery status of transported fish. Although resting intervals employed after transportation are probably useful, they may only be effective in specific situations where the release environment allows for a time of relatively safe acclimation of fish to the novel aspects of their environment.

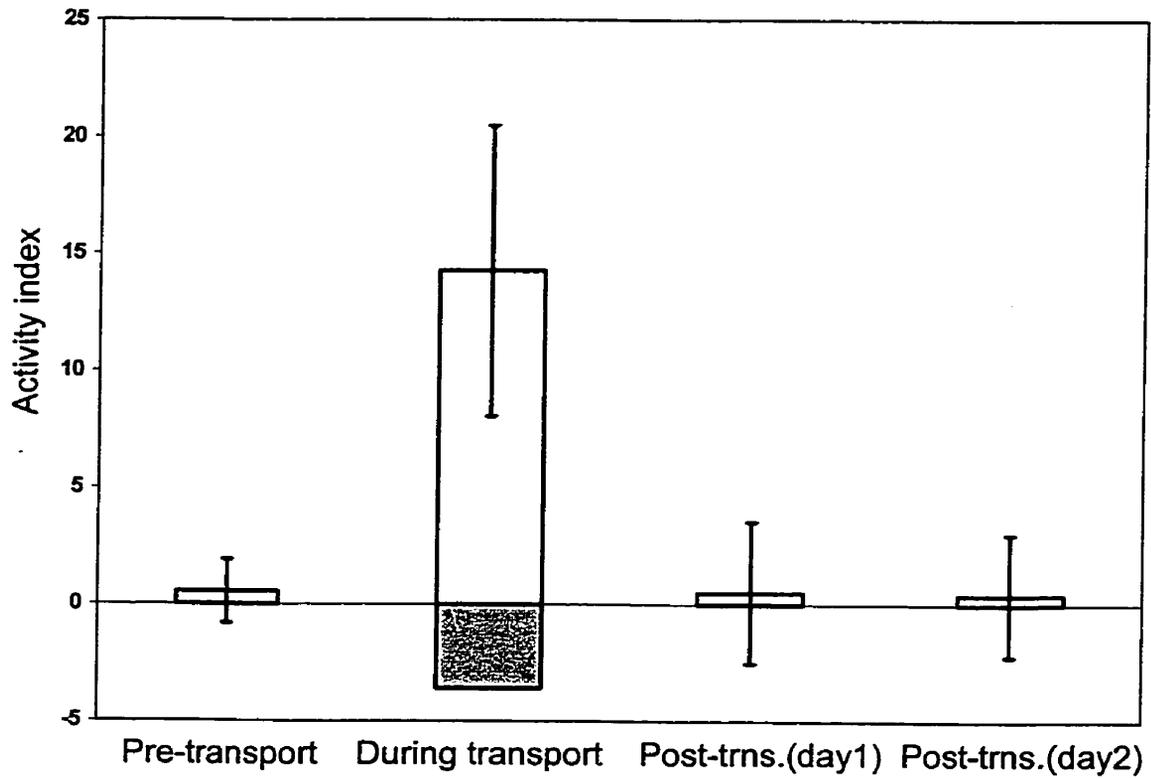
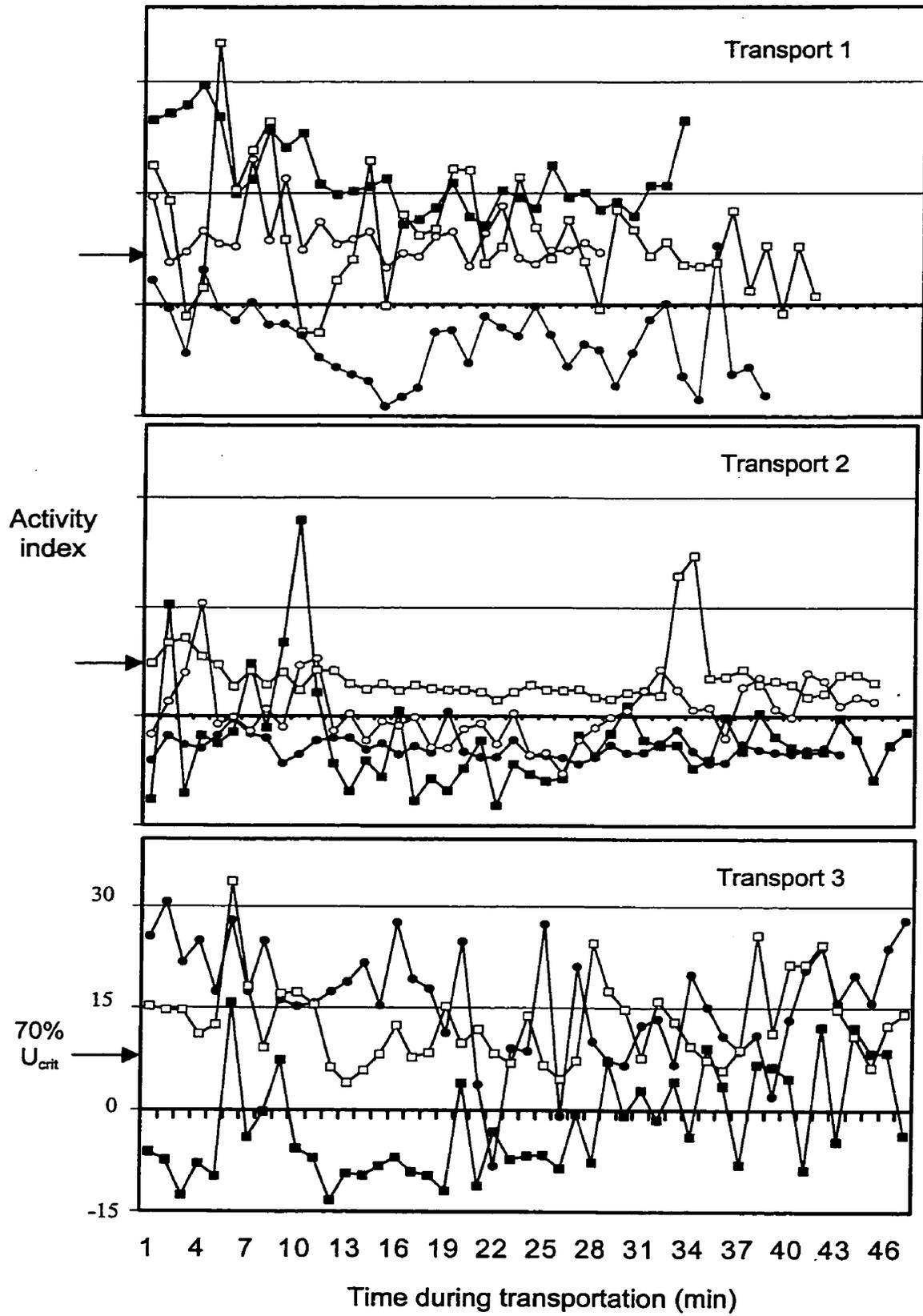


Figure 4.1 Rainbow trout activity index as recorded by EMT during pre-transport, transport, and post-transport periods (day 1 and 2). Error bars are ± 1 SEM.

Figure 4.2 Rainbow trout activity, as recorded by EMT, during a 50 minute transportation episode by truck. For the sake of clarity, error bars have been omitted. The lined data points represent the EMT activity of one individual trout. The arrows estimate the average activity index value of fish swimming at 70% U_{crit} . Calculations of 70% U_{crit} were as follows: the mean U_{crit} for all EMTtagged fish (Cooke et. al. 2000 and this study) subjected to swim trials was $0.765 \pm .13$ (m/s \pm SE). Seventy percent this value yielded a swimming speed of 0.535 m/s. Using the regression formula developed for non-rested fish ($y=24.85x - 6.54$), the activity index equivalent to a swimming speed of 0.535 m/s was calculated.



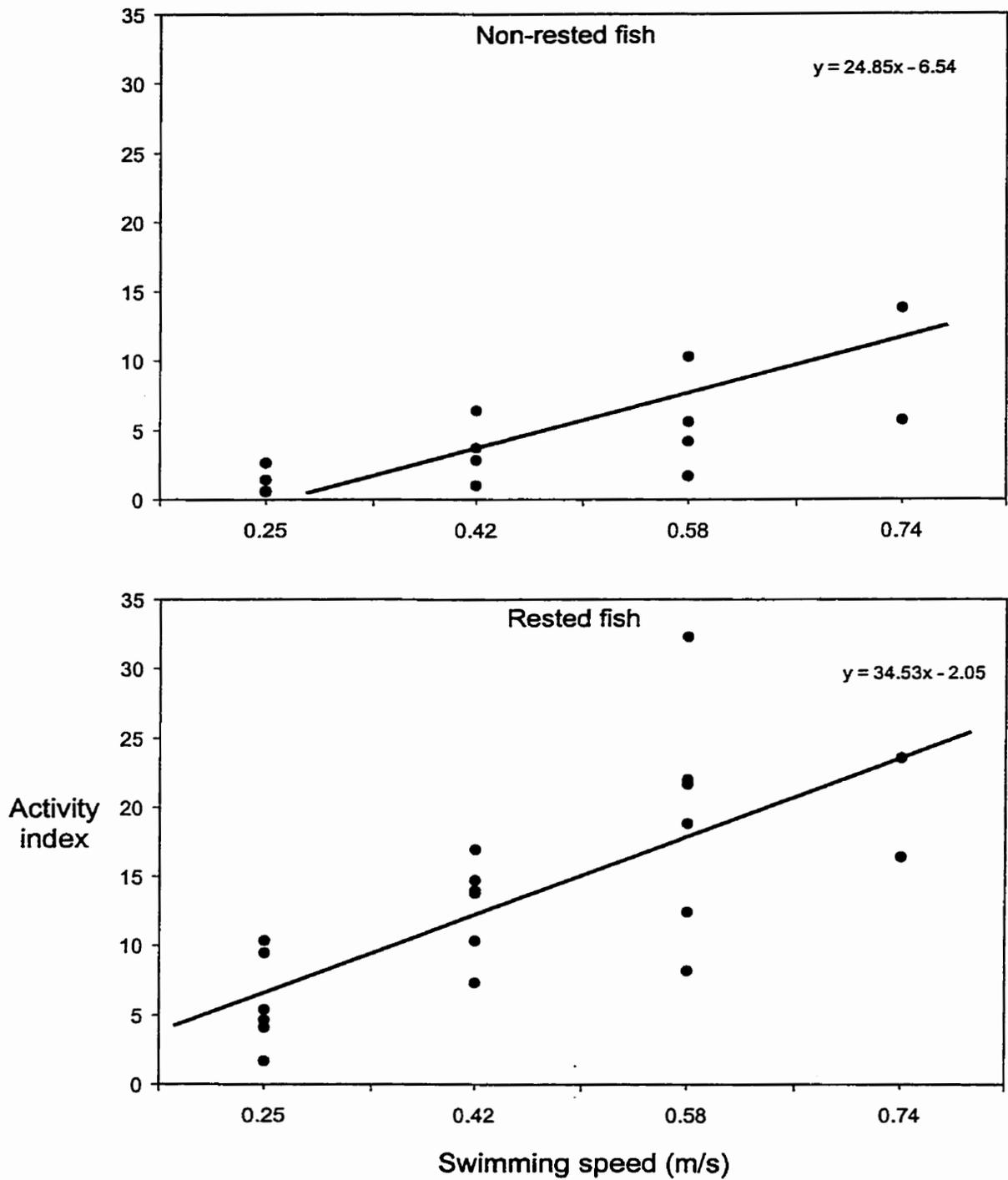


Figure 4.3 Activity index versus forced swimming speed in non-rested (n=4) and rested (n=6) rainbow trout.

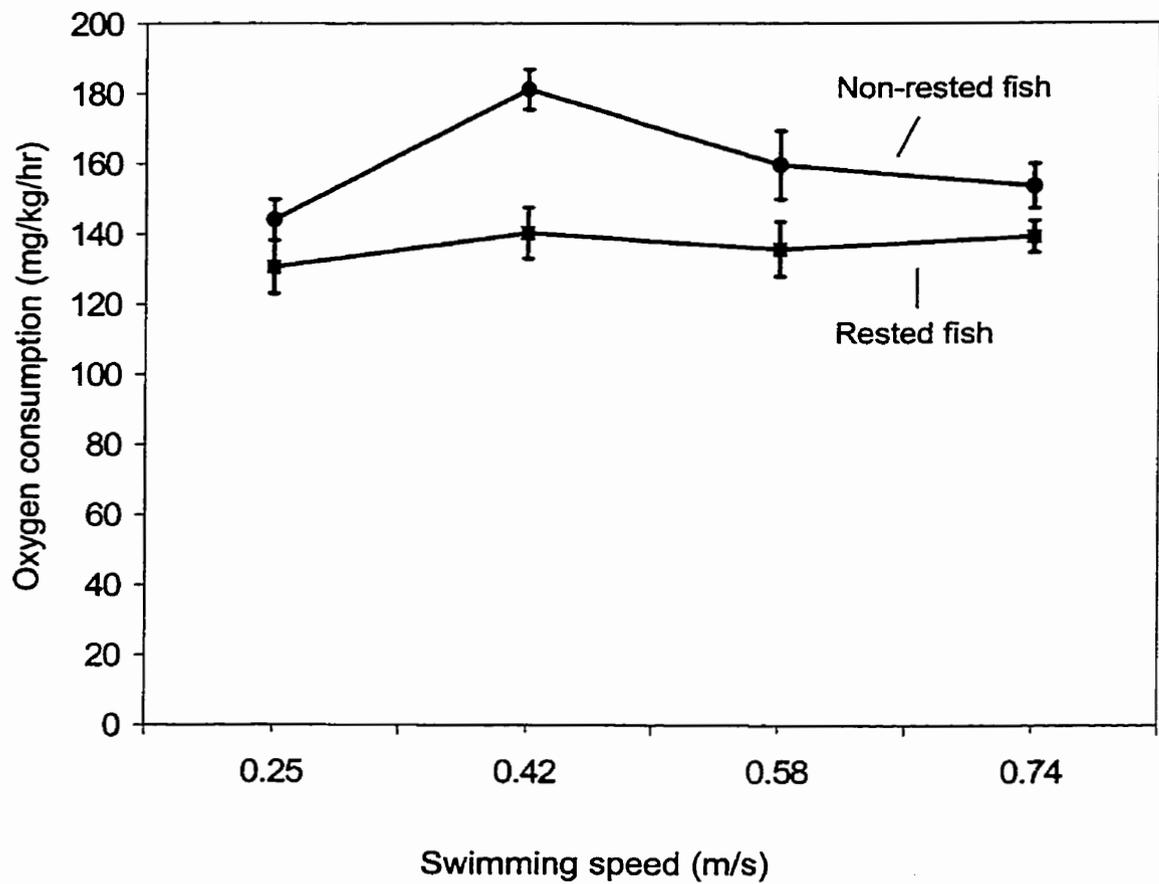


Figure 4.4 The relationship between forced swimming speed and oxygen consumption in rested and non-rested rainbow trout. Rested fish (n=6) are represented by squares, non-rested fish (n=4) are represented by circles. Error bars are ± 1 SE.

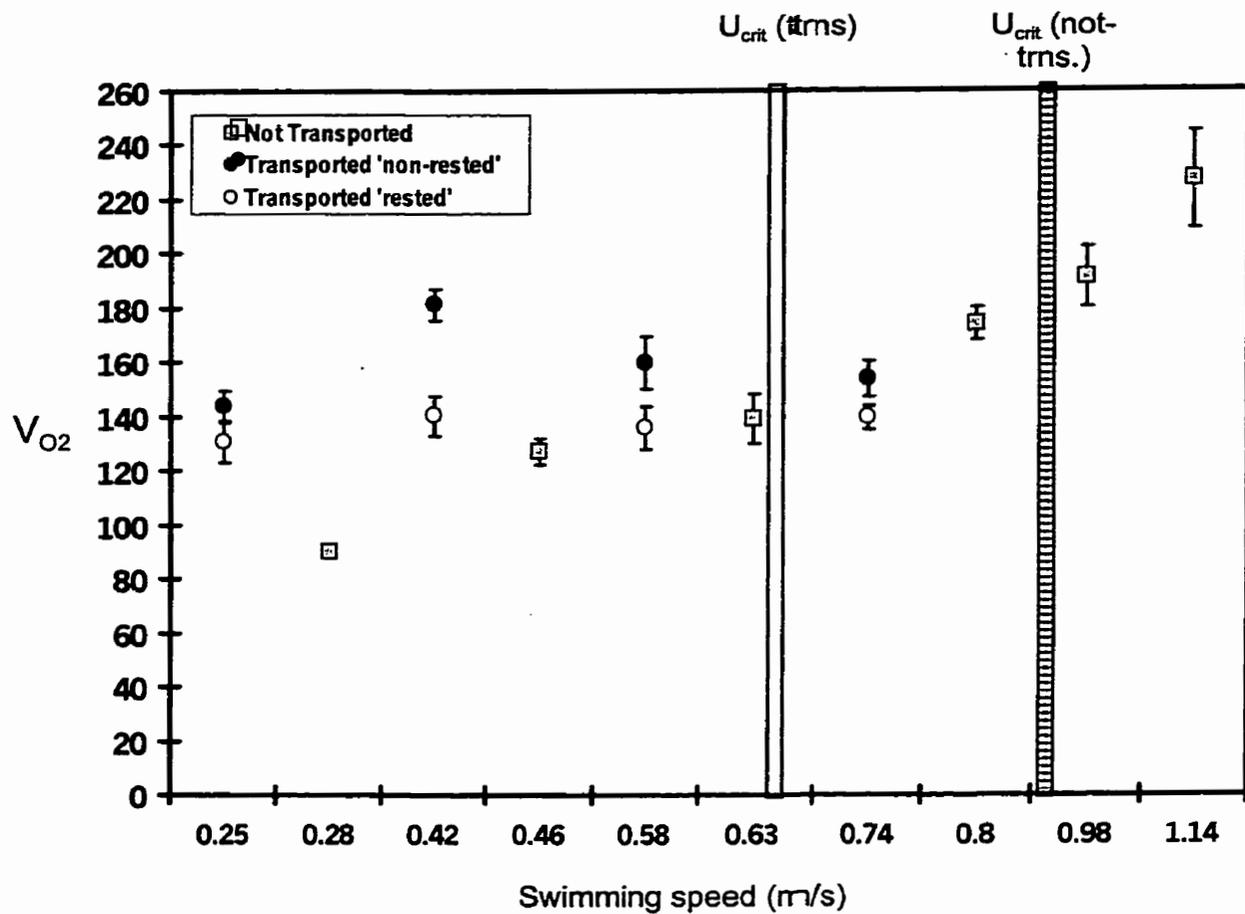


Figure 4.5 A comparison of the rates of oxygen consumption at successively increasing swimming speeds observed in non-rested, rested and fish not-transported. The shaded, vertical bar represents the mean U_{crit} of non-transported fish ($n=12$). The clear vertical bar represent the mean U_{crit} of transported fish (rested and non-rested, $n=10$). Data points plotted are the actual values measured in each study, so the x-axis is an overlay of both scales used in each study. Units of V_{O_2} are mg/kg/hr.

Table 4.1 Literature estimates of oxygen consumption in rainbow trout during forced swim trials in relation to the volume of the respirometer chamber and the mass of the fish tested.

Temp (celsius)	RV (litres)	FM (grams)	FM/RV (g/l)	O ₂ Interval (min)	V _{O₂} (mg/kg/hr)	Source
12	48	525	10.9	30	475	Weatherley, Rogers, Pincock and Patch (1982)*
10	27.5	258	9.38	15-20	468	Dickson and Kramer (1971)
9 - 10.5	34.5	1200	34.8	10-60	371	Kiceniuk and Jones (1977)**
10	48	1086	22.6	30	337	Briggs and Post (1997a)*
8.5	120	1204	10	10	227	Cooke et al. (2000)*

The quoted estimates of V_{O₂} are the maximum values reported in each study (i.e. V_{O₂} during active metabolism). The V_{O₂} estimate given for Briggs and Post (1997a) was estimated using a swimming speed of 2.7 body lengths per second.

RV = total respirometer volume

FM = average fish mass within respirometer at time of testing

O₂ interval = the time between oxygen measurement sampling used to calculate V_{O₂}

V_{O₂} = the oxygen consumption

* EMT tagged trout were used in V_{O₂} determinations

** Cannulated trout were used in V_{O₂} determination

Chapter 5: The Effect Of Two Artificial Lighting Regimens On Fish Activity

5.1 Abstract

Artificial lighting systems used in aquacultural facilities often do not provide a natural twilight period to fish. Twilight periods influence fish behaviour, and the affects of withholding or providing a twilight period to farmed fish has not been investigated. The objective of this experiment was to compare and evaluate the swimming activity of captive rainbow trout, reared with two different artificial lighting regimens; an abrupt, "instant-on" lighting regimen that did not provide a true twilight period, and a "natural phased" lighting regimen that simulated dawn and dusk. EMT tagged fish were reared at a commercial density in rooms equipped with either "instant-on" or "natural phased" lighting. EMT signals indicated that the daily activity rhythms of fish were similar under both lighting regimens. However, the overall magnitude of fish activity was significantly greater when fish were reared under the "natural phased" lighting regimen ($p=0.02$). "Fast-start" responses were most likely to be observed from fish reared with the "instant-on" regimen ($\chi^2=9.27$, $p<0.01$), suggesting that this lighting system may be considered as an environmental stressor. The results demonstrated that the swimming behaviour of rainbow trout is reflective of and responsive to manipulations of the rearing environment. These aspects of fish activity may have further relevance for fish welfare assessments, by providing baseline information of fish behaviour under intensive culture conditions.

5.2 Introduction

Light acts as a strong stimulus for physiological and behavioural processes in many cultured fish. Light can synchronize or influence cycles of metabolism and growth (Boeuf and Yves Le Bail 1999; Duncan et al. 1999), feeding (Kadri, Metcalfe, Huntingford and Thorpe 1997; Chen, Purser and Blyth 1999) and locomotory activity (Boujard and Leatherland 1992; Cooke et al. 2000) in fish. Two twilight periods occur during each diel cycle, at dusk and dawn. Although dusk and dawn only amount to 5 % or less of the 24 hour diel cycle, it has been suggested that twilight may play a role in the behaviour and physiology of fish out of proportion to the actual time involved (Helfman 1986). During the twilight periods, fish living in the wild “change over” from daytime to nighttime activity modes. This may involve shelter-leaving or seeking behaviours (Carey and Noakes 1981; Fraser, Huntingford and Thorpe 1994), vertical changes of distribution in the water column, and migration to feeding areas (Helfman 1986). Captive rainbow trout have been shown to exhibit both nocturnal and diurnal feeding patterns (Landless 1976) as well as vertical migrations in response to cyclical environmental cues (Sanchez-Vazquez and Tabata 1998; Mork and Gulbrandsen 1994). Such sequences and rhythms of fish behaviour can be adaptive (Gerkema 1992), and in some circumstances, such as in the rhythmic occurrence of feeding, may reflect behavioural motivation or anticipation (Gerkema 1992; Noakes 1992).

In chapter 3 of this thesis, it was found that “instant-on” lighting was responsible for eliciting a series of “fast-start” responses related to avoidance or escape maneuvers in rainbow trout. As judged by the reactions of the fish, the conventional “instant-on” lighting system could be considered as a significant environmental stressor. To date, very little work has investigated the importance or consequences of providing or withholding twilight periods on the behaviour and physiology of cultured fish. Furthermore, the effect of subjecting fish to “stressful” stimuli during the twilight periods on subsequent behaviour and activity has not been examined. Since the behaviour exhibited by fish during twilight appears to be largely locomotory in nature, it is possible to use EMT to evaluate fish behaviour and activity during those periods (Briggs and Post 1997a,b).

Therefore, the objective of this experiment was to compare and evaluate the diel swimming activity of captive rainbow trout, reared with two different light regimens; namely, an abrupt, “instant-on” lighting regimen that did not provide a true twilight period, and a “natural phased” lighting regimen that simulated dawn and dusk. This use of EMT may have further relevance for fish welfare assessments, by generating “baseline” information of fish behaviour under intensive culture conditions.

5.3 Methodology

5.3.1 Experimental Animals And Animal Care

Two hundred and forty rainbow trout were randomly selected and weighed at the beginning of the study (mean weight \pm SEM = 1145 \pm 385 g). Experimental fish that were implanted with EMT transmitters over the course of the study were taken from this group (see Appendix C). All experimental protocols were carried out at the Alma Aquaculture Research Station and conducted under the approval of the Animal Care Committee of the University of Guelph (Animal Utilization Protocol 98R097).

5.3.2 Environmental Conditions

Six experimental tanks (2x2 m) were evenly distributed into two windowless rooms, which featured light-proof double doorways. The tanks were covered with an adjustable, opaque plastic grid. A programmable, automated lighting system was used to control the lighting intensity and timing in each room that contained the experimental tanks. Illumination was generated in each room with four, ceiling mounted 150 watt frosted, medium base tungsten bulbs (Sylvania - A2348-0017650B). Using a digital light meter (RCC 340 FC/Lux), the light intensity at the tank surface was measured; during daytime periods, the light intensity averaged 250 lumens at the tank-water surface. During twilight, the room illumination could be phased in or out over a 30 to 35 minute time period, mimicking natural conditions for southern Ontario at this time (Elmira, Ontario,

August 1998 to April 1999) (Table 5.1). There were no sources of light during night periods.

The light intensity during twilight and photoperiods in each room was measured and recorded every 15 seconds during the experimental trials, using a light intensity logger (Stow Away™ LI, Onset Computer Corporation). The light intensity logger recorded the exact time (HH:MM:SS) for each measurement, so the twilight, day and night periods could be later synchronized with EMT signals recorded by the SRX_400 receiver.

Water that was supplied to the 2x2 meter tanks had a temperature of 8.5 ± 0.1 C, a dissolved oxygen concentration of 10.6 mg/l (oxygen saturation of 100.8%), and a pH of 7.6 ± 0.1 . Water was delivered to the tank via a PVC spout at a rate of 22 litres per minute, which generated a circular flow within the tank. Between experimental trials, the centre drainage grid in the tanks were cleaned and flushed.

5.3.3 Experimental Protocol

5.3.3.1 Acclimation Period And Data Standardization

Two months prior to the experimental trials, all of the fish were held in indoor 2x2 meter holding tanks, under the “natural phased” artificial lighting regimen, at a bio-density of 30 kg/m³. During this time, the fish were fed a maintenance ration of trout pellets, once daily (6 Pt., Shur-Gain). The food was distributed into the

tanks over a one hour period, using a belt feeder. For each trial (six trials in total), eight to fourteen fish were randomly selected from holding tanks and implanted with EMT transmitters as described in chapter 2 of this thesis. These fish were randomly assigned to the 2x2 meter tanks located in the experimental rooms. Each tank was then stocked with additional non-EMT tagged rainbow trout until a bio-density of 30 kg/m³ was achieved. Therefore, during each trial, there were 4 to 7 EMT tagged fish in each experimental room (see Appendix C).

With the experimental tanks stocked with tagged and non-tagged fish, the lighting in each room was slowly dimmed over a 45 minute period, until the light intensity at the tank-water surface was measured at 0.2 lumens. This marked the beginning of the acclimation period. During the 5 day acclimation period, the fish were not fed, and the photoperiod was kept constant (i.e. 24 hour, low intensity room lighting). The acclimation period served to rest the fish that were implanted with transmitters, and as an attempt to standardize the immediate environmental history of the fish. The SRX_400 receiver was programmed to collect 30 EMT signals from each fish in the rooms every hour. These EMT transmitter signals were used as basal measurements to standardize the data for each fish. The highest EMT pulse intervals recorded for each fish (corresponding to the lowest level of muscle activity) were averaged and defined as basal.

5.3.3.2 Light Regimen Treatments

After the 5 day acclimation period, the lighting system was activated in each experimental room. One room was illuminated with the lighting programmed to activate according to the "instant-on" type lighting regimen. The other room was illuminated according to the "natural phased" type lighting regimen. At dawn, the lighting systems in both rooms activated at approximately the same time (Table 5.1). The illumination in the room programmed with the "instant-on" type lighting regimen was instantly at full intensity, while the illumination in the room with the "natural phased" lighting remained very dim (the tungsten filaments in the bulbs glowed slightly) (Figure 5.1). During the next 30 to 35 minute period, the lighting in "natural phased" room gradually reached full intensity. Similarly, at dusk, both lighting systems commenced deactivation at the same time, except the "natural phased" lighting took 30 to 35 minutes to extinguish completely (Figure 5.1). This continued for a total of 7 days. During this time, the SRX_400 receiver was programmed to continuously collect 15 EMT pulse intervals from each fish in each of the rooms. The fish were fed once daily (6Pt., Shur-Gain), using a belt feeder which distributed the food over an hour. Food distribution ranged between 11:00 to 13:00 h daily. At the end of the 7 day treatment period, the EMT tagged fish were netted out and euthanized, and postmortem dissections were conducted to determine exact electrode placement. For each subsequent trial, additional rainbow trout from holding tanks were selected and implanted with EMT transmitters. The room in which the "instant-on" lighting system was operating was programmed to illuminate according to the "natural phased"

lighting system, and vice versa for the other room. The lighting treatment was switched between rooms in this manner for each subsequent trial.

5.3.3.3 Telemetry Data Set

Some features of the experimental set-up caused the resultant EMT data set to be missing observations and unbalanced. EMT and light intensity records were necessarily downloaded every other day during the lighting treatments. While downloading, no telemetry signals could be recorded from the experimental rooms for 3.5 hours due to the quantity of data being downloaded. The download periods were shifted as much as possible during the experimental period in order to avoid missing data; each download period was staggered approximately every 5 hours ahead of the last, but never in a manner that prevented the recording of EMT signals during the twilight periods. Occasionally throughout the trials, power failures due to weather storms and receiver malfunctions would cause the recording of EMT signals to cease, which further fragmented some portions of the data set. Finally, some EMT transmitters, during all six trials, lost battery power and emitted no signals (the transmitters are non-rechargeable). As a result, the number of working EMT transmitters, and therefore implanted fish, varied in each room, and over each trial (4 to 7 operating transmitters per room). Fish that did not recover well from surgery and appeared to be in distress during the trials were euthanized. Hence, the mortality rate of implanted fish for the first 2 trials was approximately 15 percent, which then leveled off at 1 percent for the remaining 4 trials. This most likely reflected refinements in surgical methods. In

total, data were successfully collected from 54 healthy individuals with functioning transmitters over the entire study, and this is the largest sample size of tagged fish used in an EMT study to date. All of the above factors that could have contributed to the variability of the data were accounted for in the statistical analysis.

5.3.4 Statistical Analysis

Since the lighting treatments were assigned and applied to the entire room (i.e. the tanks were not individually illuminated), the experimental unit in this study was designated as the room (Steel et al. 1997). In the analysis (Proc Mixed, SAS®), the lighting regimens, experimental rooms, treatment days (i.e. the 7 days proceeding the acclimation period in each trial), replicates, and various interactions were tested as fixed-effects for significance. The experimental tanks and EMT tagged fish were included in the analysis as covariance parameters (random-effects). In addition, each treatment day was divided into five 'daily periods' for further analysis. The daily periods were as follows: night 1 (00:00 hr to 'begin dawn', see table 5.1), dawn (30 to 35 minute period immediately proceeding night 1), daytime (after dawn to 'begin dusk', see table 5.1), dusk (30 to 35 minute period immediately proceeding daytime) and night 2 (immediately after dusk to 23:59 hr). The duration of each daily period for both "instant-on" and "natural phased" lighting treatments was identical. The activity index data obtained during each daily period was tested as a fixed-effect in the analysis.

The variance associated with the activity index was analyzed according to the mixed linear model:

$$\Upsilon_{ijklmno} = \mu + \alpha_i + \beta_j + \Phi_k + \varrho_m + \varphi_n + (\alpha\beta)_{ij} + (\alpha\Phi)_{ik} + \rho_{l(n)} + \delta_{o(lim)} + \varepsilon_{ijklmno}$$

where;

$\Upsilon_{ijklmno}$ = the activity index observed for the o^{th} fish, in the l^{th} tank, in the n^{th} room, exposed to the i^{th} lighting treatment, during the k^{th} daily period of the j^{th} day within the m^{th} replicate;

μ = the overall mean of the activity index;

α_i = the fixed effect for lighting treatment ($i = 1,2$);

β_j = the fixed day effect ($j = 0,1,2,3,4,5,6,7$);

Φ_k = the fixed daily period effect ($k = 1,2,3,4,5$);

ϱ_m = the fixed replicated effect ($m = 1,2,3,4,5,6$);

φ_n = the fixed room effect ($n = 1,2$);

$(\alpha\beta)_{ij}$ = the fixed effect for lighting treatment by day interaction;

$(\alpha\Phi)_{ik}$ = the fixed effect for lighting treatment by daily period interaction;

$\rho_{l(n)}$ = the random effect for tank nested within room;

$\delta_{o(lim)}$ = the random effect for fish nested within tank, room and lighting treatment;

$\varepsilon_{ijklmno}$ = the experimental error.

Significant differences in daily periods were further elucidated using Tukey's pairwise comparisons (Steel et al. 1997).

Additional analysis focused on the swimming activity of fish in response to the precise moment of light activation in each room (i.e. the dark-light transition), for both “instant-on” and “natural phased” lighting regimens. Data collected for each fish (EMGi; ms) was inspected for recordings that spanned the dark-light transition periods. EMT recordings that met these criteria were isolated and compiled in a separate file. Since abrupt light-dark transitions may elicit “fast-start” reactions in fish (Chapter 3), the new data set was analyzed for light-induced swimming responses, according to criteria derived from EMT data indicative of “fast-start” swimming (Chapter 3; Appendix D). As such, the swimming responses of individual fish during the dark-light transition were categorized as follows: 1) Positive response = indicative of “fast-start” activity 2) Negative response = data not indicative of “fast-start” activity 3) Unclear response = elevated fish activity, but data not indicative of “fast-start” activity. Finally, a chi-square analysis was performed on the categorical data (Steel et al. 1997). The SRX_400 telemetry receiver could only scan and collect EMT signals from one tagged fish at a time. As a result, a record of only one dark-light transition response per day for each lighting treatment was possible, providing that the receiver was scanning in the correct room (2 rooms per trial) at time of light activation.

5.4 Results

Immediately after dusk, during the first day of light exposure proceeding the acclimation period, the activity of EMT tagged fish, on average (unadjusted

means), appeared to be influenced by the light regimens (figure 5.2). Statistical analysis revealed that the trial replicates ($F_{5,46.3}=1.05$, $p=0.40$), experimental rooms ($F_{1,46.3}=0.03$, $p=0.87$) and treatment days ($F_{7,1463}=1.66$, $p=0.12$) were all non-significant factors in affecting mean activity index levels. The overall activity levels of fish reared with the "natural phased" lighting regimen were significantly elevated above the activity levels of fish reared with the "instant-on" light regimen ($F_{1,46.3}=5.46$, $p=0.02$) (Figure 5.3). Fish reared under either lighting regimen exhibited significant daily patterns of activity ($F_{4,1462}=9.30$, $p<.0001$) (Figures 5.2 and 5.4). Pair-wise comparisons of the five daily periods (night1, dawn, daytime, dusk, night2), for both lighting treatments, revealed that fish activity was significantly elevated during dusk as compared to the daytime and night levels of fish activity (Table 5.2, Figure 5.4). Also apparent were non-significant trends in fish activity during dawn when compared to the daytime and dusk activity levels of fish (Table 5.2, Figure 5.4). The activity differences in daily period appeared most pronounced in fish reared under the "natural phased" lighting regimen (Figure 5.4), although they were not significant (treatment x period interaction, $F_{4,1463}=1.54$, $p=0.19$).

The distribution of positive, negative and unclear swimming responses of fish during the dark-light transition periods was significantly different between the lighting regimens ($\chi^2=9.27$, $p<0.01$) (Table 5.3 and 5.4). Overall, 71.4% of the positive responses (indicative of "fast-start" swimming) were observed from fish reared with the "instant-on" light regime, while the majority of the unclear or

negative responses (66.7 and 64.7%, respectively) were observed from fish reared with the "natural phased" light regime.

5.5 Discussion

It is apparent from the results that the lighting regimen had significant effects on the locomotory activity of the EMT tagged fish. The only differences between the "instant-on" and "natural phased" lighting regimens occurred during dusk and dawn. Therefore, it is reasonable to suggest that it was the experiences of fish during the twilight periods, presumably influenced by the lighting systems, that affected the overall activity levels of fish. It may be viewed that functionally, the "instant-on" light regimen does not provide a true twilight period. To date, the affects of providing or withholding a twilight period to cultured fish on their subsequent activity or behaviour have remained unstudied. The results of this experiment support previous reports that have inferred the importance of twilight periods in influencing fish behaviour (Helfman 1986; Mork and Gulbrandsen 1994), and in addition, demonstrates how this influence is manifested under captive culture conditions.

5.5.1 "Fast-start" Behaviour

The data presented in chapter 3 of this thesis show that "instant-on" lighting causes fish to elicit "fast-start" behaviour. Since the environmental conditions in both the present experiment and in the study presented in chapter 3 were identical (with exception of the translucent tank covers which reduced tank

illumination), it is probable that the “fast-start” behaviour was shown by the fish in the present experiment. The biased distribution in positive responses of fish reared with the “instant-on” lighting support this suggestion. Studies that have used lower light intensities similar to the tank illumination levels of this study have also produced “fast-starts” in rainbow trout (Mork and Gulbrandsen 1994).

Although the “natural phased” lighting regimen provided a gradual increase in light intensity to the fish, 28.6 % of the positive responses recorded were observed from fish reared with this lighting system. It may be that the change in light intensity during the dark-light transition (1.5 % of full intensity; ~ 3.75 lumens) was still sufficient to elicit a startle response in some of the fish. Mork and Gulbrandsen (1994) exposed fish to two intensities of abrupt lighting (0.6 and 150 lumens), and reported that dark-light transitions of 0.6 lumens could still elicit some aspects of “light-shock” swimming behaviour in Atlantic salmon. However, the majority of the fish in this study reared with the “natural phased” regimen either did not react or showed an elevation in swimming activity. Elevated fish activity during and after the dark-light transition might be expected, since rainbow trout locomotory activity generally increases with increasing or decreasing light levels during the twilight periods (Figure 5.4; Mork and Gulbrandsen 1994).

5.5.2 Overall Fish Activity Levels

Even though circadian activity rhythms of fish reared with either lighting regime were similar (Figure 5.4), the overall level of fish activity differed, and was greatest in magnitude for fish reared with the “natural phased” lighting regimen (Figure 5.3). Cooke et al. (2000) also observed that activity levels of rainbow trout, reared at 3 densities, could differ in magnitude, but simultaneously demonstrate similar circadian rhythms. In that study, differences in overall activity levels were most likely caused by density related, conspecific interactions, as well as other physical disturbances or stressors. Similarly, it is suggested that overall differences in activity levels observed in this study were influenced by the “stressful” consequences of exposing fish to abrupt light-dark transitions. Mork and Gulbrandsen (1994) found that when rainbow trout were subjected to abrupt lighting, fish activity first increased, and then subsequently slackened. Similarly, the results presented from chapter 3 of this thesis show that fish activity subsequently decreases to low levels after “fast-starts” were elicited. On average, this behaviour could at least result in lowered levels of fish activity during twilight periods. The majority of fish reared with “natural phased” lighting did not perceive the dark-light transitions as startling (as judged by fish response distributions), and therefore their activity levels would not be expected to be lowered in a similar fashion. If “fast-start” responses temporarily disturb or alter feeding rhythms or other cyclic events (Sanchez-Vazquez and Tabata 1998), then it is conceivable that fish activity levels could be lowered as a result.

Providing or withholding twilight periods (via lighting regimen) may also have influenced the overall level of fish activity. Both Mork and Gulbrandsen (1994) and this study show that light-dark transition periods are the time of greatest locomotory activity for rainbow trout. The present results also show that the relative change in activity, from daytime to dusk, was more than doubled for fish reared under the "natural phased" regimen as compared to the "instant-on" light regimen (Figure 5.4). During the twilight periods, both wild and captive fish undergo vertical migrations; fish swim to the surface at night and down to the tank bottom during the daytime (Mork and Gulbrandsen 1994; Fernö, Huse, Juell and Bjordal 1995; Sanchez-Vazquez and Tabata 1998). The nature of such behaviour is controversial, and many factors have been considered (light intensity, water temperature, predator avoidance, foraging behaviour) (Sanchez-Vazquez and Tabata 1998). No study, however, has examined the effect of providing or withholding a true twilight period on the vertical migrations of fish. Fernö et al. 1995 found that vertical migratory behaviour of Atlantic salmon, reared with natural lighting, could be explained by a trade-off between light-induced surface avoidance and food attraction. This type of migratory behaviour, which is dependent upon varying intensities of light, may be quite different than the "light-shock" induced migrations inherent to "instant-on" type lighting systems (e.g. Mork and Gulbrandsen 1994). It is possible, therefore, that overall locomotory activity differences associated with the lighting regimens, at least during the twilight periods, are influenced by an increased expression of vertical migratory activity in fish reared with "natural phased" lighting. Additional studies

are needed in order to understand the effects of such behaviour on nocturnal and diurnal fish activity.

5.5.3 Daily Activity Rhythms

In this experiment, significant diel activity patterns of fish were observed (Figures 5.2 and 5.4) regardless of the lighting regimen employed. In general, the activity levels of fish were lower during the daytime than during the twilight periods. These results are in agreement with the findings of Cooke et al. (2000), who found similar EMT activity patterns for rainbow trout that were stocked at 30 kg/m³ and fed at midday. Locomotory circadian rhythms of rainbow trout are thought to be influenced by temperature (Landless 1976), feeding (Boujard and Leatherland 1992), light-dark cycles (Mork and Gulbrandsen 1994; Sanchez-Vazquez and Tabata 1998) and perhaps feeding method (i.e. demand feeder, belt feeder etc.) (Sanchez-Vazquez and Tabata 1998). A combination of those factors, as well as vertical migratory behaviour, could be responsible for the activity rhythms of fish observed in this study.

5.5.4 Welfare Implications

Changes of spontaneous swimming activity in response to stressors are among the first symptoms observed in fish (Scherer 1992). States of depressed or elevated levels of activity were observed in fish reared with the "instant-on" lighting regimen. The high level of positive responses (indicative "fast-start" swimming) suggests that the "instant-on" lighting regime may act as an

environmental stressor; therefore it is recommended that other technologies, such as gradual, phased-in lighting controllers, supplant this type. The presumably lower levels of overall activity shown by fish reared with the “instant-on” regimen may be indicative of additional behavioural effects, which should be the subject of future research.

With few exceptions, information describing how fish apportion their time in aquacultural environments is lacking (Baras and Lagardère 1995), and consequently, it is difficult to discriminate between normal and abnormal activities. An understanding of how key behavioural variables change can allow for the identification of preference or tolerance margins of fish, and the real-time monitoring of fish activity in response to environmental variables, as was demonstrated in this experiment, may represent one of the most relevant steps in improving aquaculture management and husbandry conditions. Environmental preference tests (tests that require animals to choose between two or more different options or environments) have been used for an impressive variety of purposes in animal welfare research (Fraser and Matthews 1997). This type of testing could possibly be a feasible avenue for fish welfare research, providing that the causes and consequences for fish when performing or not performing different behaviours are understood (see Mench and Mason 1997). The results from this study may be valuable from the perspective of understanding the “normal” behaviour of fish, and how they are altered by environmental changes and conditions found in aquaculture facilities.

5.6 Conclusions

Both “instant-on” and “natural phased” lighting regimens affect the activities of rainbow trout by providing environmental conditions that may induce or inhibit certain behaviours. For example, “fast-start” behaviour may have been induced and normal, dusk behaviours may have been inhibited due to the abrupt intensity transitions inherent to the “instant-on” light regimen. The “instant-on” lighting regime may act as an environmental stressor, and it is therefore recommended that other technologies supplant this type. While it is not clear if the effects of light regimen on overall fish activity levels actually impacts fish welfare, it is useful to observe that the daily activity rhythms observed in wild fish are still expressed by domesticated, hatchery reared fish, and are reflective of artificial manipulations to the environment. An understanding of those relationships will be necessary for the design of preference tests, which are likely to be of value for fish welfare assessments.

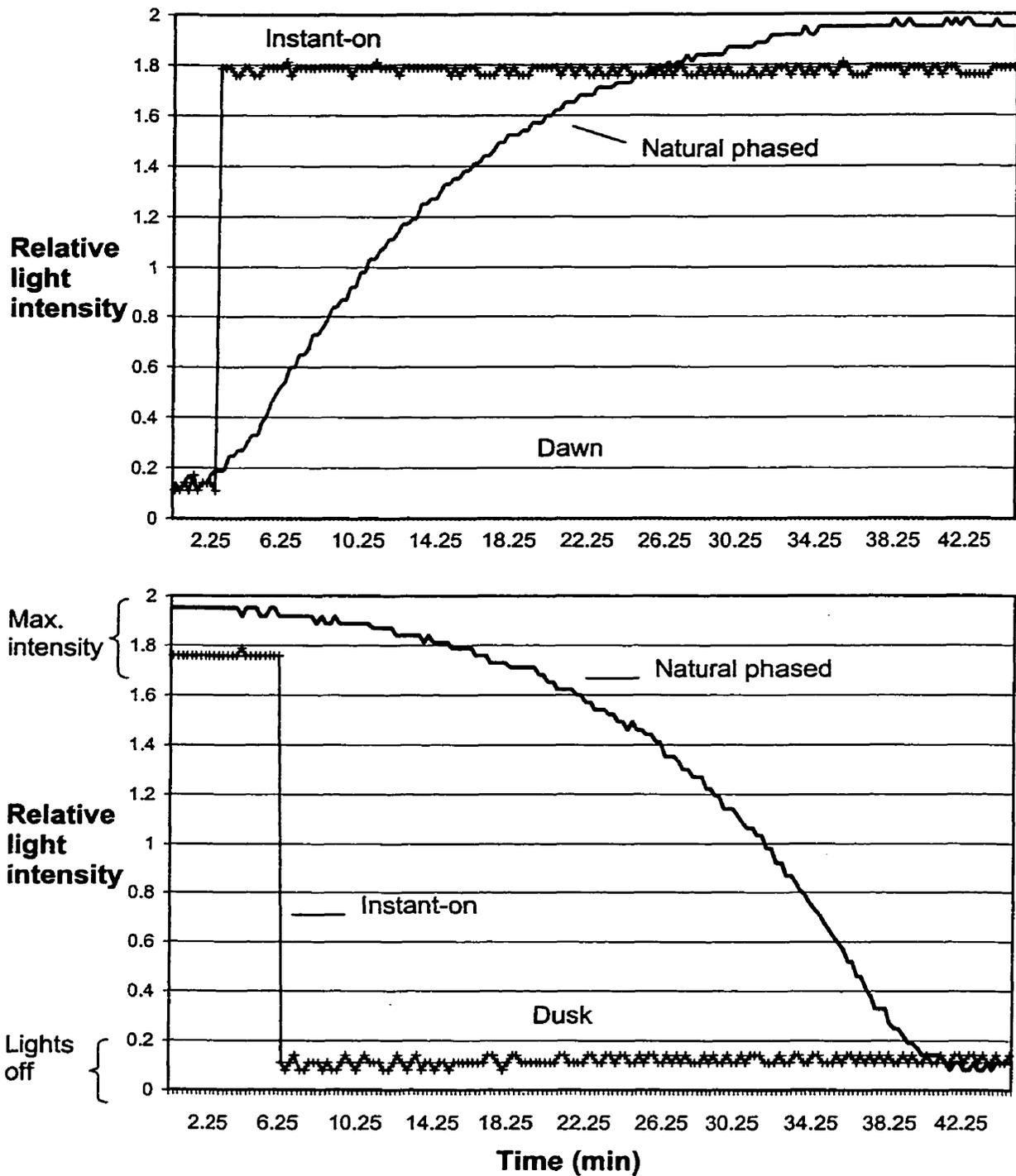


Figure 5.1 Relative light intensity during twilight for instant-on and natural phased lighting regimens. A light-logger located in each treatment room recorded the illumination level every 15 seconds. The light logger does not measure in true lumens, but gives a relative measure of light intensity. Differences in the maximum light intensity measured by the light-logger are reflective of variation in logger calibration, and not of actual light intensity.

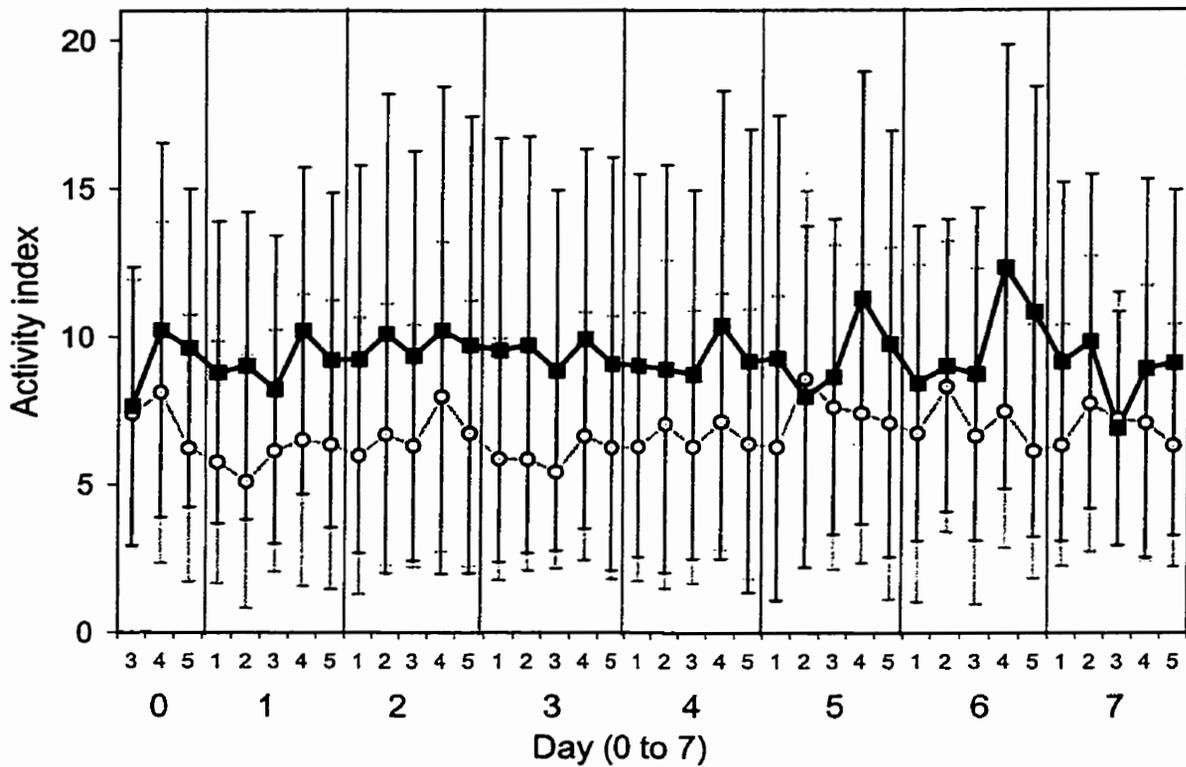


Figure 5.2 The effect of "instant-on" and "natural phased" lighting regimens on fish activity over the treatment period (unadjusted means). Black squares represent the mean activity index of fish reared with the "natural phased" regimen, gray circles represent fish activity reared under the "instant-on" regimen. The numbers directly below the x-axis represent the daily periods: 1) night1, 2) dawn, 3) daytime, 4) dusk, 5) night2. The error bars are ± 1 standard deviation.

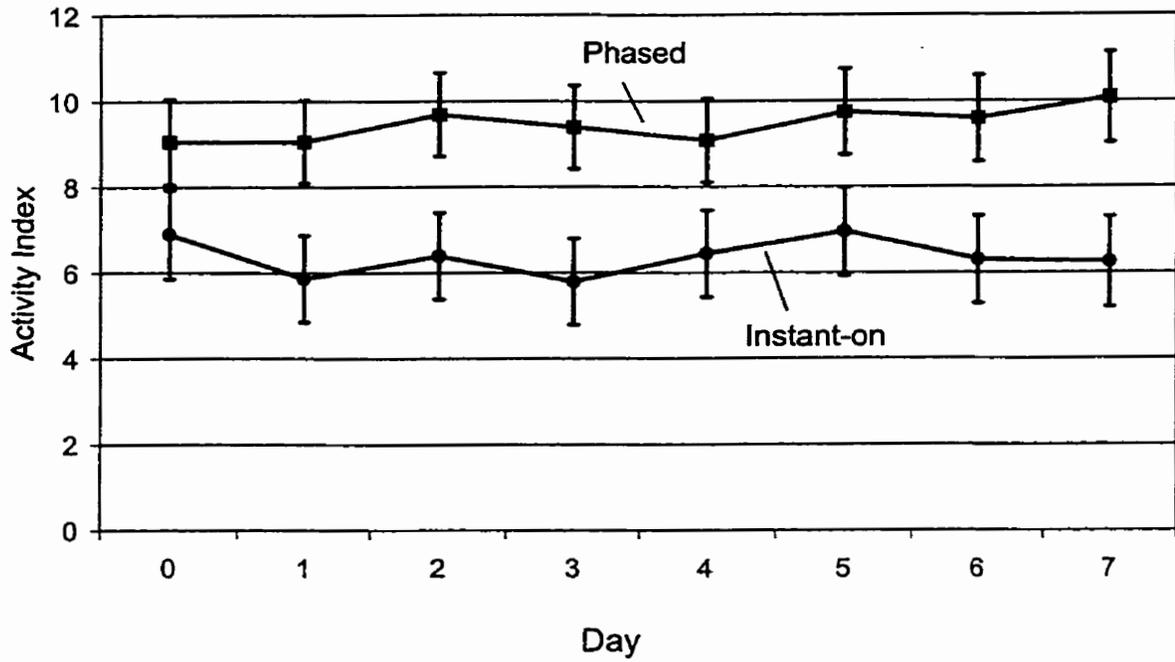


Figure 5.3 The effect of lighting regimen on fish activity over the seven day treatment period. Squares and circles represent fish activity under the "natural phased" and "instant-on" lighting regimens, respectively. The data points are least-square means, ± 1 SE.

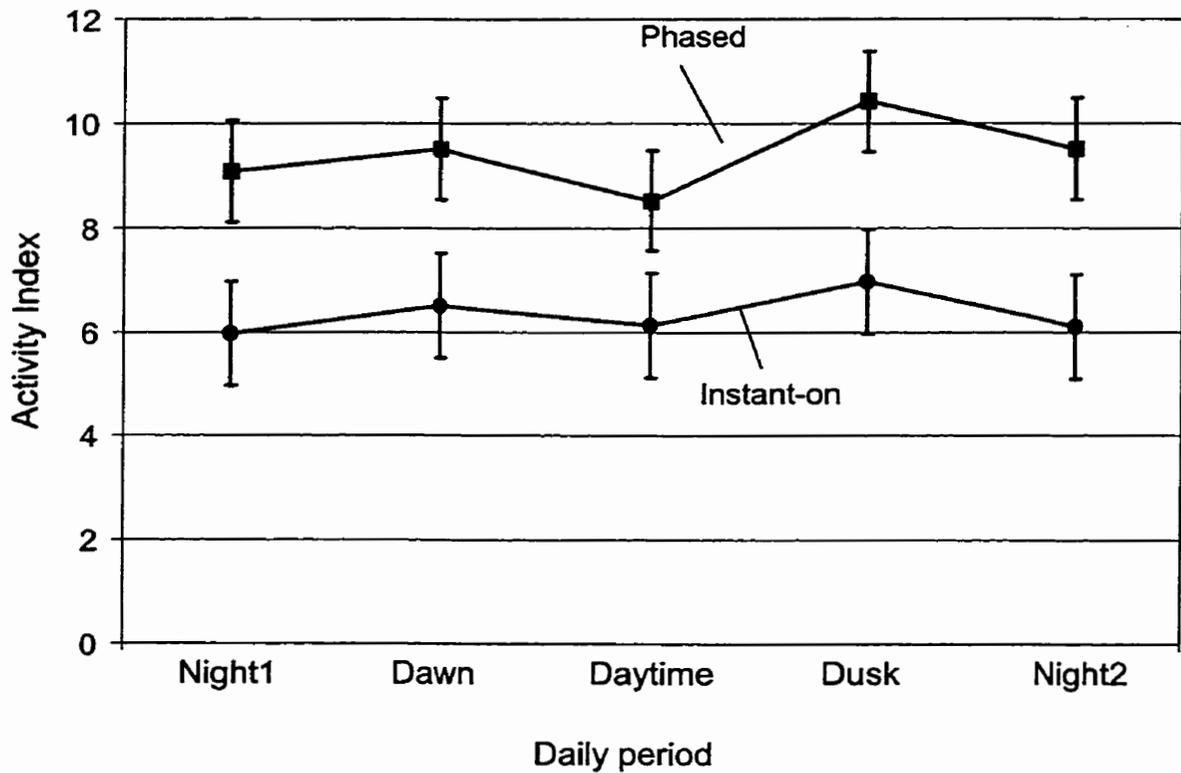


Figure 5.4 The effect of lighting regimen on fish activity during twilight, night, and daytime periods. Squares and circles represent the activity of fish reared under the "natural phased" and "instant-on" lighting regimens, respectively. The data points are least-square means, ± 1 SE.

Table 5.1 Photoperiods used during each trial replicate.

Replicate	Date	Begin Dawn	Begin Dusk	L:D
1	Sept. 98	06:00	18:30	12.5 : 11.5
2	Sept. 98	06:10	18:15	12.1 : 11.9
3	Nov. 98	07:10	17:00	9.2 : 14.8
4	Dec. 98	07:50	16:45	8.9 : 15.1
5	April 99	05:50	19:00	14.2 : 9.8
6	April 99	05:30	19:15	14.8 : 9.2

Begin Dawn = Time of "natural phased" light regimen activation.

Begin Dusk = Time of "natural phased" light regimen deactivation.

The "instant-on" lighting activated or deactivated within ± 20 minutes from the "natural phased" regimen time listed in the table. The light-dark ratio (L:D) used for both lighting regimens mimicked the local natural environment. Lighting regimens were not adjusted for 'daylight savings'.

Table 5.2 Differences in daily period least square means.

Daily period	Daily Period	P-value
night1	Dawn	0.3277
night1	Daytime	0.9621
night1	Dusk	<.0001*
night1	night2	0.7523
Dawn	Daytime	0.0719
Dawn	Dusk	0.0713
Dawn	night2	0.9505
Daytime	Dusk	<.0001*
Daytime	night2	0.2838
Dusk	night2	0.0035*

• significantly different (Tukey-Kramer pairwise comparisons)

Table 5.3 Swimming response of individual fish at time of “instant-on” light activation at dawn.

Recording Time (sec)	EMT Values	Response	Recording Time (sec)	EMT Values	Response
3	1	Unclear	21	9	Positive
29	0	Negative	14	2	Unclear
12	3	Positive	19	0	Negative
16	0	Negative	22	3	Positive
3	2	Unclear	23	0	Negative
9	4	Positive	18	7	Positive
23	8	Positive	24	4	Positive
30	0	Negative	15	4	Positive
22	8	Positive	19	1	Unclear
18	8	Positive	35	1	Unclear
19	0	Negative	17	2	Unclear
22	4	Positive	19	4	Positive
19	8	Positive	34	0	Negative
9	3	Positive	16	3	Positive
25	11	Positive	27	0	Negative
19	3	Positive	30	7	Positive
19	11	Positive	27	3	Positive

Total observations: 34
 Positive responses: 20
 Negative responses: 8
 Unclear responses: 6

Data presented are derived from continuous EMT recordings of fish that were being scanned for signals as the “instant-on” lighting activated in the room i.e. the dark-light transition).

Recording time (sec) = the duration over which signals from a fish were being recorded

EMT Values = the number of significant activity spikes (EMT signals observed to be less than 338 ± 14 ms of pre “instant-on”) during the recording time

Positive response = indicative of “fast-start” fish activity

Negative response = data not indicative of “fast-start” fish activity

Unclear response = elevated fish activity, but data not indicative of “fast-start” activity

Table 5.4 Swimming response of individual fish at time of “natural phased” light activation at dawn.

Recording Time	EMT Values	Response	Recording Time	EMT Values	Response
21	2	unclear	24	2	unclear
9	0	negative	19	0	negative
28	0	negative	24	0	negative
19	3	positive	21	10	positive
25	0	negative	17	4	positive
23	2	unclear	30	3	positive
17	3	positive	19	2	unclear
22	0	negative	16	5	positive
28	0	negative	16	2	unclear
26	0	negative	23	4	positive
27	1	unclear	24	0	negative
27	2	unclear	29	0	negative
26	0	negative	16	2	unclear
17	0	negative	27	9	positive
31	2	unclear	28	1	unclear
26	0	negative	27	0	negative
25	2	unclear	25	0	negative
24	0	negative			

Total observations: 35
 Positive responses: 8
 Negative responses: 16
 Unclear responses: 11

Data presented are derived from continuous EMT recordings of fish that were being scanned for signals as the “natural phased” lighting activated in the room (i.e. the dark-light transition).

Recording time (sec) = the duration over which signals from a fish were being recorded

EMT Values = the number of significant activity spikes (EMT signals observed to be less than 338 ± 14 ms of pre “natural phased”) during the recording time

Positive response = indicative of “fast-start” fish activity

Negative response = data not indicative of “fast-start” fish activity

Unclear response = elevated fish activity, but data not indicative of “fast-start” activity

Chapter 6: Assessing the Welfare Status Of Farmed Fish

6.1 Developing A Fish Welfare Index

In search of a practical approach to assess the welfare of farmed animals, a number of investigators have proposed or developed various welfare indexing schemes. Indices of welfare are thought to be important when monitoring or trying to improve the “quality of life” of captive animals, as they may allow for the objective assessment of welfare in a manner that makes clear which rearing practices or activities are acceptable and which are not. In addition, welfare information assembled in this format may be useful for framing animal welfare legislation (Broom and Johnson 1993). A complicating factor, however, is that indexing schemes are often based on different conceptualizations of animal welfare, and this invariably results in contradictory assessments. All welfare indices use some sort of biological indicator, ranging from measures of impaired growth and reproduction, to adrenal cortex activity or the frequency of certain behaviours. Biological indicator scores have been used to identify the position of an animal on a welfare scale as being better or worse (Broom and Johnson 1993). Little agreement exists, however, on the relative importance of each biological indicator when assessing welfare problems. There are, however, unifying themes amongst the different welfare indices. Most evaluations consider the set of standards proposed by the Farm Animal Welfare Council, known as the “Five Freedoms”. The “Five Freedoms” are as follows: 1) freedom from thirst, hunger and malnutrition, 2) freedom from discomfort, 3) freedom from pain, injury and disease, 4) freedom to express normal behaviour and 5) freedom from fear

and distress. The “Five Freedoms” concept can be used to evaluate, in a systematic and comprehensive fashion, the welfare of animals in different environments (Webster 1995). Also prevalent in most welfare indices is the concept that welfare is a measurable characteristic of animals that varies over a range, rather than being something which either exists or does not exist (Broom and Johnson 1993).

The welfare concepts, arguments and data presented in this thesis will justifiably influence the fundamental ideas factoring into the design of the fish welfare index that is to be developed in this chapter. The experiments have been designed to investigate and develop the use of EMT for use in aquaculture, and demonstrated that a number of key variables can be measured with this technology that are likely to be useful to assess the welfare of farmed fish. These variables, both behavioural and physiological in nature, are compatible with the unifying themes underlining other welfare indices. Implied in the introductory chapter of this thesis was that any method used to evaluate fish welfare should integrate a variety of indicators. Traditionally in the fisheries sciences, the roster of biological indicators has included haematological or metabolic measures, as well as performance or challenge tests. The relevance of these indicators to welfare has remained unexamined, but more importantly, many of them only give information about one level of biological organization. According to Webster (1995), the value of using multiple indicators is akin to the navigator’s art of triangulation; that is, “one attempts to get an approximate fix on the problem or

position from at least three completely different directions, thereby creating the smallest possible triangle of uncertainty". The use of EMT provides a means to assess both the behavioural and physiological responses of fish to aquacultural environments and activities from a different angle. Furthermore, this assessment can occur within a known context in which welfare inferences may be derived.

The fish welfare index that is developed here requires that a variety of biological indicators are utilized, and that the information obtained from those indicators are interpreted within an appropriate context (i.e. with reference to a fishes cognitive state and what it perceives). This means that an accurate accounting of the welfare state (i.e. good or bad) of fish will not only entail the use of various biological indicators for assessment, but will also depend on additional "environmental variables" on which the welfare of fish are assumed to depend (e.g. physical environment, social conditions). It is argued here that the environmental variables either create or highly influence the perceptions that fish have of their situation (e.g. Ross and Watten 1998). This link between the fishes environment, its cognitive state and the biological indicators should form the basis of fish welfare assessment. This implies that fish welfare indices should consist of more than just a management or environmental evaluation. This type of welfare evaluation system has precedent; Dr. Temple Grandin, a designer of livestock handling facilities, regularly conducts animal welfare assessments that are based upon the linkage between management conditions and an animals perception of those conditions (e.g. Grandin 1996).

Conceptually, the proposed fish welfare index consists of three sets of assessment factors (Table 6.1). These are 1) the biological indicators, 2) the environmental variable(s) and, 3) the "context" (i.e. the relevance of the indicators to welfare). While all 3 factors are required in this scheme, it is obviously the "context" that is fundamental to making accurate assessments of fish welfare. The environmental variables will influence the fishes perception of its environment, and therefore determine the context in which its physiological or behavioural activities are to be interpreted. The environmental variables can originate intrinsically (factors pertaining to the fish themselves) or can be extrinsic (abiotic factors) (see Klontz 1995). Only after the environmental variables have been assessed can the biological indicators provide information on the impact of a situation that is suspected to affect welfare.

Theoretically, the relationship between the assessment factors and their impact to fish welfare could be estimated by combining the assessment factors into an equation, in order to calculate a numerical welfare rating score (Figure 6.1). The advantage of the equation format is that it allows for an organized and integrated framework in which the relevant welfare criteria can be assessed in an objective way. Therefore, the numerical scores derived from multiple welfare assessments could be directly comparable. Three biological indicator categories are listed in equation 1 (Figure 6.1). The scores associated with the categories are derived from an evaluation process, in which individual biological indicator values (e.g.

cortisol or behavioural assay test results) are interpreted and ranked as being indicative of a positive, negative or neutral welfare state (stages 1, 2 and 3; Figure 6.1). The interpretation process must take into account the environmental variable(s) (e.g. social structure, light regimen, tank design), especially those for which empirical information exists with regard to fish behaviour and physiology. The resultant scores for each individual biological indicator, which range from -3, -2, -1, 0, +1, +2 and +3 (see figure 6.1 for score descriptions), are then summed in the appropriate category sub-equation (equations 2, 3 and 4; figure 6.1). At this stage, a weighted factor (WF1, WF2, WF3) is introduced into the calculation. The weighted factor is a numerical representation of species-specific cognitive ability, with particular reference to pain, fear and psychological stress. If it is assumed that all the biological indicator categories have the same weight, then the final numerical welfare score, calculated with equation 1, will range between -9 and +9 (where +9 is the best welfare scenario and -9 is the worst). The welfare index equation described above was adapted from a multicriteria decision-making model designed to assess aquacultural sustainability (Bahrami 2000).

To facilitate an understanding of the biological indicators in this indexing scheme, selected examples mentioned in Table 6.1 will now be commented upon. "Fast-starts" have been experimentally recorded during feeding (Canfield and Rose 1993), are elicited by "instant-on" lighting regimens (Chapter 3), and possibly during transportation by truck (Chapter 4). The context in which "fast-starts" are

performed by fish in these three situations are different. Since feeding is a “rewarding” activity for fish, the “fast-starts” in this context are likely to be indicative of a neutral or positive cognitive state, and therefore the estimated impact on welfare is probably negligible or good. On the other hand, “fast-starts” in a context of an unanticipated or unrewarding stimulus, such as abrupt lighting, may be perceived by the fish as a neutral or, arguably a negative experience. Therefore, the estimated impact on welfare would be either negligible or not good. In the example of transportation, “fast-starts” are elicited under novel and unpredictable circumstances, which were preceded by a physical handling episode. The “fast-starts” may therefore be regarded as an indicator of a situation that negatively impacts the welfare of the fish. In this particular example, the analysis would greatly benefit by the inclusion of evidence from a different angle: Schreck et al. (1995) demonstrated that under certain circumstances, plasma cortisol was indicative of how fish perceived a transport episode. The accuracy of the estimated welfare impact could be strengthened if cortisol measures were included in this case.

Reduced activity levels in fish may be caused by abrupt light regimens (Chapters 3 and 5, this thesis), and also as a response after a sufficient number of aggressive encounters with conspecifics (Winberg and Nilsson 1993). Again, the context in which fish activity is lowered differs. Abrupt lighting will reduce activity levels in two possible ways; by not providing the correct environmental conditions for certain behaviour to be expressed, or by inducing the fish into

“fast-start” behaviour, of which there is a resulting recovery period characterized by reduced activity. Constant aggression leads to physiological changes in subordinate fish which are thought to reduce their activity levels, and possibly lead to diseased states (Pickering and Pottinger 1989). Lack of behavioural expression or reduced activity in fish as a result of abiotic influences, such as with lighting, has not been adequately studied, and consequently it is difficult to determine the actual impact on welfare. If the disruption of normal behaviour was great enough to alter other variables, such as feed intake, then this might indicate a negative effect on welfare. The reduced activity of subordinate fish however, is likely to be indicative of welfare problems in those individuals, as it is related to unresolvable negative social conditions exacerbated by culture conditions. A general lack of control and predictability, restricted access to food, and a constant threat of aggressive actions from dominant individuals are all factors that could be manifested as reduced locomotory activity in fish (Øverli et al. 1999).

In summary, it is suggested that the fish welfare index developed here may accurately define and estimate the impact of aquacultural practices to welfare. This is primarily because it calls for a number of biological indicators, from which the results are interpreted in a context that is relevant to welfare. The “estimated impact” may be used as a type of score, which takes into account the distribution of positive, neutral or negative welfare factors. The culmination of these scores

and comparisons of score distribution may enable the evaluation of specific conditions or entire aquacultural facilities from a welfare perspective.

6.2 Limitations Of The Welfare Index

The “welfare index”, as proposed, takes into account both biotic and abiotic factors that can be used to determine what is likely or not likely to impact fish welfare. The index cannot, however, provide a purely objective assessment of fish welfare. As previously demonstrated, the biological indicators are a source of quantifiable and objective information characterizing the physiological or behavioural status of the animals. However, the welfare conclusions that are drawn from this information are based on value judgments (Duncan and Fraser 1997). Therefore, the relative importance that is placed on the different elements of welfare are likely to vary among societies and societal sectors. This will lead to different conclusions about the “quality of life” of fish, and consequentially, the definition of acceptable or unacceptable rearing practices or activities.

6.3 General Conclusions

Farmed fish are sentient animals, and it is therefore appropriate to consider their welfare. In some respects, fish are very different from other vertebrates used in agriculture because of their adaptations for aquatic life, and their “lower” status on the evolutionary scale. Therefore, it has been especially difficult to determine what they can experience and consequently how animal welfare should relate to them (FAWC 1996). The arguments and experiments presented in this thesis

have shed some light on these issues, and have demonstrated that the aquatic environment in which fish live need not be a limiting factor when devising appropriate welfare assessment investigations. This is especially true for studies that take into account a paramount indicator of welfare, behaviour. Although EMT has not traditionally been used as a behavioural indicator, it was determined in this thesis that EMT signals were an accurate measure, or proxy, of swimming behaviour in fish reared in captive culture conditions. In particular, the “fast-start” responses of rainbow trout could be identified via EMT signals. Since the “fast-start” response is elicited in a number of contexts, the experiences of fish (both positive and negative) in a variety of aquacultural rearing situations could possibly be assessed with EMT technology. This was apparent when the EMT signals of the fishes response to a transportation episode was recorded. EMT may have additional relevance for fish welfare assessments, by providing “baseline” information of fish behaviour under intensive culture conditions. This was demonstrated when the activity of fish in response to artificial lighting regimens was documented. Furthermore, EMT can be used in some situations as a metabolic indicator, providing the means to estimate and assess the energetic output associated with different swimming behaviours in response to husbandry procedures. When fish must devote a portion of their energy budget to cope with “stressors” or physical disturbances, they may have less energy available for other, important life processes (Schreck 1982). This information may be relevant in assessing the “scope of activity” of farmed fish, which can have important management implications. EMT can be

used to determine the “scope for activity” of fish, providing that the calibration of EMT signals to oxygen consumption yields a positive relationship (Cooke et al. 2000). The utility of the “scope of activity” concept to fish welfare is not fully understood, and requires further study.

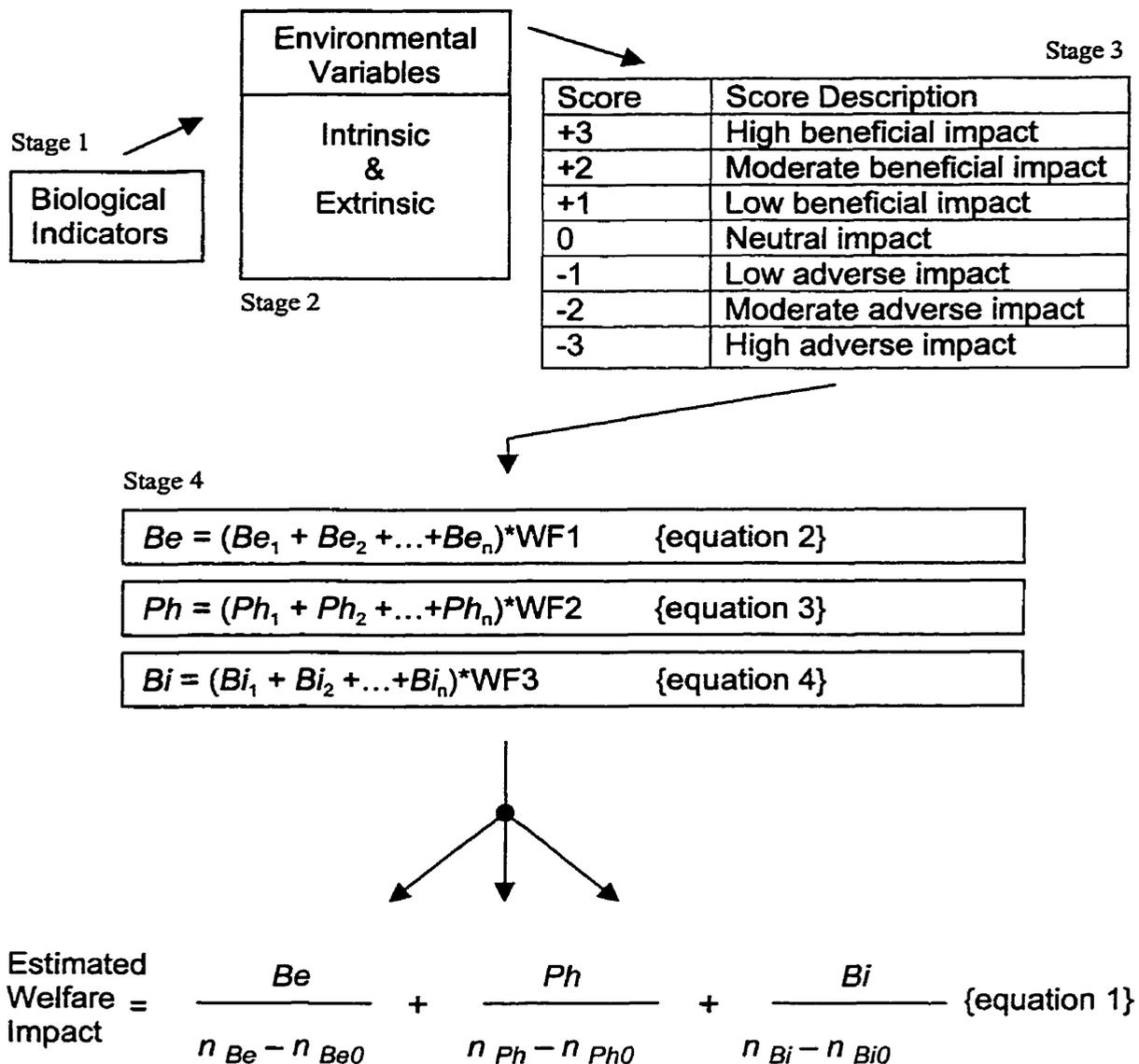
When people farm beef cattle, or poultry, they are usually dealing with differing strains of a domesticated animal, which may vary to some degree in terms of cognitive function and behavior. Hence, the welfare issues that apply to poultry, for example, will sometimes deviate because of the animal per se. When farming fish species however, a great deal of animal variation is involved. Fish species can be divided into three, major evolutionary groupings, which have been subject to divergence at an early stage in their evolutionary history (Hildebrand 1995). There are at least 20,000 species of teleost fish, and the select few that are farmed sometimes show extreme diversity in habits and habitat. Therefore, variability in brain structure and functions are to be expected (Munro and Dodd 1983). In addition, different species of farmed fish are also in various stages of domestication (Pottinger and Pickering 1997). Therefore, the welfare issues when farming catfish, for example, are probably not going to be the same as when farming rainbow trout or relatively non-domesticated Arctic charr. This implies that species-specific welfare research is needed. Since EMT is a tool adaptable to many farmed species of fish, physiological and behavioural evaluations of these species may be accomplished using this technology and should be the subject of future work.

Aquaculture facilities utilize a number of rearing technologies that need to be examined from a fish welfare perspective (for examples, see Grizzle, Chen, Williams and Spano 1992; Wedemeyer 1997; Bhujel 2000). These include high density culture, various feeding strategies, genomic manipulations, handling methods, transportation and slaughter techniques. By developing tools to evaluate these procedures, objective information can be acquired that will help to determine and define the limits of acceptable captive conditions in which farmed fish live and the procedures that fish are subjected to. This is necessary not only to ensure the high quality expected of the industry, but also in keeping with the ethical viewpoints we take with other terrestrial farm animals.

Table 6.1 Assessment factors used for estimating the welfare state of farmed fish.

Biological Indicators	Environmental Variables	"Context"	Index
Behavioural Fast-starts Elevated activity Reduced activity Physiological "stress" hormones lactic acid Performance And Other Measures U_{crit} Metabolic rate	Intrinsic Feeding Aggression Ontogeny Density Extrinsic Containment system Lighting regimen Water quality Grading Transportation	Positive Neutral Negative	Estimated Welfare Impact

The list of biological indicators has been limited to those that have been previously discussed in various chapters of this thesis.



where,

Be = behavioural category indicator score

Ph = physiological category indicator score

Bi = Other biological indicator score (e.g. U_{crit} , V_{O2})

$n(Be, Ph, Bi)$ = total number of indicators used for each score

$n(Be0, Ph0, Bi0)$ = total number of indicators for with a neutral (0) rating

Figure 6.1 A theoretical welfare indexing system. Biological indicator results are assessed, interpreted according to environmental variables and scored. The scores are summed in the appropriate equation, and multiplied by any weighted factors (WF), which relate to species-specific welfare considerations. Thus, behavioural, physiological and other biological indicator scores may be combined to give a numerical estimate of welfare.

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Appendices

**Appendix A: Meristics of fish from which EMT signals were recorded
(as described in chapter 3).**

Replicate tank	Weight (g)	Fork length (mm)
1	744	370
1	1010	430
1	1500	470
2	970	410
2	1080	435
2	970	411
3	1043	427
3	725	395
3	1210	420

Appendix B: Meristics and treatment designation of fish from which EMT signals were recorded (as described in chapter 4).

Treatment	Weight (g)	Fork length (mm)
non-rested	1021	472
non-rested	830	389
non-rested	1503	465
non-rested	1387	435
rested	1806	520
rested	1686	475
rested	796	385
rested	1026	390
rested	1560	465
rested	978	410

Appendix C: Meristics and treatment designation of fish from which EMT signals were recorded (as described in chapter 5).

Treatments are listed as: "instant-on" = 1; "phased" = 2

Replicate	Treatment	Weight (g)
1	1	1360
1	1	1256
1	1	1016
1	1	1296
1	2	1175
1	2	1220
1	2	1310
1	2	975
2	1	1150
2	1	1210
2	1	1375
2	1	950
2	2	1405
2	2	1095
2	2	1080
2	2	970
2	2	1230
3	1	1300
3	1	1485
3	1	1210
3	1	985
3	2	1050

3	2	1100
3	2	1200
3	2	1005
4	1	1035
4	1	1340
4	1	1060
4	1	1220
4	2	1375
4	2	1410
4	2	1295
4	2	1230
4	2	980
5	1	1200
5	1	1235
5	1	1340
5	1	925
5	1	1275
5	2	1170
5	2	970
5	2	1395
5	2	1160
6	1	1270
6	1	1090
6	1	975
6	1	1210

6	1	1050
6	2	1260
6	2	1370
6	2	1290
6	2	1120
6	2	1010
6	2	990
6	2	1065

**Appendix D: Selection criteria for responses featured in tables 5.3 and 5.4:
EMT signals (ms) of "Fast-start" swimming.**

Replicate #	1	2	4	5	8
Average Pre "lights-on" EMT value (i.e. pre-dawn)	1904.88	1707.118	2073.135	1788.67	2243.18
Continuously recorded EMT signals (ms) of "Fast-start" swimming during the first 30 seconds after "lights-on"	1868.1	1848	2111	1938	2308
	1070	1443	2157	1676	2201
	1201	1380	2144	1677	1880
	1573	1400	2137	1296	1429
	1095	1129	2104	1546	1710
	1887	1239	1803	1108	2091
	1873	1293	1292	1799	2182
	2016	1482	1730	1774	1803
	1860	1728	1462	1540	2005
	1767	1622	1258	1282	1716
	1517	1425	1551	2117	2009
	1712	1538	1628	1702	2128
	1083	553	1362	1372	1836
	1387	1076	1028	1241	1920
	1122	1638	1229	1123	1885
1506	1597	1422	1589		
1019	1238	1561	1917		
1330	1338	1962.8	1599		
Average of EMT signals during the first 30 seconds of "lights-on"	1493.672	1395.421	1624.84	1572	1940.2

EMT values shown are representative of "fast-start" swimming responses (source: data from Chapter 3). On average, EMT signals during the "lights-on" period were 338 ± 14 (ms \pm SEM) lower than pre "lights-on" EMT signals (i.e. activity spikes). The average recording time, spanning the dark-light transition periods (Table 5.3 and 5.4), was 14 - 15 seconds. An average occurrence of 3 "activity spikes" is present during the first 14 to 15 seconds of "fast-start" EMT recordings (above table). Therefore, during the dark-light transition periods recorded in lighting regimen trials (Chapter 5), an occurrence of 3 or more "activity spikes" was interpreted as being indicative of "fast-start" swimming.