BEHAVIOURAL AND PHYSIOLOGICAL RESPONSE OF OVERWINTERING BROOK TROUT (*Salvelinus fontinalis*) TO INSTREAM FLOW MANIPULATIONS FROM THE CANADIAN ROCKY MOUNTAINS

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Abstract

As anthropogenic demands on water resources intensify instream flow needs are becoming an increasingly important area of study, particularly over winter months during which time little is known about the behaviour and physiology of fishes. This thesis addresses the implications of water withdrawal from a small *in situ* stream on brook trout (*Salvelinus fontinalis*) during the winter of 2007 and 2008 in the Rocky Mountains. Water was withdrawn from one of two stream enclosures reducing the discharge by 50% and 75%, for 4hrs daily. Behaviour was monitored using radio transmitters that were externally attached to the small trout. Changes in physiology were monitored by measuring stress hormone levels and by measuring predicted body composition parameters using bioelectrical impedance analysis. Trout reacted to water withdrawal by being more active, but this change in behaviour did not elicit detectable changes in physiology.

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1.1 Objectives

The intention of this literature review is to consider instream flow needs (IFN) of overwintering stream resident fish populations and means by which to quantify their behaviour and physiology. The following five objectives will be investigated in this chapter:

1) Review instream flow needs

2) Identify the feeding, movement and habitat preferences of overwintering fish

3) Review the utility of cortisol as an indicator of stress

4) Review the use of radio telemetry to determine fish behaviour and habitat preferences

5) Review the use of bioelectrical impedance analysis (BIA) as a non-lethal means by which to measure proximate body composition of fish

1.2 Instream Flow Needs

1.2.1 Overview

The harnessing of water systems has sacrificed the ecological integrity and self sustaining productivity of countless rivers (Poff *et al.*, 1997). Only a small fraction of America's rivers remain free-flowing (Benke, 1990). Because of the widespread impacts that anthropogenic activity can have on water flow, it is pertinent to understand and

evaluate IFN in making water management decisions to accommodate economic use while protecting ecosystem functions (Poff *et al.*, 1997).

IFN is a flow determination that is developed to meet a stated ecosystem objective. There are many methods used to facilitate the development of IFN prescriptions in use today but they are often limited by their relative simplicity (Castleberry *et al.*, 1996). In order to formulate a holistic concept of IFN, it is important to develop a spatial and temporal framework that evaluates the structure and function of the riverine system (Annear *et al.*, 2004). Changes in flow affect the natural flow regime, biotic and abiotic diversity, and connectivity of rivers, and this ultimately affects the behaviour and physiology of stream resident fish populations.

The following section will provide a brief overview of IFN, including its relevance, the implications of altering flow regime, tools and techniques to determine IFN, the challenges and shortcomings of developing IFN parameters, and related research on the behavioural and physiological responses of fish to changes in flow.

1.2.2 Relevance of Addressing Environmental Flow Needs

Human modification of freshwater resources has, and continues to have, widespread detrimental impacts on aquatic ecosystems throughout the world (Freeman & Marcinek, 2006; Maddock, 1999). As human populations grow and per capita water consumption increases, it is predicted that anthropogenic effects will continue to intensify in the coming years (Richter *et al.*, 2006); in addition, Gibson *et al.* (2005) predict that inconsistent flow caused by anthropogenic manipulations are likely to be compounded by climate change trends.

There are five components of the flow regime: magnitude, frequency, duration, timing, and rate of change (Poff *et al.*, 1997). The artificial regulation of river flow can alter these components, threatening the survival of natural processes and species within the river (Poff *et al.*, 1997). Understanding the implications of altering natural flow regimes on aquatic environments is crucial in order to mitigate or reverse the detrimental effects of the flow manipulation (Marschall & Crowder, 1996), in turn ensuring the long term ecological integrity of streams and rivers.

1.2.3 Implications of Regulating Flow

Three dominant aspects of river health that can be affected by regulated flow are: natural variable flow, biotic and abiotic diversity, and connectivity.

Natural variable flow

The regulation of flow regime can threaten stream habitat stability (Bain *et al.*, 1988), not strictly because of increasing or decreasing flows, but because of the unpredictable nature of the temporal change in flow. Maintaining the natural flow regime variability is crucial in sustaining the ecological health of a river (Poff *et al.*, 1997). Past IFN assessment tools did not consider natural flow patterns, but rather, focused on setting a minimum flow target (Annear *et al.*, 2004; Poff *et al.*, 1997). Meeting a minimum flow requirement is not adequate in maintaining the ecological integrity of a river because many aquatic species depend on hydrological variability (Richter *et al.*, 1997). The life cycles of many aquatic plants and animals have adapted to avoid or exploit natural flow

changes. Alterations to the seasonal timing of flow events can therefore cause these organisms to suffer a fitness cost (Lytle & Poff, 2004).

Biotic and abiotic diversity

Anderson *et al.* (2006) argued that to successfully subscribe IFN for a particular river, it is necessary to have a thorough understanding of the basic aquatic ecology within the river system. Richter *et al.* (2006) support this argument emphasizing the importance of evaluating the inter-relationships between flow and physical habitat, water chemistry, energy supplies, and species interaction, in order to develop flow management restoration recommendations. IFN prescriptions have traditionally focused on the physical and chemical entities of a river, with the assumption that by managing these entities the health of the river system would be maintained; although biological components are being increasingly recognized as crucial in maintaining the health of an ecosystem (Norris & Thoms, 1999). Flow recommendations cannot address the needs of a single species or issue alone without compromising the river system as a whole. To preserve the viability of the larger river ecosystems, IFN assessments need to include feedback among both biotic and abiotic components, and connectivity between upstream and downstream habitats (Anderson *et al.*, 2006).

Connectivity

Change in flow has the potential to disrupt longitudinal and lateral connectivity of river systems, influencing aquatic species' ability to move freely (Bunn & Arthington, 2002). Longitudinal barriers can disrupt the migration paths of fish potentially inhibiting

their ability to complete their lifecycle (Bunn & Arthington, 2002). The restriction of lateral expansion of flow onto flood plains inhibits the contribution of organic matter inputs and nutrients (Norris & Thoms, 1999), and limits the development of new habitats including secondary channels and oxbow lakes (Richter *et al.*, 2006).

1.2.4 Instream Flow Assessment Tool and Techniques

As anthropogenic activity is increasingly changing water systems, there is a heightened awareness of the importance of developing a more holistic concept of IFN that closely mimics the natural hydrograph (Jeffres *et al.*, 2006). IFN have traditionally been defined on the basis of meeting the needs of one particular species or issue (Acreman & Dunbar, 2004). Evaluating IFN has since matured to take a more holistic look at incremental methods in which aquatic habitat viability is assessed (Anderson *et al.*, 2006; Maddock, 1999; Poff *et al.*, 1997; Stalnaker *et al.*, 1995). An emphasis is now being placed on the conceptual strength of incorporating site specific knowledge to determine flow allocations (Acreman & Dunbar 2004); however it is important to consider the uncertainties associated with different instream flow methods (Castleberry *et al.,* 1996; VanWinkle *et al.*, 1997).

There are a number of methods used to help develop IFN parameters. These methods have been assessed in detail (Acreman & Dunbar, 2004; Ahmadi-Nedushan *et al.*, 2006; Annear *et al.*, 2004; Annear & Conder, 1984; Bain *et al.*, 1988; Cavendish & Duncan, 1986; Hardy, 1998; Irvine *et al.*, 1987; Jowett, 1997; Orsborn, 1977; Richter *et al.*, 1997; Richter *et al.,* 2006; Tharme, 2003), but for the purpose of this chapter, only a very brief description will be given of the most commonly used methods.

Reiser *et al.* (1989) surveyed agencies in North America to determine what methods were most commonly used to determine IFN. At that time, the preferred methods used in Canada were the Tennant method and the Instream Flow Incremental Methodology (IFIM). The Tennant method is a hydrological method that sets minimum flow requirements based largely on historical flows (Tennant, 1976). This method has been criticized as being overly simplistic (Anderson *et al.*, 2006; Stalnaker *et al.*, 1995) and is consequently being phased out and replaced with other hierologically based assessment methods including the Natural Flow Paradigm (Poff *et al.*, 1997), the Range Variability Approach (Richter *et al.*, 1998), the Dundee Hydrological Regime Assessment Method (Black *et al.*, 2005), and Indicators of Hydrologic Alteration (Richter *et al.*, 1996).

IFIM takes an incremental approach to evaluating IFN incorporating hydrology, as well as biology, water quality and connectivity (Annear *et al.*, 2004; Bovee, 1982) and remains a dominant tool for predicting flow requirements (Sale & Otto, 1991). IFIM was developed by the US Fish and Wildlife Service as a method in which to quantify the physical aquatic habitat as a function of stream discharge over time and space (Stalnaker *et al.*, 1995). IFIM often incorporates the Physical Habitat Simulation System (PHABSIM) which is a computer based model that simulates hydrologic habitat suitability for target species. PHABSIM's underlying assumption is that the predicted Weighted Usable Area (WUA) is linearly related to fish abundance (Nuhfer & Baker, 2004). These assessments produce a flow versus physical habitat relationship within a specified reach for the target species (Maddock, 1999).

1.2.5 Evaluating IFN for Fish: Challenges and Shortcomings

The evaluation of IFN can be onerous because of the complexity of water systems coupled with a limited comprehensive knowledge base in defining river health. IFN studies need to develop this knowledge base by studying how changes in stream flow affect all involved species and life-stages, throughout all seasons, by acquiring site-specific knowledge that will ultimately address implications for the river system as a whole.

Addressing all affected species

Instream flow incremental methodology has been criticized for its tendency to focus on flow requirements for 'important' fish species, namely imperiled or game species (Acreman & Dunbar, 2004; Poff *et al.*, 1997). Freeman and Marcinek (2006) emphasize the importance of quantifying the responses of a range of aquatic fauna to flow alterations. Because IFIM focuses strictly on physical habitat as the limiting factor (Sale & Otto, 1991), this method can be deficient if the target species and life stage do not have specific or measurable habitat requirements (Hardy, 1998).

Considering implications across all seasons

The extent to which winter water extraction affects aquatic habitat is relatively understudied (Brown *et al.*, 2001; Cunjak, 1996; Heggenes & Dokk, 2001; Huusko *et al.*, 2007). Changes in flow during winter months may not be as well researched as fluctuations during other months simply because there is not as much awareness of aquatic habitats at this time of the year, and because it can be more difficult to undertake

field research in the winter months because of the restrictions of winter conditions, namely ice cover. Alfredsen and Tesaker (2002) drew attention to the fact that more winter research is critical because winter may be the limiting period for fish production in a water body, particularly in steep or small streams.

It is often assumed that summer IFN also apply to winter; although little is actually known about the ecology of riverine environments during winter months. There is a general lack of understanding of the behaviour and habitat needs of overwintering fish (Annear *et al.*, 2002). In order to build a successful IFN management system it is important to have information of summer and winter fish behaviour (Rimmer *et al.*, 1983). Baltz *et al.* (1991) expressed the importance of including seasonal shifts in microhabitat use into instream flow models.

The differences in IFN of fish over summer and winter are likely related to priority shifts. During winter months feeding becomes a means to sustain a minimal level of metabolic activity not for growth, as in summer (Cunjak & Power, 1987b). Access to food in the winter at near freezing temperatures is likely a low priority habitat selection criterion for most stream resident species, instead habitat is selected primarily on the need to circumvent adverse instream conditions (Cunjak, 1996). In the summer there is less emphasis on minimizing risks, instead there is an emphasis on maximizing benefits. Summer discharge and nutrient level has been found to be positively related to growth rates of adult Artic grayling (*Thymallus arcticus*) (Deegan *et al.*, 1999). Reduced summer discharge caused by drought has been shown to cause deterioration in condition of brook trout (*Salvelinus fontinalis*), related to spatially-limited food resources (Hakala & Hartman, 2004). Anthropogenic flow reduction has also been found to reduce brook trout

growth rate in the summer coinciding with a reduction in input of invertebrate drift to pools (Harvey *et al.*, 2006), demonstrating that high provision habitats are important for summer growth.

Incorporating site-specific knowledge

Frimpong *et al.* (2005) affirmed that it would be beneficial for managers to be able to evaluate streams without field measurements or observations; however, because of the complexity and variability of water systems, it is important to have a comprehensive understanding of the ecology of individual streams (Anderson *et al.*, 2006). Many factors need to be considered in defining the health of a river ecosystem including: discharge, structure of channel and riparian zone, water quality, channel management, level of exploitation, and barriers to connectivity (Acreman & Dunbar, 2004). It is difficult to effectively evaluate all of these components without physical observations. Acreman and Dunbar (2004) noted the importance of incorporating site-specific knowledge in determining IFN, although they recognized that this knowledge may not be readily available or consistent among biologists, hydrologists, historians, and local stakeholders.

Evaluating the entire river system

The evaluation of IFN entails considering the interplay of many variables that can be influenced over large areas. Because of the nature of ecosystems the dynamic ecological roles of flow components must be considered in order to develop a holistic description of IFN. Changing flow regime can ultimately disrupt components of the

aquatic ecosystem directly and indirectly. Indirect influences can be seen as the ripple

effects of the direct influence of water withdrawal; for instance reducing flow in the

summer can result in an increase in water temperatures (Richter *et al.*, 2006), which can

force cool water species to move to cooler habitats. The extent of the indirect disruption

is difficult to quantify because the repercussions of a single alteration to a river can

spread, temporally and spatially, over an unknown time and distance. Anderson *et al.*

(2006) concluded that the temporal and spatial dynamic feedbacks between system

components cannot be adequately captured in commonly used management methods.

1.2.6 Research on Behavioural and Physiological Responses to Changes in Flow

The effects of flow fluctuations on fish has predominantly been studied through

the monitoring of behavioural and physiological changes of local fish populations

(Armstrong *et al.*, 1998; Arnekleiv *et al.*, 2004; Berland *et al.*, 2004; Flodmark *et al.*,

2002; Freeman *et al.*, 2001; Halleraker *et al.*, 2003; Harvey *et al.*, 2006; Heggenes, 1988;

Hickey & Diaz, 1999; Robertson *et al.*, 2004; Shirvell, 1994; Vehanen *et al.*, 2000).

Manipulating flow has been most commonly studied in relation to hydropeaking where

the natural flow regime is disrupted by both increasing and decreasing discharge (Berland

et al., 2004; Bunt *et al.*, 1999; Curry *et al.*, 1994; Freeman *et al.*, 2001; Jeffres *et al.,*

2006; Robertson *et al*., 2004; Saltveit *et al.*, 2001; Scruton *et al.*, 2005; Valentin *et al.*,

1996; VanWinkle *et al.*, 1997).

Behavioural changes induced in fish by changes in flow regime

Jeffres *et al.* (2006) tracked the movement of Saremento sucker (*Catostomus occidentalis*) and hitch (*Lavina exilicauda*) in the spring in a large river that was regulated by a hydro dam. They found that the suckers showed a response to the changes in flow, whereas the hitch remained relatively stationary, demonstrating that some species are more sensitive to changes in flow than others. Robertson *et al.* (2004) simulated a hydropeaking operation and found that the only detectable difference in activity of Atlantic salmon parr (*Salmo salar*), elicited by the increased flow, was reduced nocturnal activity during winter indicating that effects of hydropeaking can vary dielly.

Heggenes (1988) studied the effects of discharge on brown trout (*Salmo trutta*) in a small stream during summer months and discovered that emigration increased slightly with an increase in discharge but habitat use did not change. In contrast, Pert and Erman (1994) found that rainbow trout (*Oncorhynchus mykiss*) habitat preferences shifted to deeper and faster water during short term increases in discharge from a hydropeaking operation in the summer. They also found that individuals within the population responded differently, which emphasizes the importance of not assuming that the most used habitat is the optimal habitat for the species as a whole because optimal habitat is often subjugated by a few dominant individuals (Pert & Erman, 1994).

Robertson *et al.* (2004) monitored the stranding of Atlantic salmon parr after high winter water discharges. They determined that as water levels were reduced back to base flows, stranding rates were very low. Halleraker *et al.* (2003) also studied the stranding of fish.

They examined factors that affected the stranding of brown trout by manipulating the dewatering of an artificial stream and concluded that stranding rates are lower in warmer temperatures, in darkness, and with a slower rate of water removal; whereas Saltveit *et al*. (2001) concluded that the stranding of salmon parr and brown trout was most common during daytime in winter, perhaps because of reduced swimming capacity in the winter.

Physiological changes induced in fish by changes in flow regime

Alfredsen and Tesaker (2002) argued that reduced flow can have maladaptive effects on feeding, swimming capacity, and competition between fish. For instance, rainbow trout that were in small streams in which summer flows were reduced by 75-80% for six weeks had significantly slower growth rates than the control fish, although survival rates were not affected within the sampling period (Harvey *et al.*, 2006). In addition to changes in growth rates, cortisol levels have also been studied to help determine stress levels in fish exposed to reduced rates of flow. Arnekleiv *et al.* (2004) studied brown trout exposed to a single dewatering in an artificial stream and found that cortisol levels increased substantially during dewatering but returned back to baseline within a 24hr period. Flodmark *et al.* (2002) identified a similar cortisol peak response in juvenile brown trout; in addition, they determined that after repetitive daily cyclical fluctuations by the fourth day of dewatering cortisol levels returned to base levels. Chronic stressors do not maintain cortisol at peak levels despite the continued administration of the stressor (Wendelaar Bonga, 1997). The fish may still be responding to the stressor but cortisol levels can no longer be used as a reliable indicator, instead the

implications of the stressor will be apparent in other physiological ways, including growth rates (Mommsen *et al.*, 1999).

1.2.7 Summary

Alterations of the quality and timing of river flow can cause critical changes to the ecological integrity of a river system (Poff *et al.*, 1997). Anthropogenic influences are changing the duration, timing, and magnitude of flows. Because of the naturally variable and interlinked nature of aquatic ecosystems, evaluating IFN is multifaceted. Most instream flow assessment techniques incorporate one or more flow components of riverine ecology (hydrology, geomorphology, water quality, connectivity and biology) but the expertise to evaluate all components is seldom available. For the ease of resource managers, the ideal method for determining flow requirements would be straightforward and standardized, but defining IFN parameters is inherently complex and multidimensional, analogous to the nature of rivers themselves. Challenges in addressing IFN can be exacerbated by a paucity of knowledge of how riverine components are affected by changes in flow. "Scientists need to develop and implement monitoring methods that will realize the potential of adaptive management, and develop the basic biological knowledge that will provide a more secure foundation for decisions that must balance instream and consumptive uses of water" (Castleberry *et al.,* 1996, p. 21). The further study of IFN is imperative in order to provide a more comprehensive understanding of the implications of altering flow and, in turn, building a foundation of knowledge for resource mangers to draw from.

1.3 Overwintering Stream Resident Fish

1.3.1 Overview

There is scarcity of knowledge regarding the behaviour and habitat preferences of overwintering fish (Brown *et al.*, 2001; Cunjak, 1996; Heggenes & Dokk, 2001; Huusko *et al.*, 2007), despite the fact that winter may be a restrictive time of the year for fish production (Alfredsen & Tesaker, 2002; Annear *et al.*, 2002; Jackson *et al.*, 2001). Extrapolating from the limited research that has been conducted, it is apparent that there is a shift in behaviour in stream fishes during winter months. The following section will provide an overview of overwintering stream fishes, including winter feeding, habitat preferences, activity levels and risk of mink predation.

1.3.2 Winter Feeding

Fish have a reduced capacity to process food in the winter. Winter feeding is restricted because low water temperatures limit digestion and reduce gastric evacuation rates (Cunjak *et al.*, 1987). Stomach analysis of stream resident brook trout and brown trout indicate that, despite the reduced capacity, they do continue to feed throughout the winter (Cunjak & Power, 1987b; Simpkins & Hubert, 2000), albeit at reduced levels (Keast, 1968). Trout are generally visual feeders; therefore surface ice can also cause reduced winter feeding because of restricted light penetration (Annear *et al.*, 2002). A limited food supply resulting from a decrease in the number of drifting invertebrates may also inhibit winter feeding (Bjornn, 1971; Waters, 1962). During periods of low water temperature salmonids primarily rely upon stored fat (Pottinger *et al.*, 2003).

Annear *et al.* (2002) found a significant decline in trout weight despite continuous feeding throughout the winter study period. Cunjak and Power (1987b) also reported a decrease in condition factor in brook and brown trout from early winter to spring. Younger salmonids have been shown to deplete their lipid reserves disproportionately faster than older fish (Mason, 1976). Cunjak *et al.* (1987) determined that there was a loss in lipid content of brook trout over the winter, but the loss was not to the extent that protein stores had to be mobilized; however early winter energy depletion of lipids may limit endurance with exposure to atypical environmental perturbations (Cunjak *et al.*, 1987). Webster and Hartman (2007) hypothesized that declines in overwintering brook trout populations were related to mortality caused by decline in energy stores, but discovered that mean fat levels changed very little over winter and that the declining populations were more likely related to the activity of anglers or to emigration from the system.

1.3.3 Winter Habitat Preferences

Overwintering strategies of riverine trout are dependent on stream morphology, local hydrology, and life stage (Cunjak & Power, 1986b). Winter habitat selection focuses on physical habitat features that lessen energy expenditures (Cunjak, 1996; Heggenes *et al.*, 1993) and that circumvent adverse instream conditions (Cunjak, 1996). Ideal winter salmonid habitats include a combination of slow velocity water (Alfredsen & Tesaker, 2002; Baltz *et al.,* 1991; Chisholm *et al.*, 1987; Huusko *et al.,* 2007), deep water (Annear *et al.,* 2002; Cunjak, 1996; Hartman, 1965; Muhlfeld *et al.*, 2001; Tschaplinski & Hartman, 1983), and overhead cover (Bustard & Narver, 1975; Cunjak & Power,

1986b; Heggenes *et al.*, 1999; Huusko et al., 2007; Jakober *et al.*, 1998). Cunjak and Power (1987a) suggested that in small streams, deep water may not be a primary winter habitat selection criterion in itself but rather is selected because of its associated cover and/or low water velocities. Muhlfeld *et al.* (2003) recognized the characteristics of preferred daytime winter habitat but cautioned resource managers to consider the importance of more complex habitats during winter to include shallow areas along stream margins that fish may use as night habitat, or that juvenile salmonids may prefer (Griffith & Smith, 1993). It is important to preserve complex habitat because the relative value of winter habitat varies between fish size and species, in addition habitat suitability is not static over the course of the winter, varying between the cooling of early winter, mid winter and the warming of late winter (Cunjak, 1996).

"Winter-warm" microhabitats are found in areas of groundwater discharge and can provide a thermal refugia from ice events (Cunjak, 1996; Cunjak & Power, 1986b). Brown and Mackay (1995) demonstrated that in the winter cutthroat trout moved to areas less affected by anchor and frazil ice, namely deep pools and areas where the water temperature was warmer. Cunjak and Power (1986b) found trout in large aggregations in areas of groundwater discharge where water was warmer. Preference of warm habitats varies between species; brown trout prefer to aggregate downstream from ground water discharge whereas brook trout prefer to be close to the point source of groundwater discharge (Cunjak, 1996).

1.3.4 Winter Movement

Upon finding favourable habitat, stream fishes tend to remain relatively sedentary over the winter months (Brown, 1999; Chisholm *et al.,* 1987; Hartman, 1963; Jakober *et al.*, 1998; Scruton *et al.,* 2005); although some fish are forced to move if habitats become unstable as subsurface ice accrues (Brown, 1999; Brown & Mackay, 1995; Brown *et al.*, 2001). When winter movement does occur there does not appear to be a particular trend in upstream or downstream movement (Brown & Mackay, 1995). Movement during winter ice events must be energetically costly increasing chances of mortality (Brown, 1999).

In the winter, trout species have been documented to show elevated levels of visual pigment (Allen *et al.*, 1982), facilitating a change in diurnal patterns in which the fish tend to hide in the day and become more active at night (Annear *et al.,* 2002; Heggenes & Dokk, 2001; Huusko *et al.,* 2007; Jakober *et al.*, 2000; Meyer & Gregory, 2000). Preferred night habitat typically includes low velocity water with fine substrate close to river banks (Heggenes *et al.,* 1993). Muhlfeld *et al.* (2003) found that in the winter bull trout moved from day to night locations, a median distance of 86m, from deep water with cover to shallower water at dusk. Ovidio *et al.* (2002) determined that in the winter brown trout were the most active at dusk.

Fish exhibit less territorial behaviour in the winter than in other seasons (Brown & Mackay, 1995; Cunjak & Power, 1986b; Hartman, 1963; Heggenes et al., 1993) and are more likely to form aggregations (Brown, 1999; Brown & Mackay, 1995), which tend to increase in size with decreasing water temperatures (Brown & Mackay, 1995). Heggenes *et al.* (1993) suggest that fish populations may be more regulated by abiotic

factors such as habitat availability than by social interactions that play an important role in regulating summer populations. Muhlfeld *et al.* (2001) found that with the existence of adequate winter habitat, namely deep slow moving pools with ample cover, small trout were not forced to migrate at the onset of winter.

1.3.5 Winter Mink Predation

It has been demonstrated that the preferred food of mink (*Mustela vison*) during the winter and spring is fish (Gerell, 1967). Fish may be more vulnerable at this time because of their reduced swimming capacity at low water temperatures. During other seasons crayfish, birds, rodents and amphibians are found to dominate the mink's diet (Gerell, 1967). Heggenes and Borgstrom (1988) found increased mortality of brown trout and juvenile Atlantic salmon when mink were present and they observed mink along the stream bank more often during autumn and winter than in spring and summer. They also found that there was a high mortality rate in small stream fishes because of mink predation, most commonly coinciding with low discharge. Both low flow and limited cover decreases the possibility to avoid mink predation (Heggenes & Borgstrom, 1988). Lindstrom and Hubert (2004) studied winter mink predation on radio tagged fish and found that 8% of the tagged cutthroat (*Oncorhynchus clarki*) and 28% of the tagged brook trout were killed by mink over the winter.

1.3.6 Summary

Patterns of trout activity change in winter and can vary diurnally in different
habitat types (Ovidio *et al.*, 2002). In the winter trout have to allocate their energy
differently by adopting strategies that minimize energy loss (Heggenes *et al.*, 1993).
Mortality caused by winter starvation is unlikely, but rather is more likely caused by
compounding variables such as predation, stress or disease (Annear *et al.*, 2002). In order
for fish to optimize their survival over winter they should perform low risk activities,
have large energy stores, and migrate to areas with favourable habitat (Huusko *et al*.,
2007). Complex mixes of habitat types are important to provide suitable winter habitat
for overwintering trout (Jakober *et al.*, 1998). In the winter there is a reduction in feeding,
aggression, and activity levels of stream resident fishes and a tendency to take refuge in
low velocity habitat to maximize energy conservation, and protected cover to minimize
predation.

1.4 Stress Response in Fish

1.4.1 Overview

Fish are in intimate contact with their environment and as such have lower
threshold to stressors than most mammals (Wendelaar Bonga, 1997). Cortisol is an
important indicator of stress in fish, consequently cortisol levels have been extensively
studied to determine stress responses to a range of circumstances (Barton, 2002). The
majority of studies addressing stress induced changes in cortisol levels in fish have
focused on aquaculture; although other stressors have been studied including exposure to

toxins, and changes in flow regime. The implications of these stressors are largely dependent on the magnitude, duration, and frequency of the stressor (Barton, 2002). The following section will provide an overview of the physiology of stress hormones, the study of cortisol as a stress indicator and the effects of stress on fish including changes in stress response, and its implications.

1.4.2 Physiology of Stress Hormones in Fish

When a fish is exposed to a stressful environmental stimulus the hypothalamic-pituitary-interrenal (HPI) axis initiates a biochemical pathway of reactions resulting in the release of cortisol. Corticotrophin-releasing hormone (CRH) is released from the hypothalamus in the brain signaling for the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary. Circulating ACTH is responsible for stimulating the interrenal cells in the anterior head kidney to synthesize and release cortisol (Barton *et al.*, 2002). Corticosteroid hormones together with catecholamines facilitate the ability of a fish to cope with a stressor in a number of ways: by acting on the vascular system to increase the heart rate and blood flow to the gills, by acting on the immune system to decrease white blood cells, by affecting metabolism decreasing liver glycogen and increasing plasma glucose, and by affecting hydromineral homeostasis increasing water excretion (Hontela, 1998). The principal corticosteroid produced by teleosts is cortisol (Bentley, 1976; Mommsen et al., 1999). Cortisol acts primarily on the gills, intestine, and liver of fishes (Wendelaar Bonga, 1997) and is the principal salt regulating corticosteroid stimulating sodium transport in the kidneys, in the mucosa of the gut, and across the gills (Norris, 1996).

The magnitude of increase in cortisol levels with exposure to stress varies between species (Barton, 2002). Resting levels of cortisol are generally low; <5ng/ml in salmonids. These levels show 10 to 100 fold increases when exposed to a stressor (Wendelaar Bonga, 1997). Because of a delay in the release of cortisol, peak levels may not occur for several minutes after the acute stressor is administered and levels generally come back to resting amounts after one or more hours (Barton *et al.*, 2002). This trend has been documented in a range of species including brook trout (Biron & Benfey, 1994), brown trout (Flodmark *et al.*, 2002) and Atlantic salmon (Einarsdottir & Nilssen, 1996). Chronic stressors can maintain elevated levels of cortisol, although they do not maintain peak levels despite the continued administration of the stressor (Wendelaar Bonga, 1997). Mommsen *et al.*(1999) demonstrated that cortisol levels often drop back to base levels, regardless of the fact that the fish is still responding to the stressor.

1.4.3 Cortisol Levels as an Indicator of Stress

Circulating cortisol levels have been extensively used as indicators of stress in fish for three reasons: firstly, cortisol levels can be measured with relative accuracy and ease, through tools such as the commercially available radioimmunoassay kits; secondly, using proper sampling techniques, blood samples can be obtained without contributing to stress levels; thirdly, cortisol levels usually increase as acute stress increases (Mommsen *et al.*, 1999).

The majority of studies that have used cortisol to interpret stress response in fish have focused on aquaculture, addressing issues such as genetic predisposition within and among species and vulnerability to stressors such as handling and crowding (Barton &

Iwama, 1991; Haukenes & Barton, 2004; Jentoft *et al.*, 2005; North *et al.*, 2006; Pickering, 1993; Trenzado *et al.*, 2006). Studies have also addressed cortisol stress response to toxicants (Brodeur *et al.*, 1998; Gagnon *et al.*, 2006; Hontela, 1998; Hontela *et al.*, 1992; Levesque *et al.*, 2002; Tort *et al.*, 1996). There has been a limited number of experiments that examine cortisol levels in response to changes in flow regime (Arnekleiv *et al.*, 2004; Flodmark *et al.*, 2002).

Flodmark *et al.* (2002) studied brown trout in an artificial stream channel and associated high peaks of cortisol with the reduction of flow; although the long term cortisol response to daily fluctuations showed rapid habituation to this stressor. The highest peaks were noted two hours following 30 minutes of dewatering. Pre-exposure rates of cortisol were achieved six hours after the stressor was administered. By using an artificial stream Flodmark *et al.* (2002) were able to control many extraneous variables, although the lab setting had limitations, including a restricted channel length (21m) and potential overcrowding of fish within the constrained area. Arnekleiv *et al.* (2004) used a similar method as that used by Flodmark *et al.* (2002) and verified a spike in cortisol levels with dewatering followed by a return to resting levels of cortisol. Arnekleiv *et al.* (2004) suggest that in order to establish more reliable conclusions regarding fluctuations in cortisol levels, it would be beneficial to repeat the dewatering method but instead of using artificial streams, cortisol levels should be monitored in fish in their natural habitats over different seasons.

1.4.4 Stress and its Effects on Fish

> *Stress responses*

The intensity level of a stressor that a teleost responds to is far lower than can be detected by terrestrial animals (Wendelaar Bonga, 1997). The response to a stressor can vary depending on its magnitude and duration. Barton (2002) described three interrelated responses of stress in fish: primary, secondary, and tertiary response. The primary response is marked by an increase in catecholamines and corticosteroids. The secondary response is marked by metabolic and cellular changes, as well as osmoregulatory disturbance and changes in immune function. The tertiary response is related to changes in the whole animal, including growth, resistance to disease, and behaviour (Barton *et al.*, 2002).

> *Implications of stress*

Barton (2002) argued that stress itself is not detrimental to the fish; it is not until stress levels start to compromise physiological response mechanisms that the health of the fish is put at risk. Acute responses to stressors may extend the fish's normal adaptive ability. In contrast, chronic stress can inhibit the fish's performance and threaten their survival (Davis, 2006). Wedelaar Bonga (1997) explains that the stress response to chronic stress can lose its adaptive value and become dysfunctional. This can result in tertiary maladaptive responses such as reduced capacity for growth, inhibited reproduction and decreased immunocompetence, increasing susceptibility to disease (Barton & Iwama, 1991; Schreck, 2000), including bacterial and fungal pathogens (Pickering & Pottinger, 1989).

1.4.5 Summary

Studies examining cortisol levels in fish have used a wide range of experimental approaches to contribute to the overall understanding of stress in fish. They have demonstrated that the action of cortisol is not consistent among all species of fish, or even within a species. Cortisol levels are affected by different types, magnitudes, and rates of stressors, and cannot always be used as an exclusive indicator of stress.

Stress responses allow terrestrial and aquatic vertebrates to cope with a stressor in order to maintain homeostasis (Barton, 2002); although, there can be a metabolic cost associated with stress, which is most notable in prolonged or repeated stress (Barton & Iwama, 1991). Chronic stress can lead to the impairment of growth, reproduction, and development (Schreck, 2000). As human populations grow, it is inevitable that anthropogenic effects on aquatic habitat will intensify in the coming years (Richter *et al.*, 2006), potentially increasing the frequency and severity of a range of stressors in fish. It is important to continue to study the effects of these stressors on fish in order contribute to an overall holistic understanding of the implications of altering aquatic environments.

1.5 Radio Telemetry

1.5.1 Overview

Radio telemetry has been used in a wide range of fisheries research to study the location, behaviour, and physiology of free-ranging fish (Winter, 1996). It allows for the continuous monitoring of fish from a passive perspective (Bridger & Booth, 2003) and can be applied over a wide range of species and size classes. The type of transmitter

selected and how it is attached is a significant consideration in telemetry studies, because the presence of the transmitters may adversely affect the fish, potentially causing a change in their behaviour and physiology. There are some limitations associated with different transmitter attachment methods as well as the transmitter size selected. The following section will provide an overview of telemetry in fisheries research, methods of transmitter attachment, including internal and external attachment, and means to determine optimal tag/body weight ratio of transmitters.

1.5.2 Telemetry in Fisheries Research

Radio telemetry provides a means to pinpoint individual fish location at any given time providing immediate positional and behavioural data (Alfredsen & Tesaker, 2002; Lucas & Baras, 2000; Murchie *et al.*, 2008). Studies using telemetry allow for the collection of a large amount of data, albeit on a limited sample size because of cost restrictions. Radio telemetry has been used to determine various aspects of behaviour and physiology including: responses to hydropeaking (Berland *et al.*, 2004; Bunt *et al.*, 1999; Robertson *et al.*, 2004; Scruton *et al.*, 2003; Scruton *et al.*, 2005), seasonal variation in movement and habitat preferences (Annear *et al.*, 2002; Bettinger & Bettoli, 2004; Bridger *et al.*, 2001; Brown & Mackay, 1995; Brown *et al.*, 2001; Curry *et al.*, 2002; Jakober *et al.*, 1998; Muhlfeld *et al.*, 2001; Ovidio *et al.*, 2002), diurnal shifts of habitat preferences (Muhlfeld *et al.*, 2003; Young, 1995), and spawning migration behaviour (Arnekleiv & Ronning, 2004; Cooke *et al.*, 2006a; Cooke *et al.*, 2006b).

1.5.3 Methods of Transmitter Attachment

Transmitters can be attached in three ways: surgical implantation in the peritoneal cavity, intragastric insertion, or external attachment.

Surgical implantation

Surgical implantation of transmitters has been employed in numerous studies (Bauer & Schlott, 2006; Berland *et al.*, 2004; Bettinger & Bettoli, 2004; Brown & Mackay, 1995; Brown *et al.*, 2001; Brown *et al.*, 2000; Bunt *et al.*, 1999; Curry *et al.*, 2002; Jakober *et al.*, 1998; Muhlfeld *et al.*, 2001; Muhlfeld *et al.*, 2003; Ovidio *et al.*, 2002; Robertson *et al.*, 2004; Scruton *et al.*, 2003; Scruton *et al.*, 2005; Young, 1995). With the exception of the antenna, the bulk of the surgically implanted transmitter is contained internally, thus the transmitter cannot be entangled in vegetation, is less likely to cause abrasion than the external transmitter, and will not cause drag (Winter, 1996). Surgically implantation keeps the transmitter near the fish's centre of gravity (Bridger & Booth, 2003) so it does not encumber the fish's balance. Bridger and Booth (2003) suggested using this method for long term studies (>20 days).

The principal limitations of surgical implantation are that it takes longer than the other methods, requires a longer recovery period and is more likely to cause infection (Winter, 1996). It also requires more skill and practice to execute than the other two methods (Winter, 1996). It is possible for transmitters to be lost through the incision if suture threads come loose, or to be expelled through the anus or body wall (Bridger & Booth, 2003).

Intragastric implantation

Intragastric insertion has been applied in some research studies (Cooke *et al.*, 2006a; Mellas & Haynes, 1985; Ramstad & Woody, 2003), but is not as commonly used as surgical implantation. The limitations of intragastric insertion are that transmitters can be difficult to insert, can rupture the esophagus or stomach (Winter, 1996) and are often regurgitated after implantation (Mellas & Haynes, 1985). Feeding can decline with intragastric insertion potentially because of decreased available stomach volume (Bridger & Booth, 2003).

Like surgical implantation, gastric implantation keeps the transmitter near the centre of gravity of the fish (Bridger & Booth, 2003). Using this method transmitters cannot be entangled in vegetation, they are less likely to cause abrasion, and will not cause drag (Winter, 1996). Unlike surgical impanation, intragastric implantation is a quick procedure that requires very little habituation time (Winter, 1996). Mellas and Haynes (1985) found that gastric insertion was the most successful method of transmitter attachment in white perch (*Morone americana*). Jepsen *et al.* (2001) studied cortisol levels in chinook salmon (*Oncorhynchus tshawytscha*) smolts after surgical and gastric implantation of radio transmitters and found that 24 hours after implantation both groups had higher cortisol levels than the control group; although seven days after implantation cortisol levels were back to baselines. There was no clear indication that surgical implantation was more or less stressful than gastric insertion.

External attachment

External attachment has been used in select research studies (Arnekleiv &
Ronning, 2004; Herke & Moring, 1999), as they can be quickly attached (Cooke, 2003),
with a rapid recovery. The use of external transmitter facilitates studies on small fish or
early life stage when the size of the body cavity precludes the use of internal transmitters
(Cooke, 2003). The method can also be used on fish that are spawning or feeding
(Winter, 1996).

A significant drawback to using an external attachment method is that the
transmitters can increase drag on fish potentially affecting swimming speed (Steinhausen
et al., 2006; Winter, 1996) and can result in greater energy expenditures and shorter
exhaustion times (Mellas & Haynes, 1985). External transmitters can also become
entangled in aquatic vegetation ultimately increasing mortality (Ross & McCormick,
1981; Thorstad *et al.*, 2001). This method of attachment can cause irritation of the dermal
layers beneath the transmitter (Herke & Moring, 1999; Winter, 1996), however dermal
irritation from external attachment can be minimized by simply reducing the amount of
material that is in contact with the fish (Crook, 2004).

Herke and Moring (1999) successfully attached large external radio tags to
northern pike (*Esox lucius*) without apparent influence on movement behaviour and with
minimal tissue abrasion. Cooke (2003) applied the external attachment method using
small transmitters on rock bass (A*mbloplites rupestris*) to monitor how the presence of
the transmitter affected parental care activity and found negligible effects; concluding
that externally attached radio transmitters provided a viable means in which to study

small fish movement without altering their behaviour. In contrast, Counihan and Frost (1999) found that external transmitters significantly affected the swimming performance of juvenile white sturgeon (*Acipenser transmontanus*). External transmitters will eventually be shed by the fish (Mulcahy, 2003) which is an advantage for the well-being of the fish but may be a limitation if the transmitters are shed before the experiment is completed.

1.5.4 Tag/Body Weight Ratio

Many researchers have set a 2% maximum threshold for tag/body weight ratio based on a general suggestion by Winter (1996). Jepsen *et al.* (2005) caution that it is insufficient to assume that a 2% tag/body weight ratio is adequate across all species and life stages. They also suggested that the size, position and shape of the transmitter must be considered, particularly in external attachment. When selecting transmitter weight it is important to evaluate the buoyancy regulating mechanism of the fish and therefore the ability of the fish to adjust to the additional mass (Jepsen *et al.*, 2005).

Some studies have demonstrated how the behaviour of fish is changed with the attachment of transmitters. Lafrancois *et al.*(2001) found that when the externally attached tag/body weight ratio of sea bass (*Dicentrarchus labrax*) reached 4% available metabolic energy was compromised, therefore they recommended that the tag ratio should be below 2.9% to 4% in order to limit the detrimental effects of tagging. Counihan and Frost (1999) demonstrated that external ultrasonic transmitters significantly affected the swimming performance of juvenile white sturgeon using a tag ratio that did not exceed 1.25%. Ross and McCormick (1981) studied yellow perch (*Perca flavescens*) and

largemouth bass (*Micropterus salmoides*) and also found that the 2% rule of thumb was not adequate, suggesting that transmitter weight, in water, should not exceed 1.5%.

Other studies have demonstrated that transmitters had no notable effect on the experimental fish. Jepsen *et al.*(2001) studied chinook salmon smolts using transmitters that ranged from 1.3 to 3.5% tag/body mass ratio and did not find any indication that it is more stressful for the fish with the higher tag ratios. Brown *et al.* (1999) implanted transmitters into rainbow trout using a larger tag/body weight ratio ranging from a 6 to 12% in air and found that the transmitters did not adversely affect the swimming performance of the trout.

1.5.5 Summary

Radio telemetry enables researchers to determine the long term and long range positioning of individual fish elucidating information about their behaviour and physiology (Lucas & Baras, 2000). Telemetry can provide a means in which to help formulate instream flow needs of stream resident fish based on their behaviour; however it is important to ensure that the transmitter is not affecting normal physiology or behaviour. Mellas and Haynes (1985) cautioned researches in selecting attachment methods of transmitters, suggesting that external, gastric, and surgically implanted transmitters all have associated risks and that a method should be chosen based on what information needs to be collected and the habitat type under study. Mulcahy (2003) suggested using the smallest lightest transmitter that still provides the desired strength and duration.

1.6 Bioelectrical Impedance Analysis

1.6.1 Overview

Bioelectrical impedance analysis (BIA) has been commonly used as a safe, inexpensive rapid and portable method to determine proximate body composition (Kushner, 1992). BIA measures the resistance and reactance of a given substance, providing insight into compositional properties of the substance. Because BIA is a non-lethal measure, it is an important tool in researching changes for body composition. The following section will provide an overview of the principles of BIA, how BIA is measured, and research that has implemented BIA for use on fish.

1.6.2 BIA Principles

BIA measures the conduction of a low current through soluble ions in intracellular and extracellular fluids of the body (Schoeller & Kushner, 1989). BIA can be used to determine proximate body composition because of the differences in impedance between fat mass and fat free mass. In a series pathway impedance is equal to the vector sum of resistance (R) and reactance (Xc); impedance $= (R^2 + Xc^2)^{0.5}$ (Liedtke, 1997).

Resistance is the inverse of conduction. Ohms Law states that resistance is proportional to the voltage drop of an applied current as it passes through a substance [R (ohms) = applied voltage drop (volts)/current (amps)] (Kushner, 1992). Body tissues containing large amounts of water and electrolytes are highly conductive and therefore provide a low resistance pathway; whereas materials such as bone and fat are poor conductors and correspond to high resistance (Liedtke, 1997).

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Reactance, also referred to as capacitive reactance, is a measure of the opposition to alternating current arising from the presence of capacitors within a circuit. A capacitor is made up of two or more conducting plates isolated from each other by a dielectric used to store the charge of electrons (Kushner, 1992). In tissue, reactance is a measure of the opposition to an alternating current by the cell membrane. Cell membranes are composed of a bi-layer of polar proteins and phospholipids separated by a core of nonconductive lipids, that acts as a capacitor (Kushner, 1992). When an electrical signal is introduced into a tissue, a small amount of the signal is leaked through protein channels to charge the inside of the cell membranes. Cell membranes act as capacitors and cause the electrical current to lag behind the applied voltage resulting in a measurable effect determined by the resistance of the dielectric (Liedtke, 1998).

Body composition can be simplified into two components: fat, and fat free mass, where fat includes the extractable lipid mass and fat free mass includes water, protein and minerals that can be further divided into intracellular fat free mass and extracellular fat free mass (Schoeller & Kushner, 1989). Within fat free mass, the aqueous space around the cell acts as a resistor and the cell acts as a capacitor (Liedtke, 1998). By measuring BIA on a biological cylinder (eg. cucumber), He *et al.* (2003) determined that the electrical current does not just pass through the periphery, but rather passes through the entire subject.

Reactance decreases with an increase in the extracellular water (ECW) / intracellular water (ICW) ratio (Segal *et al.*, 1987) and it has been suggested that ECW/ICW ratio increases with malnutrition (Kushner, 1992). Barbosa-Silva *et al.* (2003)

stated that, in humans, malnutrition is typified by changes in cellular membrane integrity and alterations in fluid balance. Generally high reactance indicates better cell membrane integrity and health (Liedtke, 1997). When the cell dies, reactance becomes zero (Liedtke, 1998) indicating a breakdown of cell membrane integrity.

1.6.3 Measuring Instrument

The RJL impedance analyzer (RJL Sytems, Detroit, MI) is manufactured to determine the body composition of human subjects but can be adapted for use on other animals. The RJL impedance analyzer delivers 800μA alternating current at 50 KHz. The device is tetrapolar composed of two signal electrodes which introduce the alternating current and two detecting electrodes which measure the voltage drop, directly measuring the vectors of resistance and reactance in series (Liedtke, 1997).

1.6.4 Use of BIA on Trout

There is extensive literature citing the use of BIA on humans (for a review of BIA use on human subjects see: Kyle *et al.*, 2004) but studies have also been conducted on other animals including wild mammals (Barthelmess *et al.*, 2006; Bowen *et al.*, 1998; Hilderbrand *et al.*, 1998; Hundertmark & Schwartz, 2002; Rutter *et al.*, 1998), livestock (Berg *et al.*, 1997; Daza *et al.*, 2006; Kraetzl *et al.*, 1995; Velazco *et al.*, 1999), and to a lesser extent, on fish (Bosworth & Wolters, 2001; Cox & Hartman, 2005; Duncan *et al.*, 2007; Webster & Hartman, 2007; Willis & Hobday, In Press). The principal concept of BIA is that the tested body is an isotropic conductor with a uniform cross-sectional area

and length (Kushner, 1992). The bodies of most mammals are divided into several cylindrical segments; whereas a fish has a relatively geometric configuration and therefore, BIA can be more easily applied to a fish's body shape.

Initial documentation of the use of BIA in fish was for the purpose of predicting carcass yield and composition of farmed catfish (*Ictalurus punctatus*) (Bosworth & Wolters, 2001) and later in farmed juvenile cobia (*Rachycentron canadum*) (Duncan *et al.*, 2007). Both studies found strong linear relationships between impedance measures and proximate analysis variables: total body water, dry mass, fat-free mass, total body protein, total body ash and total body fat mass. Cox and Hartman (2005) tested the use of BIA to determine the proximate body composition of wild and hatchery brook trout. They also found strong correlations between composition parameters and impedance measurements and validated these measures with a number of other species of fish. Webster and Hartman (2007) applied the regression models built by Cox and Hartman (2005) to measure changes in body composition of overwintering brook trout populations in the Appalachian Mountains in West Virginia as well as to brook trout exposed to winter conditions in a lab. They did not find significant changes in protein or fat composition over the nine week experiment. Willis and Hobday (In Press) used BIA to measure relative condition of bluefin tuna (*Thunnus maccoyii*) during conventional tagging at sea and suggest that composition determined by a impedance index, using reactance, was a good indicator of nutritional health.

1.6.5 Summary

BIA can provide a non-invasive means in which to determine proximate body composition of fish and other animals. By measuring the voltage drop between signal electrodes and adjacent detecting electrodes, direct measures of resistance and reactance of the whole body can be determined. These values of impedance are measures of conduction and capacitance that provide insight into proximate body composition parameters. Proximate body composition parameters that have been determined using BIA measures include: body fat, fat free mass, body protein, body water (ECW and ICW), and more broadly, nutritional health. BIA is a useful tool because the equipment is portable, the procedure is simple, and the results are reproducible (Kyle *et al.*, 2004).

1.7 Conclusion

Instream flow needs are becoming an increasingly important area of study as anthropogenic demands on water intensify. In order to develop a holistic concept of IFN it is pertinent to address hydrology, geomorphology, water quality connectivity and biology (Annear *et al.*, 2004), paying extra attention to areas where a scarcity of knowledge exists. Huusko *et al.* (2007) point to the importance of evaluating the impacts of flow regulation on overwintering salmonids. The ability to effectively study behavioural and physiological responses of stream resident salmonids to reduced winter flows can be facilitated with the use of radio telemetry to locate fish and BIA to measure the proximate body composition of fish.

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2. Behavioural and Physiological Response of Overwintering Brook Trout (*Salvelinus fontinalis*) to Instream Flow Manipulations from the Canadian Rocky Mountains

2.1 Abstract

As human populations grow and water use increases, the anthropogenic effects of manipulating instream flow will continue to intensify. It is pertinent to evaluate and understand instream flow needs (IFN) to effectively manage stream and river systems. Assessing IFN across all seasons, including winter, is necessary in order to provide a holistic understanding of flow manipulations. To date few studies have focused on the effects of water withdrawal on overwintering fish populations. During the winter of 2007 and 2008, the response of brook trout (*Salvelinus fontinalis)* to experimental flow manipulations was studied in a small stream in Kananaskis Country, Alberta. Brook trout behaviour and physiology was monitored, in response to reduced flow, using two *in situ* experimental channels located in a small spring-fed stream. Experimental treatments consisted of a 4hr daily water withdrawal from one of the enclosures, removing 50% of the stream discharge in 2007 and 75% in 2008. Activity levels and habitat preferences of the brook trout were studied using manual radio telemetry. The activity levels of the fish were found to increase when water was withdrawn from the stream and the trout showed an affinity for habitat with deeply undercut banks. In 2007 fluctuations in cortisol and glucose levels were measured to assess stress levels. The fish did not exhibit significantly elevated levels of cortisol or glucose at the end of the six week experiment. In 2008, proximate body composition was measured as a physiological indicator of stress using biological impedance analysis (BIA). There was significant weight loss, predicted total body fat (TBF) loss and predicted total body water (TBW) gain over the duration of the

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winter in all of the fish; however there was no significant difference in predicted TBW or

TBF detected between the control and experimental fish.

2.2 Introduction

The flow regulation of water systems has sacrificed the ecological integrity and

self sustaining productivity of countless rivers (Poff *et al.*, 1997). In order to minimize

impacts of artificial flow manipulation, it is necessary to understand and evaluate

instream flow needs (IFN) to accommodate economic use while protecting ecosystem

functions (Marschall & Crowder, 1996; Poff *et al.*, 1997). Moreover, IFN must be

considered across all seasons (Alfredsen *et al*. 2002). There is a limited understanding of

both the winter habitat requirements for many fish populations as well as the impact of

winter water extraction (Brown *et al.*, 2001; Cunjak, 1996; Huusko *et al.*, 2007). Indeed,

winter may be the limiting period for fish production, particularly in steep or small

streams (Alfredsen & Tesaker, 2002).

Overwintering salmonids require strategies that minimize energy expenditure

(Heggenes *et al.*, 1993; Ultsch, 1989). The energy expenditure associated with swimming

increases with decreased water temperature (Tang & Boisclair, 1995); consequently,

salmonids tend to remain relatively sedentary in the winter (Curry *et al.*, 2002; Hartman,

1963; Ovidio *et al.*, 2002). Winter activity that does occur, has been found to be largely

dependent on habitat stability (Brown, 1999).

Preferred winter salmonid habitats include areas of deep water (Hartman, 1965;

Muhlfeld *et al.*, 2001; Tschaplinski & Hartman, 1983), with low velocity (Baltz *et al.*,

1991; Chisholm *et al.*, 1987; Huusko et al., 2007) and warm temperatures provided by groundwater inputs (Brown & Mackay, 1995; Cunjak, 1996; Power *et al.*, 1999). Overwintering salmonids also show a strong preference for instream cover (Cunjak & Power, 1986b; Heggenes *et al.*, 1999; Huusko *et al.,* 2007). Cover can provide shelter from current as well as a hiding place from predators. Coastal steelhead (*Salmo gairdneri*) have been shown to exhibit 'hiding' behaviour in the winter, in part, as a means of protection from predation (Bustard & Narver, 1975; Hartman, 1965). Predation by mink can be a major mortality factor affecting salmonid numbers in small streams (Heggenes & Borgstrom, 1988). Predation efficiency may vary with stream habitat (Heggenes & Borgstrom, 1988), for instance, overwintering fish may be more susceptible to mink predation in streams that do not become fully covered with ice (Lindstrom & Hubert, 2004), or in small streams with low discharge (Heggenes & Borgstrom, 1988).

Artificial changes in flow threaten habitat stability strongly influencing stream fish community structure (Bain *et al.*, 1988). Change in flow regime has both behavioural and physiological implications for stream fishes. There are a number of studies that have addressed the affects of flow manipulation on stream fishes (Berland *et al.*, 2004; Bunt *et al.*, 1999; Freeman *et al.*, 2001; Harvey *et al.*, 2006; Heggenes, 1988; Jeffres *et al.*, 2006; Pert & Erman, 1994; Scruton *et al.*, 2005; Shirvell, 1994), however there has been limited research on effects of flow manipulations under winter conditions in the field (Robertson *et al.*, 2004; Saltveit *et al.*, 2001) or in the lab (Arnekleiv *et al.*, 2004; Halleraker *et al.*, 2003; Vehanen *et al.*, 2000).

Behavioural responses of stream fishes, to flow manipulation, have been studied by monitoring fish movement and habitat preferences. Scruton *et al.* (2003) demonstrated that artificial alterations to flow affected fish activity levels differently between species, with Atlantic salmon (*Salmo salar*) moving more than brook trout (*Salvelinus fontinalis*). Robertson *et al.* (2004) and Berland *et al.* (2004) found minimal effects of flow manipulation on Atlantic salmon parr; whereas Armstrong *et al.* (1998) found that Atlantic salmon showed an increase in exploratory behaviour during flow reduction. Juvenile brown trout (*Salmo trutta*) movement has been shown to increase in response to simulated high flows, and displacement was greatest during high stream flows at low temperatures in the winter (Vehanen *et al.*, 2000). In contrast, brown trout activity levels have been observed to remain unchanged in response to summer hydropeaking (Bunt *et al.,* 1999). Change in flow has been shown to elicit shifts in habitat preference (Armstrong *et al.*, 1998; Pert & Erman, 1994; Shirvell, 1994).

Physiological responses of stream fishes in response to flow manipulation have been studied by monitoring fish growth, population abundance, and stress levels. Growth rates of rainbow trout (*Oncorhynchus mykiss*) were significantly lowered in the summer when discharge was reduced (Harvey *et al.*, 2006), and an increase in the number of major discharge fluctuations downstream of a dam has been shown to correlate with mortality of winter-stocked trout (Annear *et al.*, 2002). A stress response, indicated by elevated cortisol levels, has often been found to accompany dewatering (Arnekleiv *et al.*, 2004; Flodmark *et al.*, 2002; Thomas *et al.*, 1999); however, cortisol has been shown to return to base levels following four days of repetitive daily dewaterings, despite exposure to continued dewatering events (Flodmark *et al.*, 2002). Chronic stressors do

not maintain cortisol at peak levels despite the continued administration of the stressor (Wendelaar Bonga, 1997). The fish may still be responding to the stressor but cortisol levels can no longer be used as a reliable stress indicator, instead the implications of the stressor may become apparent in other physiological ways, including growth rates (Mommsen *et al.*, 1999).

The effects of flow fluctuations depend on the capability of fish to respond to habitat alterations and their ability to find appropriate refugia (Valentin *et al.*, 1996). The availability of "desirable" space is the major regulator of salmonid density in winter (Chapman, 1966); therefore physiological and behavioural changes in fish caused by alterations in winter flow may be more pronounced in streams that provide less available habitat. The purpose of this study is to provide insight into the relationship between winter flow, fish habitat and individual fish fitness.

2.2.1 Objectives and Hypotheses

The following five objectives and related hypotheses will be investigated in this chapter:

1.) To determine how available habitat will be altered with a reduction in stream discharge.

> **Ha1**: Deep water with deep undercut bank habitat will be reduced and slow
>
> velocity habitat will increase with a reduction in stream discharge.

2.) To determine the preferred habitat of overwintering brook trout.

Ha2: Brook trout will prefer habitat with overhead cover, deep water, and large

substrate.

3.) To determine if overwintering brook trout activity levels will change when subjected

to flow manipulations.

    Ha3: Flow reductions will increase the activity levels of overwintering brook

    trout.

4.) To determine if there will be a change in plasma cortisol and glucose levels in brook

trout subjected to flow manipulation.

    Ha4: Flow reductions will cause an increase in plasma cortisol and glucose levels

    in overwintering brook trout subjected to flow manipulation.

5.) To assess body composition changes of overwintering brook trout subjected to flow

manipulations.

    Ha5: Brook trout percent total body fat will decrease in both control and

    experimental fish over the winter; though, the magnitude of total body fat loss

    will be greater in experimental fish.

2.3 Methods

2.3.1 Study Site

Site location

The study was carried out using *in situ* experimental channels located in a small unnamed first order stream in the Canadian Rocky Mountains (50º59'03.2"N; 115º04'48.2"W). The spring-fed stream drains into the southern end of Barrier Lake (Figure 2-1). The stream is small with a mean width of 1.5m ±0.034 (±*SE*) (range: 0.8-3m) and a mean depth of 0.21m ±0.007 (±*SE*) (range: 0.05-0.53m), with a winter discharge of approximately 45 l/s. Because the stream is groundwater fed it remains largely ice free throughout the winter. The elevation at the study site is 1425m at which point the stream has a moderate gradient (1-2%) and a NW aspect. The habitat within the stream is heterogeneous containing, riffles, runs and pools with diverse substrate and cover and is shaded by a canopy of lodgepole pine (*Pinus contorta*) and white spruce (*Picea glauca*). Brook trout species are the only fish species within the stream with the exception of an infrequent presence of a brown trout.
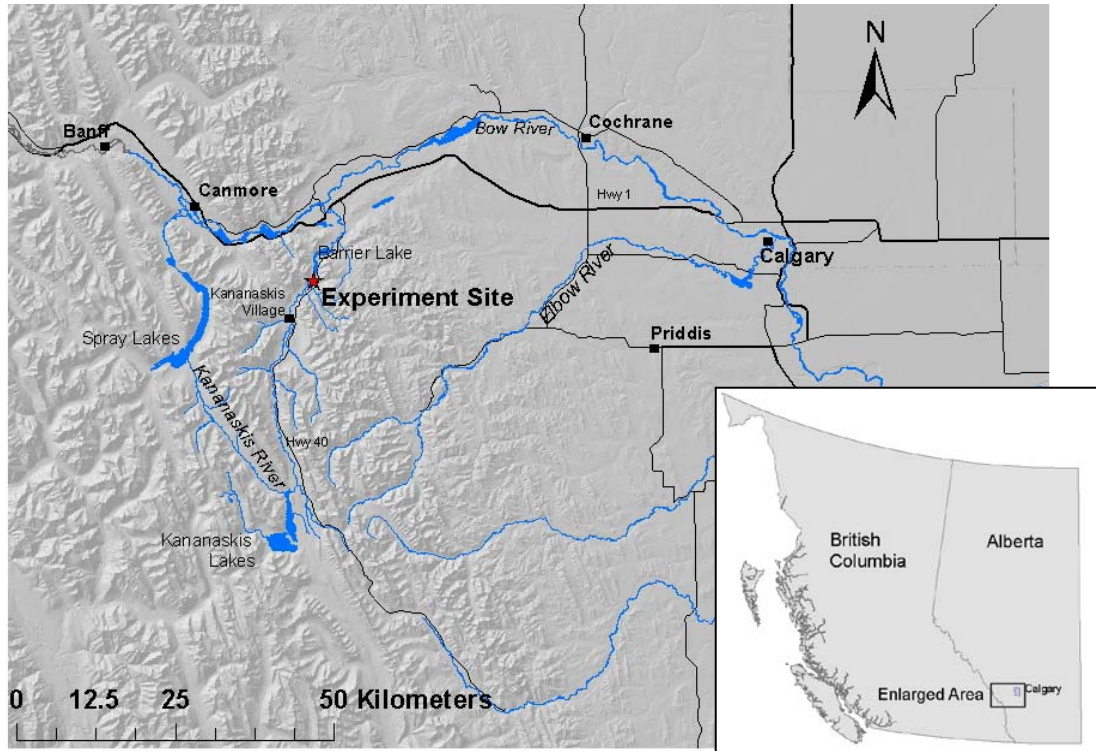
Figure 2-1 Map of the experimental site located in Kananaskis Country in the Canadian Rocky Mountains.

Site Design

Two similar 100m stretches of the stream were isolated using fences made of 6mm metal hardware-cloth and T-bar posts: a control reach (downstream, 50º58'59.6"N; 115º04'46.8"W) and an experimental reach (downstream, 50º59'03.2"N; 115º04'48.2" W). The two sites were separated by a 35m reach where the stream ran through a culvert under the Porcupine Group Campsite Road (Figure 2-2). The control site was upstream of the experimental site.
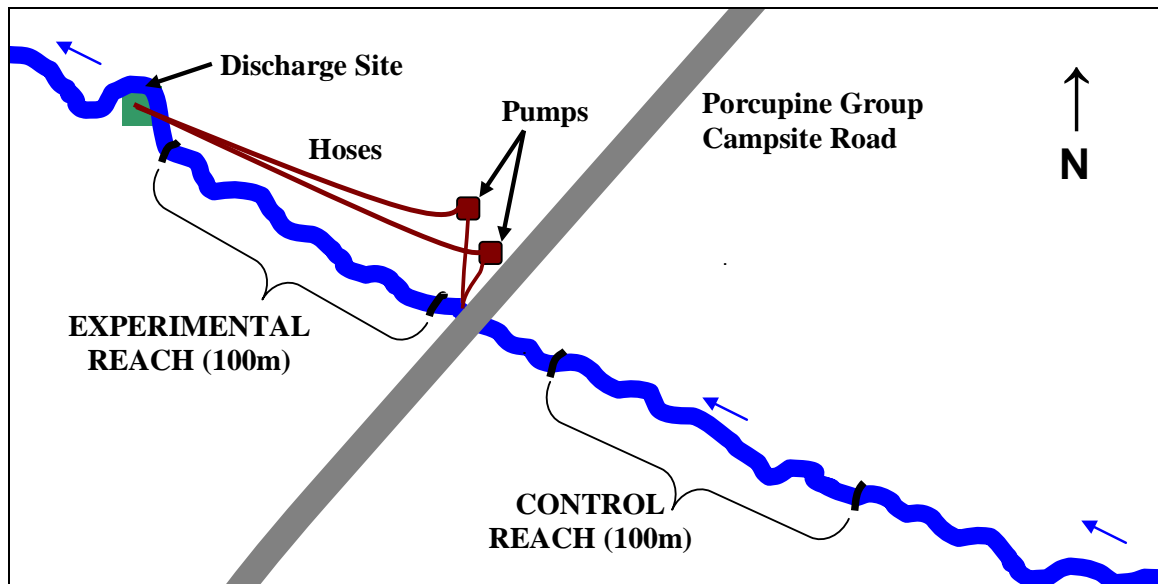
Figure 2-2 Experimental site layout depicting the position of the control and experimental reaches. Small arrows indicate stream-flow direction. Scale ~1:2500

2.3.2 Timeframe

The experiments were conducted during the winters of 2007 and 2008. In 2007 the experiment ran for six weeks, commencing on Jan 22 and finishing on Mar 4 and was divided into three two-week treatment regimes. In 2008 the experiment ran for seven weeks, commencing on Jan 23 and finishing on Mar 12 and was divided into seven one-week treatment regimes.

2.3.4. Sampling

Fish capture

In 2007, after the experimental sites were isolated with fish fences, the enclosures were electrofished on January 20th using a backpack electrofisher. The stream was also electrofished above and below the experimental sites in order to capture enough fish of

adequate size for the study. A total of 58 brook trout were captured for sampling with a mean weight of 22.0g ±1.5 (±*SE*) and mean length of 131mm ±3.1 (±*SE*).

In 2008, brook trout were introduced to the study site from Canmore Creek (51º 04' 47.3" N; 115º 22' 46.4" W; elevation: 1375m) in order to ensure that the experimental fish used in 2008 were unaffected by the 2007 experiment. Fish were captured for relocation from Canmore Creek on January 14th using a backpack electrofisher. A total of 70 brook trout were captured with a mean weight of 35.8g ±1.8 (±*SE*) and length of 159mm ±2.9 (±*SE*). Before the fish were relocated to the experimental site on January 15th, the experimental reaches were isolated with fish fences and the two reaches were electrofished, in three passes, to remove all resident brook trout.

Sample treatments

Sampling treatments and sample sizes were slightly different in 2007 and 2008 (Table 2-1).

Table 2-1 Summary of the number of brook trout sampled in 2007 and 2008 and the number of fish within each sampling treatment

	2007		2008	
Sampling treatment	Control	Experimental	Control	Experimental
Blood sample taken	22	19	0	0
Bioelectrical impedance analysis	0	0	35	35
Radio transmitter externally attached	10	10	15	15
Tagged	10	12	20	20

Blood sampling

Blood samples were taken in the 2007 field season in order to determine plasma cortisol and glucose levels. All fish that were large enough to get a sufficient blood sample from (≥100mm), were bled at the end of the experiment; a total of 41 fish. Fish

were first anaesthetized using a 40ppm solution of clove oil with ethanol as an emulsifier (Keene *et al.*, 1998). After being anaesthetized, blood was taken from the caudal vessels using heparinised 1ml plastic syringes. The blood was transferred to a 0.5ml centrifuge tube and was kept cool until it was centrifuged. Plasma samples were temporarily stored in liquid nitrogen until being transferred to a -80°C freezer were they were held for analysis.

The plasma samples were thawed in the lab and cortisol levels were measured in duplicate (ng/ml plasma) with a radioimmunoassay kit. Glucose levels were also measured in samples containing a sufficient amount of plasma. Glucose was measured in duplicate (mg/ml plasma) in a spectrophotometric assay (510 nm) using the GOD-PAP reagent.

Bioelectric impedance analysis

In 2008, BIA was determined by measuring resistance and reactance of the fish using a tetrapolar, Quantum X bioelectrical body composition analyzer (RJL Systems, Detroit, Michigan). The analyzer was adapted for use on fish by using 28 gauge 12mm stainless steel needle electrodes (Grass Telefactor, West Warwick, Rhode Island). After the fish were anesthetized and patted dry, they were placed on a dry paper towel. The electrodes were inserted ipsilaterally into the fish midway between the lateral line and the dorsal midpoint (Cox & Hartman, 2005). The distance between the two inner detecting electrodes was measured and resistance and reactance readings were taken. Resistance readings ranged from 445-766Ω with a mean of 607Ω ±15.09 (±*SE*), and reactance readings ranged from 120-197Ω with a mean of 153Ω ±3.55 (±*SE*). Readings were taken

on 70 fish before the experiment commenced and again on 54 recovered fish after the experiment was completed.

Attachment of transmitters

Brook trout that weighed >20g were selected for the external attachment of radio transmitters ensuring that the weight of the transmitter did not exceed 2.5% of the total weight of the fish (Table 2-2). Following the advice of Brown *et al.* (1999) the suggested 2% tag/body mass ratio (Winter, 1996) was not considered a strict rule, but instead, the smallest possible tag/body weight ratio was used by selecting the smallest available transmitters with sufficient battery life in order to minimize any behavioural or physiological responses the fish could have to the transmitter. The coded radio NanoTag transmitters (model NTC-3-M; Lotek Wireless Inc., Newmarket, Ontario) weighed 0.5g in air with dimensions of 7.2mm x 7.0mm x 14.5mm. The transmitters had a 10s burst rate with a 64 day 'calculated operational' longevity and a 51 day guaranteed longevity.

Table 2-2 Summary of brook trout length (fork length; mm), weight (g), and tag ratio (transmitter weight to the corresponding fish weight; %) in 2007 (*n=20*) and in 2008 (*n=30*).

	Minimum	Maximum	Mean	*SE*
Winter 2007				
Length	131.0	189.0	155.1	±3.4
Weight	20.7	60.1	33.1	±2.0
Tag ratio	2.4	0.8	1.6	±0.1
Winter 2008				
Length	160.0	198.0	180.4	±2.0
Weight	31.1	78.7	49.7	±1.2
Tag ratio	1.6	0.6	1.1	±0.0

The NanoTag transmitters were attached externally to 20 fish in 2007 (coded: 11-30; frequencies: 150.280-150.460kHz) and 30 fish in 2008 (coded: 11-40; frequencies:

148.380-151.890kHz). The transmitters were manufactured with a small straw (10mm in length) attached to the side of each transmitter, secured in place by heat shrink wrap. The straw on the transmitter was threaded with 0.225mm diameter non-absorbable nylon suture thread (Supramid, S. Jackson Inc.; Alexandria, VA). Two small gauged sewing needles were then threaded through each end of the suture thread. The transmitter was attached by simultaneously pushing the threaded needles through the dorsal musculature of the fish and then through the holes in a plastic back plate situated on the opposite side of the dorsal fin. The back plate was 1.5mm x 4mm x 16.5mm and was used to prevent tissue abrasion by the thread.

Measuring and tagging

Each fish sampled was measured (FL) and weighed. Fish that were not fitted with external transmitters, but were >100mm in length, were tagged with Floy FTF-69 Fingerling Tags (Floy Tag Inc., Seattle, Washington) that were attached to the anterior of the dorsal fin through the dorsal musculature. In 2007, 22 fish were tagged with Floy tags, 10 in the control reach and 12 in the experimental reach. Tag retention was poor (27% retention) in 2007, so the attachment method was modified in 2008. In 2008, the tags were not ordered threaded on needles as they were in 2007; instead they were ordered loose and were threaded with 0.225mm diameter non-absorbable nylon suture thread (Supramid, S.Jackson Inc.; Alexandria, VA) onto a smaller needle. In 2008 a total of 40 fish were Floy tagged, 20 in the control reach and 20 in the experimental reach. Tag retention rate of captured fish in 2008 was 100%.

Relocation and recovery

After weights and lengths were taken and the transmitters and tags were attached, the fish were placed in large bins of fresh stream water and were monitored to ensure that they retained full equilibrium evident from their upright position and their activity and swimming behaviour. Visual observations suggested that there was no difference in fish behaviour before or after transmitters were attached or between the fish with transmitters and those without. After the fish were fully recovered from the anesthetic, they were placed into the fenced reaches of the stream. In 2007 a total of 46 resident fish were released back into the control and experimental reaches. They were given 36 hours to recover from sampling before water withdrawal treatments commenced. On January 15th 2008 a total of 70 fish were transported in 125 L aerated water barrels to the study area from Canmore Creek and released into the control and experimental reaches. The fish were given a week to recover from sampling before the water withdrawal treatments commenced.

2.3.5 Tracking

Movement and habitat selection was monitored using manual radio telemetry. A receiver with a hand held H antenna (Lotek Wireless Inc., Newmarket, Ontario) was used to track the externally attached coded transmitters. Each radio tagged fish was located three times daily: at 8:30 before water withdrawal, at 11:30 during water withdrawal and again at 14:30 after water withdrawal. Linear longitudinal movement was determined relative to the previous position occupied. The fish within the control and experimental sites were tracked concurrently.

Labelled stakes were placed in the ground every two metres along the banks of the control and experimental reaches. Pilot tests suggested that transmitters could be located within 0.5m; therefore the position of each fish was measured to within 0.5m by using the stakes as location markers. In 2007, 20 fish were tracked over 42 consecutive days from January 22nd to March 4th, yielding ~126 observations for each fish. In 2008, 30 fish were tracked over 50 days from January 22nd to March 11th, yielding ~150 observations per fish.

2.3.6 Water Withdrawal

Pumps and hoses

Two trash pumps were used to withdraw water from the stream to reduce discharge. In 2007 water was withdrawn using a 5cm 5HP Honda pump and a 10cm 8HP Rotating Right pump. In 2008 water was withdrawn using two 10cm 8HP Rotating Right pumps. The pumps were situated approximately 4m away from the stream on the right bank. Water was withdrawn with suction hoses that were positioned immediately upstream of the upper experimental fence. The water was then routed through two 91m lay-flat hoses that ran parallel to the stream to the discharge site, situated below the lower experimental fence. The water ran out onto a tarp, where the water velocity was reduced before it ran back into the stream maintaining turbidity levels and preventing scouring of the stream channel (Figure 2-2).

The 2007 experiment ran for six weeks (Table 2-3). During water withdrawal

treatments approximately 50% of the total discharge was withdrawn from the

experimental site for four hours daily, from 10:00 to 14:00.

Table 2-3 The 2007 experimental treatment regime schedule. Each treatment lasted two weeks, alternating from water withdrawal (WW) to no water withdrawal (NW). Brook trout sample size was calculated per day. Control fish *n=10*, Experiment fish *n=10*

2007 Date	Treatment	Duration (days)	Experimental sample (% of radio tagged fish remaining)	Control sample (% of radio tagged fish remaining)
Jan 20 - Jan 21	Acclimatization	1.5	100	100
Jan 22 - Feb 4	1: WW	14	100	100
Feb 5 - Feb 18	2: NW	14	100	100
Feb 19 - Mar 4	3: WW	14	100	100

The 2008 experiment ran for seven weeks (Table 2-4). During water withdrawal

treatments approximately 75% of the total discharge was withdrawn from the

experimental site for four hours daily, from 10:00 to 14:00.

Table 2-4 The 2008 experimental treatment regime schedule. Each treatment lasted one week, alternating from water withdrawal (WW) to no water withdrawal (NW) for 7 weeks. During treatments 1 and 5, only 6 days of data were collected because of weather and predator events. Brook trout sample size was calculated per day. The sample size was reduced because of predators and failing transmitters. Original sample: Control fish *n=15*, Experiment fish *n=15*

2008 Date	Treatment	Duration (days)	Experimental sample (% of radio tagged fish remaining)	Control sample (% of radio tagged fish remaining)
Jan 16 - Jan 22	Acclimatization	7	100	100
Jan 23 - Jan 29	1: WW	6	100	100
Jan 30 - Feb 5	2: NW	7	100	100
Feb 6 - Feb 12	3: WW	7	100	100
Feb 13 - Feb 19	4: NW	7	100	100
Feb 20 - Feb 26	5: WW	6	83	100
Feb 27 - Mar 4	6: NW	7	67	100
Mar 5 - Mar 11	7: WW	7	63	97

Quantifying discharge

Quantifying the amount of water withdrawal necessary to reduce stream discharge by approximately 50% in 2007 and 75% in 2008 was done in two ways. Firstly discharge was correlated with staff gauge depth. A total of four staff gauges were located within the experimental and control sites. They were monitored a minimum of three times daily to indicate approximate discharge. The revolutions per minute (RPM) of the pumps could be initially calibrated according to the readings on the experimental site staff gauges. Secondly, levels of water withdrawal were double checked using actual discharge measures determined by a current meter. Discharge was measured using a hand held Pygmy current meter (Model 625D, Gurley Precision Instruments, Troy, N.Y.) at two permanent transects. The transects were located downstream of the upper and lower experimental fish fences within the reach that was subjected to water withdrawal. Discharge was measured a minimum of three times daily before water withdrawal, during water withdrawal, and again after water withdrawal. The RPM of the pumps were adjusted accordingly to ensure correct discharge reduction.

2.3.7 Measuring Stream Habitat

Water temperature

Water temperature was automatically recorded every 10 minutes using four digital temperature loggers (HOBO Water Temp Pro; Onset Computer Corporation: Bourne, MA, USA). The loggers were placed upstream and downstream of the two study reaches, in the middle of the stream channel.

Air temperature

Air temperature was recorded at the stream site three times daily at 8:30, 11:30, and 14:30. These temperatures were correlated with three nearby weather stations: University of Calgary Kananaskis Field Station, Kananaskis (Nakiska Ridgetop), and Bow Valley (Provincial Park). Weather data from Bow Valley (Provincial Park) was most closely correlated with data from the stream site and was therefore used to indicate approximate hourly air temperature at the study site.

Habitat transects

To determine how available habitats changed as discharge was manipulated stream width and velocity were measured along a series of 24 transects incorporating four different macrohabitats; pools, riffles, runs with deeply undercut banks, and runs without undercut banks. Twelve transects were positioned within the control reach and an additional twelve within the experimental reach. Depth and velocity were measured at 0.2m increments along each transect. Velocity readings were taken at 0.4 of the stream depth, measured from the streambed (Gordon *et al*., 1992). The surveys where executed at four levels of discharge; 100%, 75%, 50%, and 25% of the natural stream discharge.

Habitat characteristics were recorded every meter along both the 100m control reach and the 100m experimental reach. Descriptions of the stream width, depth, substrate composition, bank shape, undercut bank depths, and instream cover were recorded. Substrate was recorded according to a modified Wentworth scale (Gordon *et*

al., 1992) as silt and sand (<2mm), gravel (2-64mm), cobble (65-256mm), and boulder (>256mm).

2.4 Results

2.4.1 Stream Habitat

Water and air temperatures

The mean water and air temperatures measured over the duration of the experiment were slightly cooler in 2007 than in 2008. However the 2008 experiment ran for an extra week into March when the temperatures were warmer. The range of water and air temperature was greater in 2008 (Table 2-5, Figure 2-3 & 2-4).

Table 2-5 Summary of water and air temperature over the 2007 and 2008 experimental duration. Temperature was measured every hour during Jan 22-Mar 5 in 2007 and during Jan 22-Mar 11 in 2008.

	Minimum	Maximum	Mean	*SE*
Winter 2007				
Water temperature (°C)	0.98	3.68	2.32	±0.02
Air temperature (°C)	-23.3	11.7	-4.9	±0.25
Winter 2008				
Water temperature (°C)	0.25	4.94	3.07	±0.02
Air temperature (°C)	-37.6	12.3	-3.9	±0.27
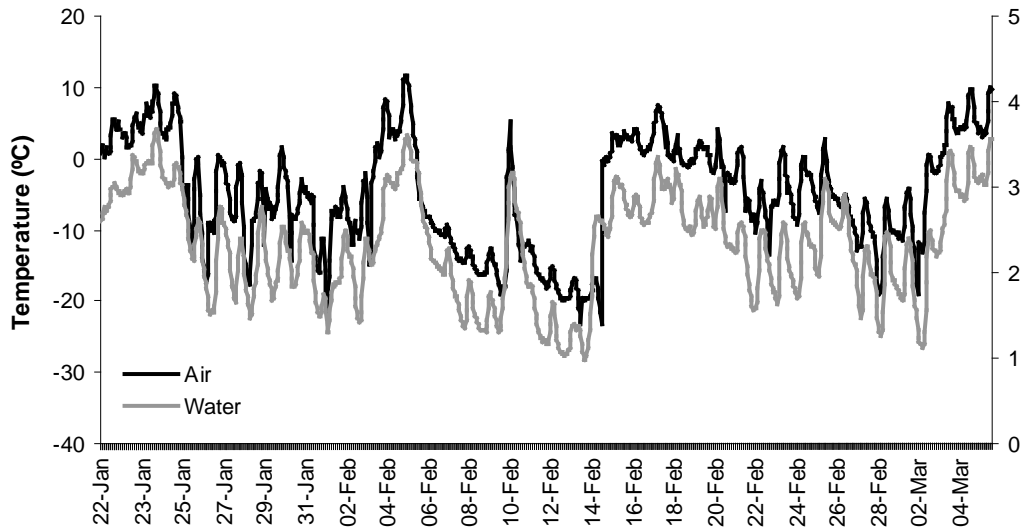
Figure 2-3 The 2007 air and water temperature of the site measued over the duration of the experiment. The water temperature is an average of temperatures recorded at the top and bottom of the control and experimental sites. Air temperature was recorded from the Bow Valley (Provincial Park) weather station (51° 4.800' N 115° 4.200' W).

Figure 2-4 The 2008 air and water temperature of the site measured over the duration of the experiment. The water temperature is an average of temperatures recorded at the top and bottom of the control and experimental sites. Air temperature was recorded from the Bow Valley (Provincial Park) weather station (51°4.800' N 115°4.200'W)

Habitat availability

As stream flow was reduced there was a reduction in the stream width, depth and the quantity of available undercut bank cover (Figure 2-5).

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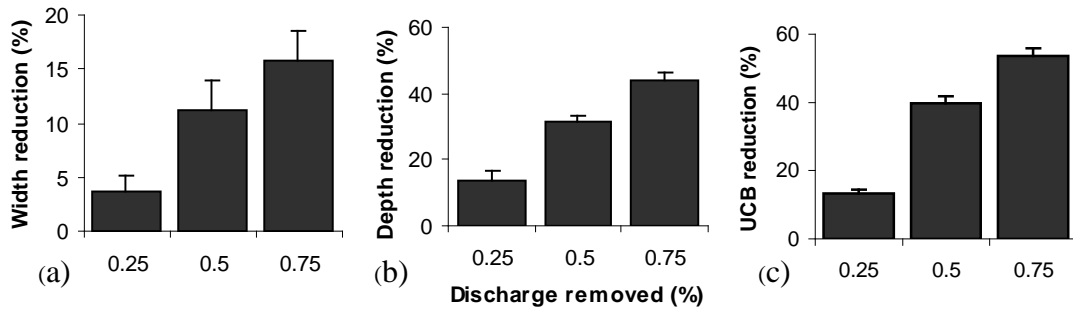
Figure 2-5 Summary of how reducing discharge affects reduction of stream (a) width, (b) depth, and (c) undercut bank (UCB) availability within the experimental reach site. *n=101*.

Water velocity was observed at different discharges to document changes in water velocity with reduced flow (Figure 2-6). Mean water velocity at base flows was 0.20m/sec ±0.03 (±*SE*) (range: 0-0.84m/sec), at 50% reduction was 0.15m/sec ±0.02 (±*SE*) (range: 0-0.78m/sec), and at 75% reduction was 0.11m/sec ±0.02 (±*SE*) (range: 0-0.58m/sec).
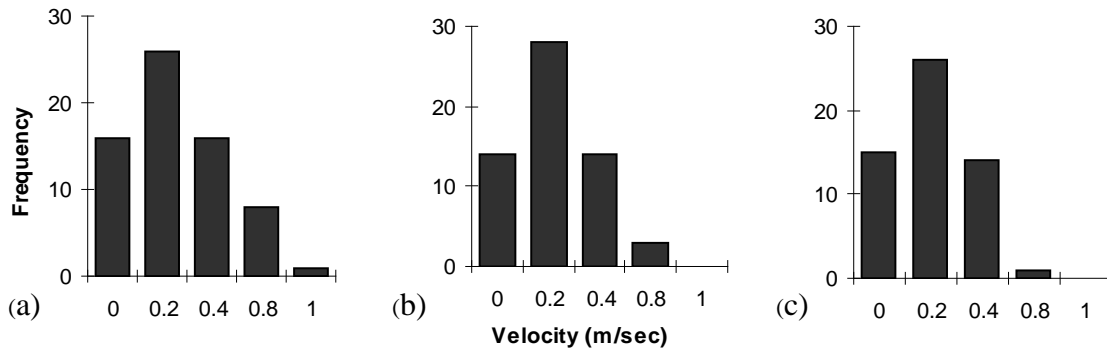
Figure 2-6 Velocity histograms illustrating frequency of velocities at: (a) base discharge, (b) 50% discharge reduction, and (c) 75% discharge reduction at the experimental stream site. Velocity measures were taken every 0.2m across the width of the stream at 12 transects; *n= 67*.

Habitat use

The stream substrate was largely composed of fines and gravel with some cobbles and boulders. Stream habitat type was primarily runs with some riffles and pools. Undercut banks and woody debris provided a large amount of cover throughout both

reaches (Table 2-6). Using t-tests, no significant difference between the amount of undercut bank cover ($t_{(198)} = 0.31,. p = .76$), boulder substrate ($t_{(198)} = 1.67,. p = .10$), or stream width ($t_{(198)} = 2.51, p = .27$) was found between the experimental or control sites; however the experimental site was significantly deeper ($t_{(198)} = 4.64, p = < .001$), with more fine substrate ($t_{(198)} = 4.42, p < .001$), more woody debris cover ($t_{(198)} = 3.15, p = .002$), less gravel substrate ($t_{(198)} = 4.50, p < .001$), and less cobble substrate ($t_{(198)} = 2.62, p = .01$).

Table 2-6 Stream habitat characteristics measured along (a) all transects n: 202, (b) control transects $n = 101$ and (c) experimental transects $n = 101$. UCB: undercut banks, WD: woody debris

	depth (m)	width (m)	% Substrate				%Instream Cover			%Habitat		
			fines	gravel	cobble	boulder	UCB	WD	boulder	riffle	run	pool
(a)	0.21	1.53	47	45	8	0.6	92	67	4	10	85	5
(b)	0.23	1.50	56	38	6	0.4	97	76	1	8	88	4
(c)	0.18	1.55	39	52	9	0.8	87	57	7	12	82	6

The habitat characteristic of the locations used by the control and experimental fish were compared to the habitat available over each reach during 2007 and 2008. Used stream positions were determined by locating the fish three times a day: before, during, and after water withdrawal over the duration of each experiment. Using t-tests there was no consistently significant difference found between available substrate, and used substrate, including fines (2007 cont: $t_{(1349)} = 1.84, p = .07$, exp: $t_{(1349)} = 1.48, p = .14$; 2008 cont: $t_{(2220)} = 0.31$ $p = .70$, exp: $t_{(1962)} = 0.25$ $p = .80$), gravel (2007 cont: $t_{(1349)} = 3.32, p < .005$, exp: $t_{(1349)} = 1.78, p = .08$; 2008 cont: $t_{(2220)} = 0.83$ $p = .40$, exp: $t_{(1962)} = 0.08$ $p = .94$), cobbles (2007 cont: $t_{(1349)} = 3.12, p < .05$, exp: $t_{(1349)} = 1.44, p = .15$; 2008 cont: $t_{(2220)} = 0.95, p = .34$, exp: $t_{(1962)} = 1.92$ $p = .06$), and boulders (2007 cont: $t_{(1349)} = 0.30$ $p = .76$, exp: $t_{(1349)} = 1.56, p = .12$; 2008 cont: $t_{(2220)} = 2.96, p < .005$, exp: $t_{(1962)} =$

1.02, $p = .31$). There was also no consistently significant difference between available

and used woody debris cover (2007 cont: $t_{(1349)} = 5.77$, $p < .001$, exp: $t_{(1349)} = 1.77$, $p =$

.12; 2008 cont: $t_{(2220)} = 0.57$, $p = .57$, exp: $t_{(1962)} = 0.86$ $p = .39$) or between available

width and used width (2007 cont: $t_{(1349)} = 2.28$, $p = .20$, exp: $t_{(1349)} = 0.68$, $p = .54$, 2008

cont: $t_{(2220)} = 0.77$, $p = .44$, exp: $t_{(1962)} = 3.71$ $p < .001$). Locations that the brook trout

used had deeply undercut banks and deep water that were found to be significantly

greater than that available (Table 2-7, Figure 2-7).

Table 2-7 Comparison of the depth of the stream and the depth of the undercut banks (UCB) (mean± *SE*) at locations that the brook trout used in comparison to locations that were available within the (a) control and the (b) experimental reach. Significance was determined using t-tests

(a) Control stream position	n	UCB depth (cm)	t	p	Stream depth (cm)	t	p
2007 Used	1250	31 ± 0.2	8.9	0.001	23 ± 0.2	2.9	.005
2008 Used	2121	28 ± 0.2	5	0.001	24 ± 0.2	4.3	.001
2007/2008 Available	101	23 ± 1.2			21 ± 0.7		
(b) Experimental stream position	n	UCB depth (cm)	t	p	Stream depth (cm)	t	p
2007 Used	1250	42 ± 0.7	8	0.001	29 ± 0.2	5.1	.001
2008 Used	1863	38 ± 0.5	6.7	0.001	29 ± 0.2	5.4	.001
2007/2008 Available	101	23 ± 1.2			25 ± 0.7		

(a)   (b)
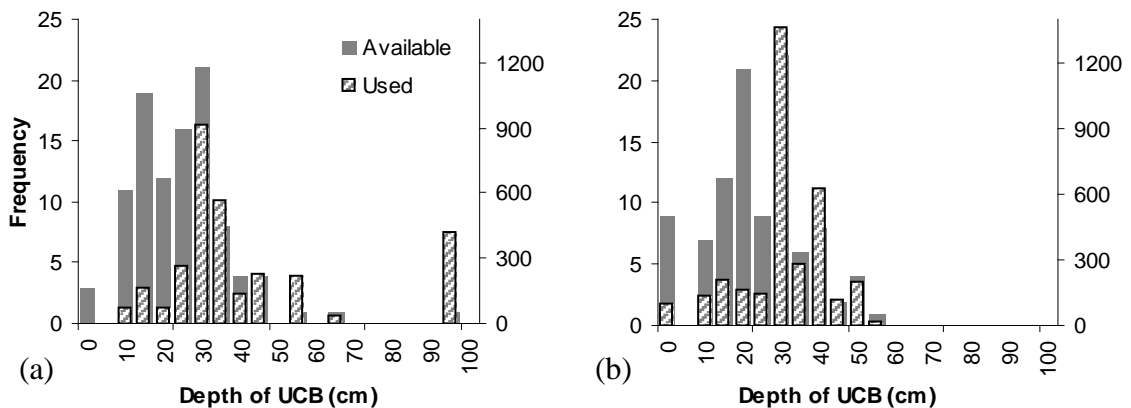
Figure 2-7 Undercut bank depth (UCB) histograms illustrating frequency of UCB cover available and the frequency of UCB cover used by the brook trout within the (a) experimental reach and the (b) control reach. Used locations were determined three times daily over 2007and 2008. Control: used cover *n=3371*, available cover *n=101*. Experimental: used cover *n=3113*, available cover *n=101*

The most used location of each individual fish was compared to their position prior to water withdrawal treatments. In 2007, nine of the ten experimental fish were found in habitat characterized by equal or deeper undercut banks in comparison to their initial location. In 2008, eight of the fifteen experimental fish were found in habitat characterized by equal or deeper undercut banks in comparison to their initial location.

2.4.2 Activity Level

Activity levels in 2007

Because of the hierarchical sampling structure, a mixed model nested ANOVA was used to compare the log-transformed distance moved of fish in the control and experimental stream sections, over the entire experiment. For this analysis, sampling days were repeated measures nested within the three treatments, with time as replication. This nested ANOVA indicated a significant difference between control and experimental fish movement ($F_{(1,78)} = 4.19$, $p = .044$). The fish in the experimental site moved significantly more over the entire experiment; however movement varied between treatments. The experimental fish moved significantly more than those in the control site during treatment one ($F_{(1,278)} = 7.93$, $p = .005$) but not during treatment two ($F_{(1,278)} < 1$), or treatment three ($F_{(1,278)} = 2.73$, $p = .10$) (Figure 2-8a). Most of the movement occurred within the first six days of the two water withdrawal treatments (Figure 2-8:1.b, & 3.b); in which the experimental fish activity levels were significantly greater than the control fish during both water withdrawal treatments: treatment one ($F_{(1,118)} = 9.93$, $p = .002$) and three ($F_{(1,118)} = 16.33$, $p < .001$).
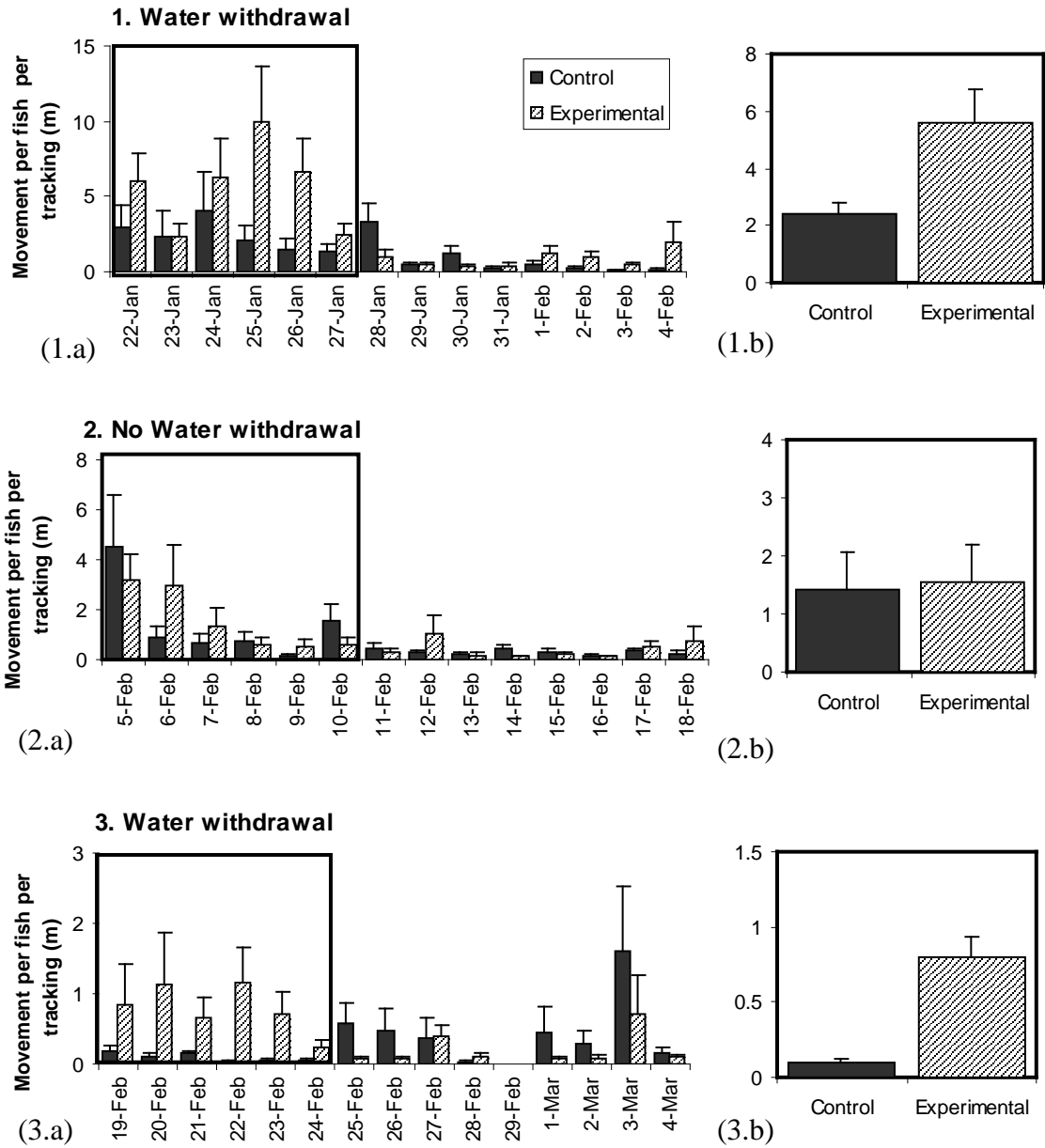
Figure 2-8 The distance individual control and experimental brook trout moved each tracking measured over (a) the two week treatment regimes: (1.a) water withdrawal; $F_{(1,278)} = 7.93$, $p = .005$, (2.a) no water withdrawal; $F_{(1,278)} < 1$, and, (3.a) water withdrawal; $F_{(1,278)} = 2.73$, $p = .10$. Mean distance moved per tracking per day of each fish measured over (b) the first six days of the treatment: (1.b) $F_{(1,118)} = 9.94$, $p = .002$, (2.b) $F_{(1,118)} = .25$, $p = .620$, (3.b) $F_{(1,118)} = 16.3$, $p < .001$. Significance was determined using log transformed movement data in a one way ANOVA. Control fish $n = 10$, Experimental fish $n = 10$.

The number of active experimental fish (move ≥ 2m) was significantly greater

than the number of active control fish at each tracking during the two combined

treatments of water withdrawal ($X^2_{(1,\ N=1680)} = 13.6$, $p < .001$), and not significantly

different during the no water withdrawal treatment ($X^2_{(1,\ N = 1840)} = 1.27$, $p = .26$).

There was a large degree of variability in individual fish movement over the

duration of the experiment. Within the control reach, the range of movement of

individual fish during each tracking was 0.2-2.4m (mean ±SE: 0.9m ±0.2). Within the

experimental reach, tracked movement ranged from 0.4-4.2m during each tracking (mean

±SE: 1.4m ±0.4). Movement showed no apparent upstream or downstream trend.

Movement was significantly related to water temperature although there was high

variance in this relationship (linear regression: $R^2 = .04$, $F_{(1,\ 248)} = 10.63$, $p = .001$).

Corrected weight loss was found to be significantly correlated with the distance

individual fish moved (linear regression, $R^2 = .24$, $F_{(1,\ 18)} = 5.74$ $p = .028$), whereby the

more the fish moved the more weight they lost.

Activity levels in 2008

A mixed model nested ANOVA was used to compare the log-transformed

distance moved of fish in the control and experimental stream sections, over the entire

experiment. For this analysis, sampling days were repeated measures nested within the

seven weeks (treatments), with time as replication. This nested ANOVA indicated that

the experimental fish moved significantly more than the control fish ($F_{(1,80)} = 78.0$, $p <$

.0001) over the duration of the entire seven week experiment. The mean daily movement

of experimental fish was significantly greater than that of the control fish during each

water withdrawal treatment (Table 2-8, Figure 2-9.1). In addition, a greater percentage of

the experimental fish were active than the control fish during the water withdrawal

treatments (Figure 2-9.2).

Table 2-8 Comparison of control and experimental brook trout movement during each treatment in 2008. Movement was calculated by determining the mean movement of each fish per tracking, measured three times daily, over each treatment. Each treatment lasted 7 days, alternating from water withdrawal (WW) to no water withdrawal (NW) for 7 weeks. Significance was determined using logged movement data in a one-way ANOVA. Control fish: $n = 15$, Experimental fish: $n = 15$

Treatment	Control move (m)	Experimental move (m)	F	df	p
1 WW	0.80	3.50	39.9	178	.001
2 NW	0.41	0.91	4.00	208	.047
3 WW	0.84	1.41	10.1	208	.002
4 NW	0.78	0.85	0.22	208	.642
5 WW	0.48	1.22	29.5	163	.001
6 NW	0.49	0.21	2.15	173	.145
7 WW	0.51	1.33	29.4	166	.001
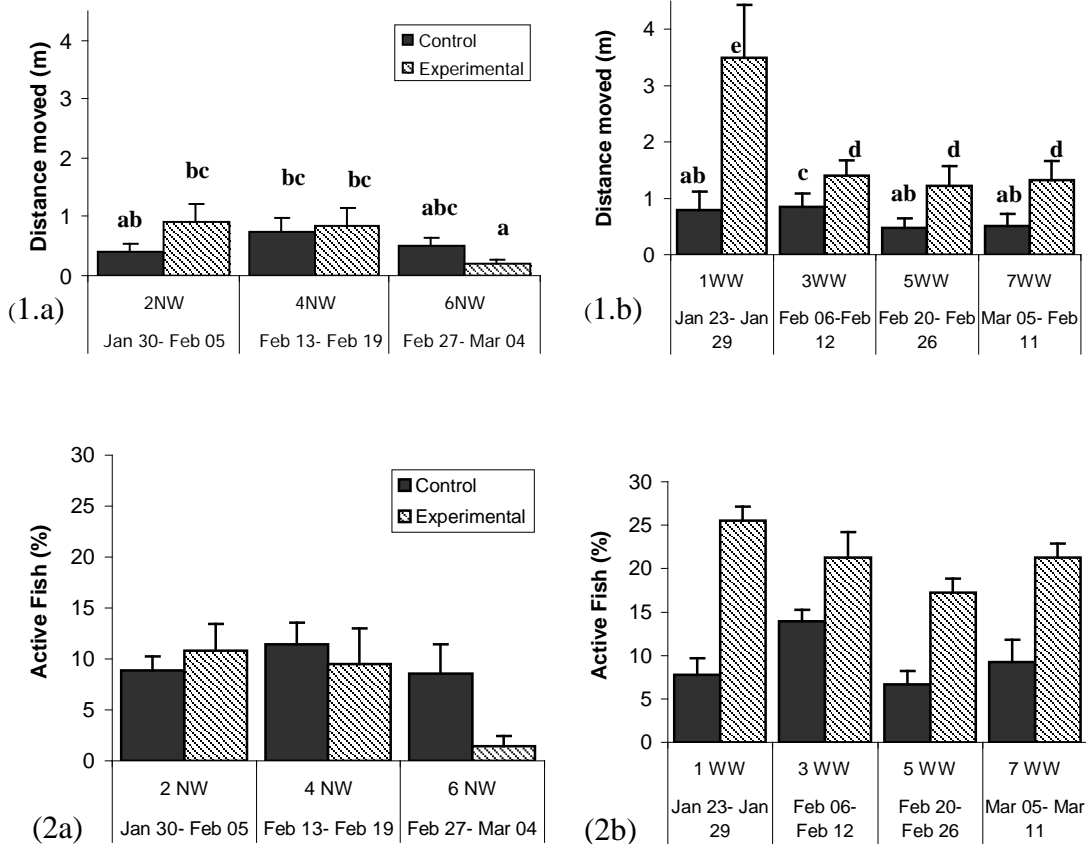
Figure 2-9 (1) Mean distance individual control and experimental brook trout moved each tracking in 2008 measured over (1a) weeks 2, 4 and 6 of no water withdrawal (NW), and during (1b) weeks 1, 3, 5,

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and 7 of water withdrawal (WW) treatments. The distance each fish moved was measured three times a day. Significance was determined using daily mean logged distance moved per tracking in an ANOVA with control/experimental fish as a fixed factor and week as a random factor: (1a) NW; $F_{(1,2)} = 25.5$, $p = .70$ (control $n = 387$, experimental $n = 336$), (1b) WW; $F_{(1,3)} < 1$, (control $n = 315$, experimental $n = 280$). Movement with different letters differ significantly ($p < .05$) according to Duncan's multiple range test. (2) Percent of the control and experimental brook trout that were active each tracking in 2008 over (2a) weeks 2, 4 and 6 of no water withdrawal (NW), and during (2b) weeks 1, 3, 5 and, 7 of water withdrawal (WW) treatments. A fish was defined as active if it moved 2m or more between each tracking. Significance was determined using Pearson Chi Square: active vs. inactive control and experimental fish: (2a) NW; $X^2_{(1, N = 2169)} = 1.47$, $p = .23$), (2b) WW; $X^2_{(1, N = 1785)} = 58.5$, $p < .001$. Control fish $n = 15$, Experimental fish $n = 15$.

There was a large degree of variability in individual fish movement over the duration of the experiment in both years. Within the control reach, the range of movement of individual fish during each tracking was 0.0-1.3m (mean $\pm SE$: 0.6m ± 0.1). Within the experimental reach, tracked movement ranged from 0.2-4.3m during each tracking (mean $\pm SE$: 1.4m ± 0.4) and movement showed no apparent upstream or downstream trend, as in 2007. Weight loss in 2008 was not found to be significantly correlated with the distance individual fish moved (linear regression; $R^2 = .01$, $F_{(1, 23)} < 1$), nor was movement significantly related to water temperature (linear regression; $R^2 = .01$, $F_{(1, 139)} = 1.4$, $p = .24$).

2.4.3 Cortisol and Glucose

There was no significant difference in the levels of cortisol or glucose between the control and experimental fish taken at the end of the experiment in 2007 (Table 2-9).

Table 2-9 Comparison of the (a) cortisol levels (mean $\pm$ SE) and (b) glucose levels (mean $\pm$ SE) of the control and experimental brook trout in 2007. Significance was determined using a one-way ANOVA.

(a)	Cortisol (ng/ml)	n	F	df	p
Control	14.1 ± 5.4	22	< 1	39	.951
Experiment	13.7 ± 4.5	19			

(b)	Glucose (mg/ml)	n	F	df	p
Control	0.67 ± 0.02	22	< 1	32	.897
Experiment	0.68 ± 0.02	12			

2.4.4 Body Composition Analysis

The fish lost a significant amount of weight over the duration of the winter of 2007 (Table 2-10a) (repeated measures ANOVA: control fish, $F_{(1,9)} = 32.4$, p < .001, and experimental fish, $F_{(1,9)} = 38.9$, $p < .001$), and 2008 (Table 2-10b) (repeated measures ANOVA: control fish, $F_{(1,28)} = 93.5$, $p < .001$, and experimental fish, $F_{(1,24)} = 62.9$, $p < .001$), although there was no significant difference between weight loss of the control and experimental fish (one-way ANOVA; 2007, $F_{(1,18)} < 1$; 2008, $F_{(1,52)} = 2.07$, $p = .156$).

Bioelectrical impedance readings indicate that the fish generally gained water content and lost lipid content over the duration of the experiment (Table 2-10b). There was a significant increase in proximate TBW (repeated measures ANOVA: $F_{(1,52)} = 7.12$, $p = .010$) of 1% and a decrease in proximate TBF (repeated measures ANOVA: $F_{(1, 52)} = 6.24$, $p = .016$) of 21% in the fish over the winter, but there was not a significant difference between the change in predicted compositional variables between the control and experimental fish (one-way ANOVA; TBW: $F_{(1,52)} < 1$; TBF: $F_{(1,52)} = 1.02$, $p = .932$).

Table 2-10 Descriptive statistics of changes in condition of the overwintering brook trout in 2007 and 2008: (a) 2007 weight loss, (b) 2008 weight loss and change in percent predicted total body water content (TBW) and total body fat content (TBF).

(a) 2007		N	Minimum	Maximum	Mean	*SE* (±)
	pre	20	22.7	60.1	33.6	2.01
Weight (g)	post	20	21.1	59.0	31.5	2.01
	diff		*-1.6*	*-1.1*	*-2.1*	*0.24*

(b) 2008		N	Minimum	Maximum	Mean	*SE* (±)
Weight (g)	pre	54	11.4	78.7	37.3	2.15

	post	54	10.6	71.6	33.5	1.87
	diff		*-0.8*	*-7.1*	*-3.8*	*0.31*
	pre	54	74.6	83.0	80.0	0.22
Predicted TBW (%)	post	54	76.9	85.0	80.7	0.25
	diff		*2.3*	*2.0*	*0.7*	*0.25*
	pre	54	-0.8	15.0	4.8	0.45
Predicted TBF (%)	post	54	-2.0	12.2	3.8	0.48
	diff		*-1.2*	*-2.8*	*-1.0*	*0.41*

2.4.5 Predation

During the fifth treatment of water withdrawal, on February 23 2008, five experimental fish with transmitters were killed by mink (*Mustela vison*). Two fish with transmitters were consumed on the bank within the experimental reach. The transmitters from these fish were left behind, one functioning and the other damaged and no longer transmitting. The mink did not eat the other three fish with transmitters, but instead removed them from the experimental reach and transported them downstream where they were cached in two separate locations. The dead fish were found, submerged in the creek, in holes dug into the undercut banks. There were an additional two dead fish found downstream that did not have transmitters but were found stashed together with the fish with transmitters; one with a Floy tag and one untagged fish. Overall in 2008, 33% (*n=15*) of the experimental transmitter fish were killed by a predator. The control fish (*n=15*) were unaffected by predation.

2.5 Discussion

Flow manipulations have been shown to affect habitat availability and activity levels of overwintering brook trout within this study. Repeated flow reductions did not

cause detectable physiological changes in the cortisol and glucose levels of the experimental fish, or TBF and TBW content, over the duration of the experiment. It was also found that mink predation resulted in significant mortality of experimental fish.

2.5.1 Habitat Availability

Within the context of this study, as water was removed from the experimental stream width and depth were reduced, thereby restricting undercut bank habitat availability. Because reducing discharge limits habitat availability, the capacity for a stream to maintain salmonid populations may be compromised with increased water withdrawal. Baran *et al.* (1995) also found that area of cover, and depth decreased with reduced discharge, in addition they noted a decrease in velocity.

A reduction in velocity could be viewed as a positive alteration to winter habitat, as trout prefer low velocity water and this preference becomes more pronounced in the winter (Heggenes & Dokk, 2001). Within this study, the mean water velocity in the experimental channel decreased when discharge was reduced because of a diminishing range of maximum velocities; however there was not a corresponding increase in low velocity habitat. The quantity of low velocity water stayed constant at 25%, 50%, and 75% flow reduction, but because the stream width and depth was reduced with water withdrawal, there was a reduction in habitat that provided low velocity water that was covered by undercut banks.

2.5.2 Habitat Use

Cover

The findings of this study indicate a strong cover preference in daytime habitat selection of overwintering brook trout, with deep water with deeply undercut banks being the most commonly selected cover. The preference for brook trout to seek covered habitat is stronger in the winter than the summer (Cunjak & Power, 1986b), and it has been noted that trout have a stronger preference for submerged winter cover than high cover (Cunjak & Power, 1987a). Following this trend, coho salmon (*Oncorhynchus kisutch*) and steelhead trout have been shown to move closer to cover as water temperatures decline in the winter (Bustard & Narver, 1975).

Depth and velocity

The present study found that overwintering brook trout were rarely seen in riffles, instead they preferred deep water habitats; however, it is important to consider that stream habitat variables tend to be intercorrelated (Young, 1995). Winter habitat selection was likely not based on depth alone as depth was found to be correlated with the available area of undercut banks, and was also associated with low velocity habitat.

Substrate

The brook trout within this study showed no preference for any particular substrate in winter. These findings coincide with Chisholm et al. (1987) findings, but are in contrast to other studies which demonstrated that cutthroat trout had a preference for

small substrate (Brown & Mackay, 1995) while juvenile Atlantic salmon preferred large substrate (Hiscock et al., 2002).

Water temperature

Because of the relatively homogenous temperature of the experimental stream selected for this study, temperature was not examined as a habitat selection variable; however the presence of fish naturally overwintering in the experimental ground-water fed stream suggests an affinity to "winter warm" water.

2.5.3 Activity Level

Using radio telemetry, control and experimental fish could be tracked multiple times daily to determine activity levels and habitat preferences of overwintering brook trout and to observe how the fish were affected by changes in flow regime.

Winter activity levels

The tendency for fish to remain sedentary in the winter was apparent within this study in both 2007 and 2008 with some fish showing virtually no movement for the duration of the experiment. The relative inactivity may be the result of low water temperatures reducing swimming performance (Hartman, 1963). There was a weak, but significant correlation between movement and water temperature in 2007 but not in 2008. Brown *et al.* (2001) also addressed the association between movement and water temperature and did not find a significant relationship between temperature and distance

moved of brown trout , white sucker (*Catostomus commersoni* Lacepede) or common carp (*Cyprinus carpio* Linnaeus).

This study focused on tracking daytime activity levels; however overwintering salmonids do exhibit a shift in diurnal activity in the winter with a propensity for daytime concealment and a tendency to become more active at night (Heggenes & Dokk, 2001; Huusko et al., 2007; Jakober et al., 2000). Within the study period, fish became more visually observable during the night. There was an apparent diurnal trend marked by some fish moving laterally out from under cover at night and back under cover during the day.

Activity levels with flow manipulation

Within the context of this study, activity levels of the experimental brook trout were significantly increased during reduced flows. In both 2007 and 2008 the experimental fish were most active during the first few days of the first water withdrawal treatment. Because the flow manipulations within each treatment were consistent in magnitude and duration throughout each experiment, the fish may have been able to adapt to the environmental perturbation. Adaptation or habituation appears to be likely in 2007 when the fish activity levels came steadily back down to control levels after six days of treatment. In 2008 flow was reduced to lower levels than in 2007. Comparable to fish movement in 2007 the highest activity level in 2008 occurred in the first week, but unlike 2007, there was no trend in reduced activity over the repeated treatments potentially demonstrating an inability to adapt or habituate to the greater magnitude of water withdrawal.

Atlantic salmon parr move significantly more at changing flows than at stable flows (Berland *et al.*, 2004), and brook trout have been shown to have elevated levels of activity during up- and down-ramping (Scruton *et al.*, 2003). In this study, visual observations were made of increased activity and exposure of the trout during the period of changing flows. These data were not quantitatively captured because the change in discharge occurred quickly and the visually active fish were often found to return to the same location after flows stabilized when tracking occurred, resulting in no recordable distance moved.

Activity levels with change in habitat

Discharge reduction changed the quantity and quality of habitat available for the brook trout within the present study. Experimental fish tended to seek out deeper undercut banks in response to flow manipulations likely for refuge. Trout have been shown to modify their habitat selection as a result of a change in flows (Bunt *et al.*, 1999; Pert & Erman, 1994). In 2007 when fish where subjected to 50% water withdrawal, 90% of the experimental fish moved to habitat characterized by equal or deeper undercut banks after water withdrawal treatments commenced. In 2008 when fish where subjected to 75% water withdrawal, only 53% of the experimental fish moved to habitat of equal or deeper undercut banks after water withdrawal treatments commenced. This discrepancy is consistent with the difference in activity levels. In 2007 activity levels declined throughout each treatment indicating that the fish may have been able to find suitable habitat, namely deep undercut banks, which permitted relative stability regardless of flow reduction. In 2008 activity levels did not decline throughout each treatment potentially

indicating that the increased intensity of the treatment resulted in the inability of some fish to find habitat unaffected by the increased levels of water withdrawal.

Stream morphology, in conjunction with magnitude of flow manipulation, can dictate habitat availability (Valentin *et al.*, 1996) and can be a central determinant of the tendency of a fish to have to move (Scruton *et al.*, 2005). If the increase in fish movement, in response to discharge reduction, is primarily the result of fish seeking out better habitat, it is likely that their level of activity would be inversely proportional to the available habitat at the reduced flows. The stream chosen for this study was relatively deep and narrow with a large amount of cover. When discharge was reduced by 75% almost half of the undercut bank habitat was still available. If the experimental stream reach had a shallow and wide lateral profile, activity levels may have increased significantly more with water withdrawal because the wetted perimeter of rivers this shape show more sensitivity to flow manipulation (Anderson *et al.*, 2006), ultimately affecting the quantity and quality of refugia. Brook trout activity levels have been shown to peak during periods of extreme high or low flows likely as a result of having to seek a new suitable habitat and activity levels have been shown to decrease when suitable refuge is found (Murchie & Smokorowski, 2004).

Trout biomass has been found to increase with habitat improvements including creation of pools and cover (Burgess & Bider, 1980). In turn it would be expected that biomass would decrease with the deterioration of habitat availability. This was not tested within the present study as the fish were enclosed, inhibiting out migration.

Variation in activity levels

Behaviour is likely highly context-dependent and there may be mobile and non-mobile salmonid individuals within one population (Hiscock *et al.,* 2002; Muhlfeld *et al.,* 2001; Pert & Erman, 1994). Variability in movement may be influenced by habitat availability (Murchie & Smokorowski, 2004). A large range in activity levels within single size classes of cutthroat trout (*Oncorhynchus clarki*) has been documented (Brown, 1999), demonstrating large intra-species variation. These findings correspond with the present study where there was a large range of brook trout activity levels in 2007 and 2008; furthermore, this range was considerably magnified in experimental fish subjected to water withdrawal, likely the result of reduced habitat. In conjunction with these findings, a large range in mobility in brown trout subjected to pulsed discharge has also been observed (Bunt *et al.*, 1999). Large variation in movement among individual fish can obscure more subtle responses to environmental conditions such as changes in discharge (Berland *et al.*, 2004).

Fish size and activity levels

In salmonid species, large body size often correlates with dominance (Mellas & Haynes, 1985). In this study, in order to minimize tag/body mass ratios, the largest fish were selected for transmitters and, by default, for monitoring behaviour; consequently it was not possible to assess how the changes in discharge were affecting the behaviour of the small less dominant fish. Scruton *et al*. (2005) hypothesized that larger fish may have a greater propensity to hold their position in their preferred habitat during exposure to

flow manipulations than smaller less dominant fish because site fidelity may be linked to social hierarchy. Larger Atlantic salmon parr have been shown to be less affected by variable flow than smaller fish (Berland *et al.*, 2004). Within the present study it is possible that the smaller trout were being more affected by flow manipulations than the larger trout; however fish tend to exhibit less aggression in winter (Hartman, 1963, 1965; Vehanen *et al.*, 2000) so dominant habitat fidelity in the winter may not be as pronounced as it would be in the summer.

2.5.4 Cortisol and Glucose

  Cortisol

  Cortisol levels were measured as an indicator of the stress response initiated by the repeated stressor of manipulated flow. In order to avoid affecting fish behaviour throughout the study, cortisol levels were only measured for analysis once at the end of the experiment and there was virtually no difference in cortisol levels between control and experimental fish at this point.

  Repeated stressors can desensitize fish, inhibiting catecholamine release (Reid *et al.*, 1998). A repeated stressor may elicit a slightly elevated level of cortisol, although the initial peak cortisol level is not maintained despite the continued administration of the stressor (Einarsdottir & Nilssen, 1996; Jentoft *et al.*, 2005; Pickering & Pottinger, 1989; Wendelaar Bonga, 1997). After ten weeks of subjecting rainbow trout to an acute daily stressor, there was an observed decrease in cortisol and glucose levels indicating habituation (Barton *et al*., 1987). Habituation, or compensation, was also observed in brown trout in response to a repeated stressor, apparent by a drop in cortisol levels back

to resting levels, despite the continued administration of the flow manipulation stressor (Flodmark *et al.*, 2002).

It is probable that the experimental fish in the present study responded in a similar way to the experimental fish in the study carried out by Flodmark *et al.* (2002) and Arnekleiv *et al.* (2004) in which there was an initial peak in cortisol levels after exposure to the stress of reduced flow. With repeated administration of the stressor there may have been greatly reduced or completely diminished elevations of cortisol levels, until no response was detectable. Sloman *et al.* (2001) also speculated that cortisol concentrations could have increased briefly but returned to basal concentrations by the time blood samples were taken in brown trout after a two week exposure to artificial drought conditions.

Because behavioural and physiological responses to stress are interconnected (Iwama, 1998), it is likely that within the context of this study, cortisol levels were increasing in parallel with activity levels. If the experimental fish did exhibit an initial spike in cortisol, like that seen in activity levels, it is probable that there would be large variation in the cortisol levels depending on the degree to which the habitat of individual fish was being affected by reduced flow.

Glucose

Within the present study, there was no detectable difference in glucose levels between control and experimental trout taken at the end of the study period. Like cortisol, glucose levels show a habituated reduction in repeatedly stressed trout (Barton *et al.*, 1987; Scruton *et al.*, 2005). It has been demonstrated that the groups of brown trout with

elevated cortisol levels also had the highest glucose levels after exposure to dewatering in an artificial channel (Arnekleiv *et al.*, 2004), in contrast, no change in blood glucose was found in brown trout exposed to a flow regime stressor despite elevated cortisol levels (Flodmark *et al.*, 2002). Within the present study it is probable that glucose levels were not significantly elevated if the fish were able to find refuge from the stressor.

Implications of the stress response

Stress responses allow a fish to cope with a stressor to maintain relative homeostasis and do not become detrimental until the fish's physiological stress mechanisms become compromised (Barton *et al.*, 2002). The rate of change and time period between a stressor (eg. a change in flow) can play a role in determining if the effects become cumulative or if the fish is able to habituate (Scruton *et al.*, 2005). In order to alleviate or minimize detrimental effects caused by stress on fish, there should be a sufficient time period between stressors in accordance with the stressors' severity and duration (Barton & Iwama, 1991). The sensitivity to stress of fish at different life phases should also be considered.

2.5.5 Body Composition Analysis

Weight

Salmonid winter feeding is restricted because low water temperatures limit appetite, digestion and gastric evacuation rates (Cunjak *et al.*, 1987; Sweka *et al.*, 2004); consequently they primarily rely upon stored fat to meet energetic demands (Pottinger *et al.*, 2003) and have been shown to lose weight while they adjust to winter (Cunjak *et al.*,

1987; Simpkins & Hubert, 2000). Within this study, in both 2007 and 2008, every control and experimental fish lost weight over the duration of the experiment. The physiological implications of flow regime manipulations may be even more pronounced in early winter as it has been shown to be a stressful period of acclimatization in which condition factor has been shown to rapidly decline (Cunjak, 1988).

Attempting to compensate for extreme flows can impose significant energetic costs (Heggenes *et al.*, 1999). In a lab setting fish exposed to fluctuating flows had reduced feeding consumption and reduced growth than those held at stable high water levels (Flodmark *et al.*, 2004). Within this study, there was a significant, albeit weak, relationship between tracked movement and weight loss in 2007; however this relationship was not significant in 2008. In both 2007 and 2008 the experimental fish did not lose significantly more weight than the control fish. Borrowing from Flodmark *et al.*'s (2004) postulations, fish may be better able to better adapt to changes in flow, in a stream that provides a complex environment than they would in the lab or in shallow low gradient rivers with inadequate habitat at low flows.

Water and fat

There was a reduction in percent TBF and, respectively, a small gain of percent TBW content of the control and experimental fish over the extent of the experiment. Cunjak and Power (1986a) also found that percent water content of brook trout increased over the winter, and lipid levels have been shown to rapidly decrease in early winter for brook and brown trout (Cunjak, 1988). Mortality of overwintering young rainbow trout has been shown to be linked to the depletion of lipid reserves (Biro *et al.*, 2004); however

salmonids are not always found to lose a significant amount of fat over the winter (Webster & Hartman, 2007) .

If the experimental fish within this study were unable to habituate to the water withdrawal stressor, and instead had to compensate by adjusting physiologically or behaviourally, it would be expected that there would be an energetic toll affecting their growth, reproduction and, potentially, survival (Scruton *et al.*, 2003). It has been demonstrated that sedentary juvenile rainbow trout maintain lipid levels, while active fish exhibit a decrease in lipid content (Simpkins *et al.*, 2003a, 2004). The experimental fish exposed to the stress of flow manipulations, did adjust behaviourally by being more active, potentially seeking out more hospitable habitats, but they did not lose significantly more weight or fat in comparison to fish not exposed to the stressor. There are a number of possible explanations for this. The stressor may have been affecting the fish in ways that were not measured, for instance inhibition of reproduction functions (eg. reduced quality of gametes (Campbell *et al.*, 1992)). The experiments lasted six weeks in 2007 and seven weeks in 2008. This may not have been a sufficient amount of time to detect a change in physiology indicative of a stress induced energetic toll, or the fish may have been able to habituate to the stressor because of the complex available habitat.

2.5.6 Predation

Predation can be a significant limiting factor of winter survival. Lindstrom and Hubert (2004) found that mink predation played a major role in winter fish mortality as at least 8% of the 25 radio-tagged cutthroat and 28% of the 25 radio-tagged brook trout were killed by mink. Significant mink predation has also been documented on

overwintering radio-tagged juvenile Atlantic salmon (Hiscock *et al.*, 2002). The seasonal

shift in salmonid diel behaviour, characterized by day time concealment (Annear *et al.*,

2002; Heggenes & Dokk, 2001; Meyer & Gregory, 2000), may be an adaptive behaviour

to minimize predation risk (Heggenes *et al.*, 1993; Jakober *et al.*, 2000; Valdimarsson &

Metcalfe, 1998). Within the present study, experimental fish were visually observed more

frequently than control fish, likely as a result of a decrease in undercut bank cover

availability. There was no control fish regularly observed in the centre of the stream

channel; whereas, four of the fifteen experimental fish with transmitters were observed

on a regular basis in the centre of the channel during tracking. Of the four regularly

exposed fish, three were killed by mink. An additional two experimental fish with

transmitters were also killed by mink predation. These two fish were also regularly

located in the middle of the stream channel, however they were usually under the cover

of a piece of large woody debris. It is apparent that the exposed fish were more

vulnerable to predation than those protected by undercut banks; and therefore is likely

that the experimental fish were more vulnerable to predation than the control fish as a

result of flow manipulation.

2.5.6 Conclusion

The ecological process of fish populations are regulated by biotic factors

including competition and predation and by abiotic factors including habitat (Heggenes,

1996; Jackson *et al.*, 2001). In the winter salmonid behavioural strategies change

(Cunjak, 1996; Cunjak & Power, 1986b; Heggenes *et al.*, 1993), whereby competition for

food and social interactions appear to play less of a role than at other times of the year and the relevance of predation and habitat becomes magnified.

There is a dynamic energetic cost associated with winter movement. The decrease in riverine habitat availability with winter flow reduction and the resulting increase in fish activity exemplifies the importance of habitat availability, particularly instream cover. Complex habitats provide important refuge for fish during disturbances (Pearsons *et al.*, 1992). Bjornn (1971, p. 436) proposed that "the amount of suitable winter cover in a stream plays a major role in regulating the number of fish that overwinter in streams with winter water temperatures below 4-5 C". Bustard and Narver (1975) also stress the importance of cover and infer that altering the winter habitat of salmonids would likely result in reduced overwinter survival. Cover plays a major role in providing refuge from predation (Valdimarsson & Metcalfe, 1998). Under natural flows, habitat availability is a limiting factor for overwintering brook trout and this limitation is exacerbated by reducing flows. To minimize adverse effects of daytime winter flow reduction it is important to have complex stream habitat at normal and reduced flows.

To summarize

(1) Because reduced flow decreased the width and depth of the stream the amount of undercut bank cover was also diminished. Mean water velocity decreased with a reduction in discharge, however the quantity of low velocity water did not increase as hypothesized and the amount of low velocity habitat that provided overhead cover was reduced. (2) Deeply undercut banks and deep water provided preferential winter habitat. (3) Pulsed deviations from the natural flow regime had a significant effect on movement

of stream resident brook trout. The duration of increased movement is likely to be dependent on the magnitude of water withdrawal. (4) There was no difference in cortisol and glucose levels between the control and experimental fish after six weeks of alternating treatments. (5) The body composition of the brook trout changed significantly over the winter with a proximate increase in TBW and decrease in TBF; however there was no significant difference of body composition between control and experimental fish.

Further research

It is important to further monitor the implications of water withdrawal during winter in conjunction with different ice conditions and channel shapes. In addition studies should examine effects of manipulating winter flow at a range of magnitudes over varying durations at different times of the day. It is also important to investigate the implications of winter disturbances on fecundity, specifically of spring spawners.

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3. Bioelectrical Impedance Analysis of Overwintering Brook Trout (*Salvelinus fontinalis*) from the Canadian Rocky Mountains

3.1 Abstract

The physiological response of brook trout (*Salvelinus fontinalis)* to experimental flow manipulations was studied during the winter of 2008. The study was carried out using *in situ* experimental channels located in a small stream from Kananaskis Country, Alberta. Experimental treatments consisted of four hour daily water withdrawals from one of the two enclosures, removing 75% of the stream discharge. Changes in brook trout proximate body composition over the duration of the eight week experiment were measured using bioelectrical impedance analysis (BIA). Regression models were developed, using body composition measures and BIA readings of resistance (R) and reactance (Xc), as a tool to predict percent total body water (TBW) and percent total body fat (TBF) of live fish. The best predictive models to determine body composition combined reactance with length to predict TBF yielding an R^2 value of 0.774 and combined reactance with Fulton's condition factor (K) to predict TBW yielding an R^2 value of 0.765. Using these models proximate body composition of the experimental fish was predicted before and after the experiment. The brook trout experienced a significant weight loss, predicted TBF loss, and predicted TBW gain over the eight week winter study; however there was no significant difference detected in predicted body composition between the fish exposed to flow manipulations and those not exposed.

3.2 Introduction

BIA measures the conduction of a current as it passes through soluble ions in intracellular and extracellular fluids of a body. BIA directly measures reactance (Xc) and resistance (R) parameters from which proximate body composition can be determined because of differences in impedance between fat mass and fat free mass (Liedtke, 1997). Resistance is equal to the pure opposition of alternating current to flow, and reactance is the opposition to flow of current caused by the capacitance of cell membranes (Kushner, 1992). Body tissues containing large amounts of water and electrolytes are highly conductive and therefore provide a low resistance pathway; whereas materials such as bone and fat are poor conductors and correspond to high resistance (Liedtke, 1997).

Reactance, also referred to as capacitive reactance, is a measure of the opposition to alternating current arising from the presence of capacitors within a circuit. A capacitor is made up of two or more conducting plates isolated from each other by a dielectric used to store the charge of electrons (Kushner, 1992). In tissue, reactance is a measure of the opposition to an alternating current by the cell membrane. Cell membranes are composed of a bilayer of polar proteins and phospholipids separated by a core of nonconductive lipids that act as a capacitors (Kushner, 1992). When an electrical signal is introduced into a tissue a small amount of the signal is leaked through protein channels to charge the inside of the cell membranes. Cell membranes act as capacitors and cause the electrical current to lag behind the applied voltage resulting in a measurable effect because of the resistance of the dielectric (Liedtke, 1998). In tissue, reactance is analogous to intracellular volume; whereas resistance is analogous with extracellular volume (Liedtke, 1998).

There is extensive literature citing the use of BIA on humans (for a review of BIA use on human subjects see: Kyle *et al.,* 2004)). Studies have also been conducted on other animals including wild mammals (Barthelmess *et al.,* 2006; Bowen *et al.,* 1998; Farley & Robbins, 1994; Hundertmark & Schwartz, 2002; Rutter *et al.,* 1998), livestock (Berg *et al.*, 1997; Daza *et al.*, 2006; Kraetzl *et al.*, 1995; Velazco *et al.*, 1999), and to a lesser extent, on fish (Bosworth & Wolters, 2001; Cox & Hartman, 2005; Duncan *et al.*, 2007; Webster & Hartman, 2007; Willis & Hobday, In Press). The bodies of most mammals are divided into several cylindrical segments; whereas fish have a relatively geometric configuration, therefore, BIA can be more easily applied to a fish's body shape because the principal concept of BIA is that the tested body is an isotropic conductor with a uniform cross-sectional area and length (Kushner, 1992).

Initial documentation of the use of BIA on fish was for the purpose of predicting carcass yield and composition of farmed catfish (*Ictalurus punctatus*) (Bosworth & Wolters, 2001) and later in farmed juvenile cobia (*Rachycentron canadum*) (Duncan *et al.*, 2007). BIA has been further developed as a means of determining condition and health of wild stocks. The relative condition of bluefin tuna (*Thunnus maccoyii*) at sea (Willis & Hobday, In Press) has been measured using BIA; in addition, BIA has been used to determine the proximate body composition of wild and hatchery brook trout (*Salvelinus fontinalis*) (Cox & Hartman, 2005). The regression models built by Cox and Hartman (2005) were further applied to overwintering brook trout *in situ* and in the lab to measure changes in body composition (Webster & Hartman, 2007). Because BIA has the capacity to detect small change in the electrical properties of a body (Foster & Lukaski,

1996), it can be used as a tool to monitor changes in body composition and therefore changes in physical condition and energy expenditure.

A change in the body composition of salmonids over winter has been associated with a winter fitness cost. A significant decline in trout weight despite continuous feeding throughout winter has been observed (Annear *et al.*, 2002), particularly in reproductive fish (Hutchings *et al*., 1999). Coinciding with weight loss, lipid loss has also been documented in overwintering brook trout (Cunjak *et al.*, 1987), and has been found to be related to post-reproductive survival of brook trout (Hutchings *et al*., 1999). Lipid loss of overwintering brook trout is generally not to the extent that protein stores have to be mobilized; however early winter energy depletion of lipids may limit endurance with exposure to atypical environmental perturbations (Cunjak *et al.*, 1987). Because winter may be a restrictive time of the year for fish production (Alfredsen & Tesaker, 2002; Annear *et al.*, 2002; Jackson *et al.*, 2001), it is important to monitor changes in fish physiology over winter months, including how winter condition is affected by compounding anthropomorphic stressors, such as instream flow manipulation.

3.2.1 Objectives

The following two objectives and related hypothesis will be investigated in this chapter:

1.) To determine if bioelectrical impedance analysis provides an accurate means in which to measure brook trout proximate body composition.

Ha1: Bioelectrical impedance analysis will provide an accurate means in which to measure proximate body composition of brook trout.

2.) To determine how the body composition of brook trout changes over winter and how these changes in composition are further affected by flow manipulations.

Ha2: Brook trout will lose weight, lose fat and gain water over the winter and these changes will be exacerbated in brook trout that are subjected to flow manipulations

3.3 Methods

3.3.1 Model Building

Thirty two brook trout were collected from two locations in Kananaskis on October 18 2007. The first sampling site was an unnamed tributary to Porcupine Creek (50º59'13.0"N; 115º05'14.0"W), and the second sampling site was at Joshua Creek (50º10'35.6"N; 114º 53'57.6"W). The creeks were fished using a backpack electrofisher. Each sampled trout was anesthetized using a 40ppm solution of clove oil, at which point their weight and length (FL) was measured (Table 3-1). BIA measures were then determined using a tetrapolar, Quantum X Bioelectrical Body Composition Analyzer (RJL Systems, Detroit, Michigan). The analyzer was adapted for use on fish by using stainless steel needle electrodes (Grass Telefactor, West Warwick, Rhode Island). The fish were placed on a dry paper towel and stainless steel electrodes were inserted ipsilaterally into the fish midway between the lateral line and the dorsal midpoint (Cox & Hartman, 2005). Resistance and reactance readings were recorded, and the distance

between the two inner detecting electrodes was measured (Table 3-1). Because the bulk electrical properties of tissue can be anisotropic (Foster & Lukaski, 1996), electrode placement was consistent in all trials.

The sampled brook trout were euthanized, individually wrapped in foil, numbered, and bagged. The fish were put on ice, and transported to the lab where they were frozen. The trout were processed in the lab to measure body composition parameters. The whole bodies were dried at 50°C in individual foil containers until a constant dry weight was reached (~48hrs). TBW was determined by calculating the difference between wet and dry weight and percent TBW was calculated relative to the total fresh body weight of the fish. Each whole dried fish was homogenized in a small grinder. Aliquot samples, containing a minimum of 1.5 grams of homogenized tissue were run using the hot Soxhlet extraction method, with petroleum ether as a solvent. The samples were run in duplicate for a minimum of three hours until the sample maintained a constant weight. TBF was determined by calculating the difference in weight of the tissue sample before and after lipid extraction and percent TBF was then calculated relative to the total fresh body weight of the fish (Table 3-1).

Table 3-1 Body composition measures of brook trout sampled in October 2007 from Kananaskis Country to create BIA regression models. TBW: total body water, TBF: total body fat. $n=32$

	MEAN	MIN	MAX	SE (±)
Length (mm)	150	110	193	4.10
Weight (g)	35	13	76	2.72
Resistance (ohms)	607	445	766	15.09
Reactance (ohms)	153	120	197	3.55
TBW (%)	78	73	80	0.32
TBF (%)	8	2	16	0.67

3.3.2 Study Site

Site location

The study was carried out using *in situ* experimental channels located in a small unnamed first order stream in the Canadian Rocky Mountains (50º59'03.2"N; 115º04'48.2"W). The spring-fed stream drains into the southern end of Barrier Lake (Figure 3-1). The stream is small with a mean width of 1.5m ±0.034 (±SE) (range: 0.8-3m) and a mean depth of 0.21m ±0.007 (±SE) (range: 0.05-0.53m), with a winter discharge of approximately 45 l/s. Because the stream is groundwater fed it remains largely ice free throughout the winter. The elevation at the study site is 1425m at which point the stream has a moderate gradient (1-2%) and a NW aspect. The habitat within the stream is heterogeneous containing, riffles, runs and pools with diverse substrate and cover and is shaded by a canopy of lodgepole pine (*Pinus contorta*) and white spruce (*Picea glauca*). Brook trout species are almost the sole species within the stream with the exception of an infrequent presence of a brown trout.
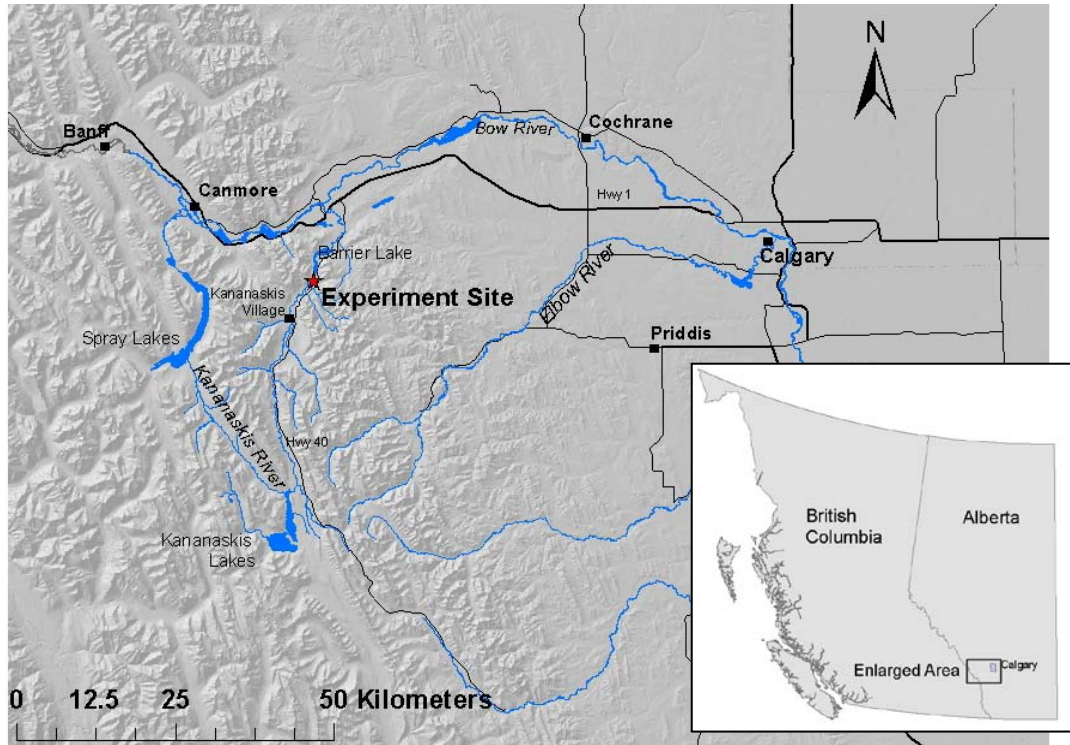
Figure 3-1 Map of the experimental site located in Kananaskis Country in the Canadian Rocky Mountains

Site design

Two similar 100m stretches of the stream were isolated using fences made of 6mm metal hardware-cloth and T-bar posts: a control reach (downstream, 50º58'59.6"N; 115º 04' 46.8"W) and an experimental reach (downstream, 50º59'03.2"N; 115º04'48.2"W). The two sites were separated by a 35m reach where the stream ran through a culvert under the Porcupine Group Campsite Road (Figure 3-2). The control site was upstream of the experimental site.
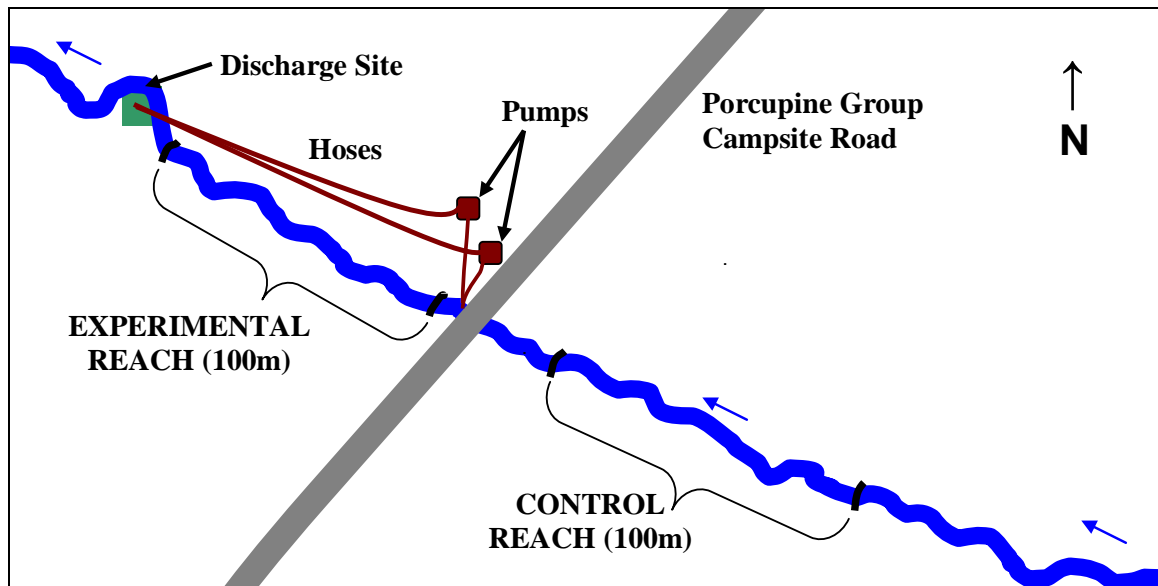
Figure 3-2 Experimental site layout depicting the position of the control and experimental reaches. Small arrows indicate stream-flow direction. Scale ~1:2500

3.3.4. Sampling

The experiment was conducted during the winter of 2008, commencing on Jan 16 and finishing on Mar 12 (Table 3-2). The experimental reaches at the study site were isolated with fish fences and the two reaches were electrofished, in three passes, to remove all resident brook trout. Brook trout were introduced to the study site from another homogeneous stream in order to ensure that the experimental fish used in 2008 were unaffected by experimental treatments that were implemented in the past at the study site. The experimental trout were captured for relocation from Canmore Creek (51º04'47.3"N; 115º22'46.4"W; elevation: 1375m) using a backpack electrofisher. A total of 70 brook trout were captured with a mean weight of 35.8g ±1.8 (±SE) and length of 159mm ±2.9 (±SE).

BIA readings were taken on the 70 sampled brook trout using the same method as that used on fish sampled to build regression models (see section 3.3.1). The fish were

transported to the study site in 125 L aerated water barrels from Canmore Creek. They were given one week to recover from sampling before the water withdrawal treatments commenced. BIA readings were taken again at the end of the experiment on the same fish to provide before and after resistance and reactance measures and ultimately before and after predicted body composition measures.

3.3.5 Water Withdrawal

Pumps and hoses

Water was withdrawn from the stream using two Rotating Right 10cm 8HP water trash pumps. The pumps were situated approximately 4m away from the stream on the right bank. Water was withdrawn with suction hoses that were positioned immediately upstream of the upper experimental fence. The water was routed through two 91m lay-flat hoses that ran parallel to the stream to the discharge site, below the lower experimental fence. The water ran out onto a tarp, where the water velocity was reduced before it ran back into the stream maintaining turbidity levels and preventing scouring of the stream channel (Figure 3-2).

Stream flow manipulation

The experiment ran for eight weeks (Table 3-2). During water withdrawal treatments approximately 75% of the total discharge was withdrawn from the experimental site for four hours daily, from 10:00 to 14:00.

Table 3-2 The experimental treatment regime schedule. Each treatment lasted a week, alternating from water withdrawal to no water withdrawal for 7 weeks. During treatments 1 and 5 only 6 days of data were collected because of weather and predator events.

2008 Date	Treatment	Duration (days)
Jan 16 - Jan 22	Acclimatization	7
Jan 23 - Jan 29	1: water withdrawal	6
Jan 30 - Feb 5	2: no water withdrawal	7
Feb 6 - Feb 12	3: water withdrawal	7
Feb 13 - Feb 19	4: no water withdrawal	7
Feb 20 - Feb 26	5: water withdrawal	6
Feb 27 - Mar 4	6: no water withdrawal	7
Mar 5 - Mar 11	7: water withdrawal	7

3.4 Results

3.4.1 Predicting Body Composition

Linear regression analysis was used to evaluate the relation between body composition measures, morphometric measures, and BIA readings. Water content and fat content were found to be inversely proportional with an R^2 of 0.82 (Figure 3-3).
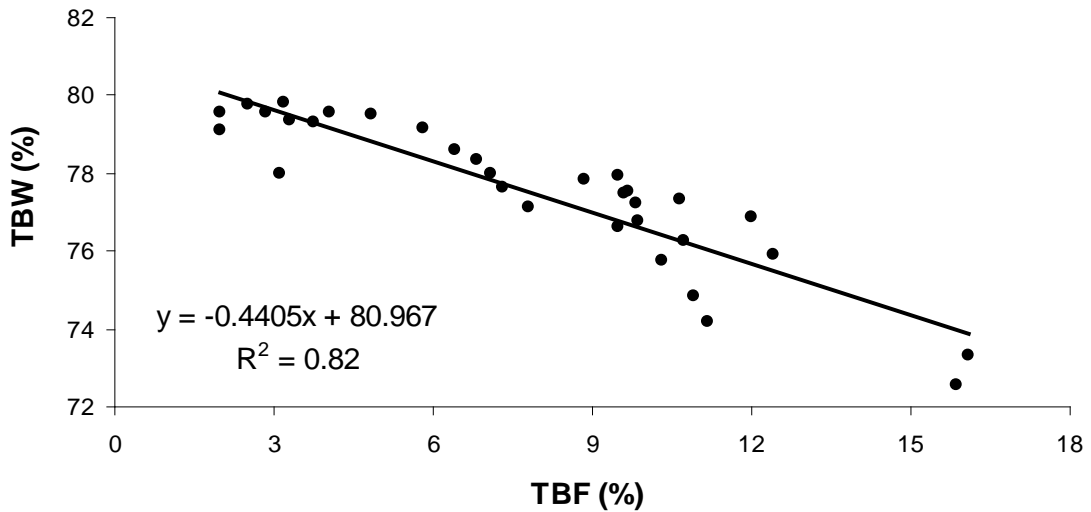
Figure 3-3 Linear relationship between percent total body fat (TBF) and percent total body water (TBW) of sampled brook trout. *n=32*

Morphometric measures of weight and length were significant independent predictors of

TBW and TBF (Table 3-3).

Table 3-3 Linear regression analysis of morphometric measures of brook trout as predictors of a) total body water (TBW) and b) total body fat (TBF). Weight (g) , Length: FL (mm), BIA length: distance between inner electrodes (mm), K: Fulton's condition factor (W/L³)*100,000. $n=32$

a) TBW

Morphometric Measure	Slope	Intercept	R^2	R^2 adj	SEE	p
Weight	0.054	75.627	.23	.20	1.69	.006
Length	0.042	71.237	.26	.24	1.65	.003
BIA length	0.053	74.666	.09	.06	1.84	.095
K	-7.321	84.610	.09	.62	1.83	.910

b) TBF

Morphometric Measure	Slope	Intercept	R^2	R^2 adj	SEE	p
Weight	-0.148	13.012	.41	.39	3.05	.000
Length	-0.114	24.897	.46	.44	2.92	.000
BIA length	-0.175	17.225	.23	.21	3.47	.005
K	11.115	-2.948	.05	.02	3.85	.217

BIA linear regression models were developed using bioelectrical impedance readings of

resistance and reactance and measured body composition. The impedance quotient (L^2/R)

was strongly related to total body weight ($R^2 = .95$) (Figure 3-4).
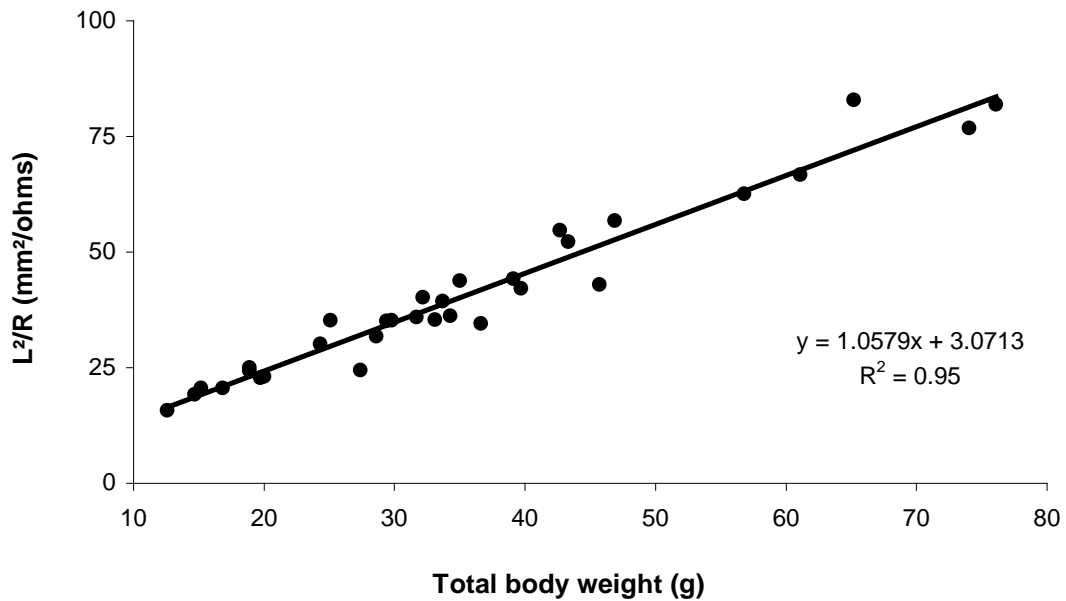
Figure 3-4 Linear relationship between total wet body weight and the bioelectrical impedance quotient, L: fork length and R: resistance of sampled brook trout. *n=32*.

Reactance was the best sole independent predictor of both TBW and TBF (Table 3-4) accounting for 72% of the total variability associated with both TBW and TBF (Figure 3-5).

Table 3-4 Linear regression analysis of impedance measures of brook trout as predictors of a) total body water (TBW) and b) total body fat (TBF). L: length (mm), R: resistance (Ω) measured in series, Xc: reactance (Ω) measured in series. *n=32*

a) TBW

Impedance Measure	Slope	Intercept	R^2	R^2 adj	*SEE*	*p*
R_m	-0.015	86.615	.473	.456	1.40	.001
Xc_m	-0.079	89.572	.719	.710	1.02	.001
L^2/R_m	0.062	75.005	.360	.339	1.54	.001
L^2/X_{cm}	0.019	74.492	.438	.419	1.44	.001

b) TBF

Impedance Measure	Slope	Intercept	R^2	R^2 adj	*SEE*	*p*
R_m	0.036	-13.824	.635	.623	2.40	.001
Xc_m	0.162	-16.921	.718	.708	2.10	.001
L^2/R_m	-0.159	14.242	.555	.540	2.64	.001
L^2/X_{cm}	-0.047	15.216	.617	.605	2.45	.001
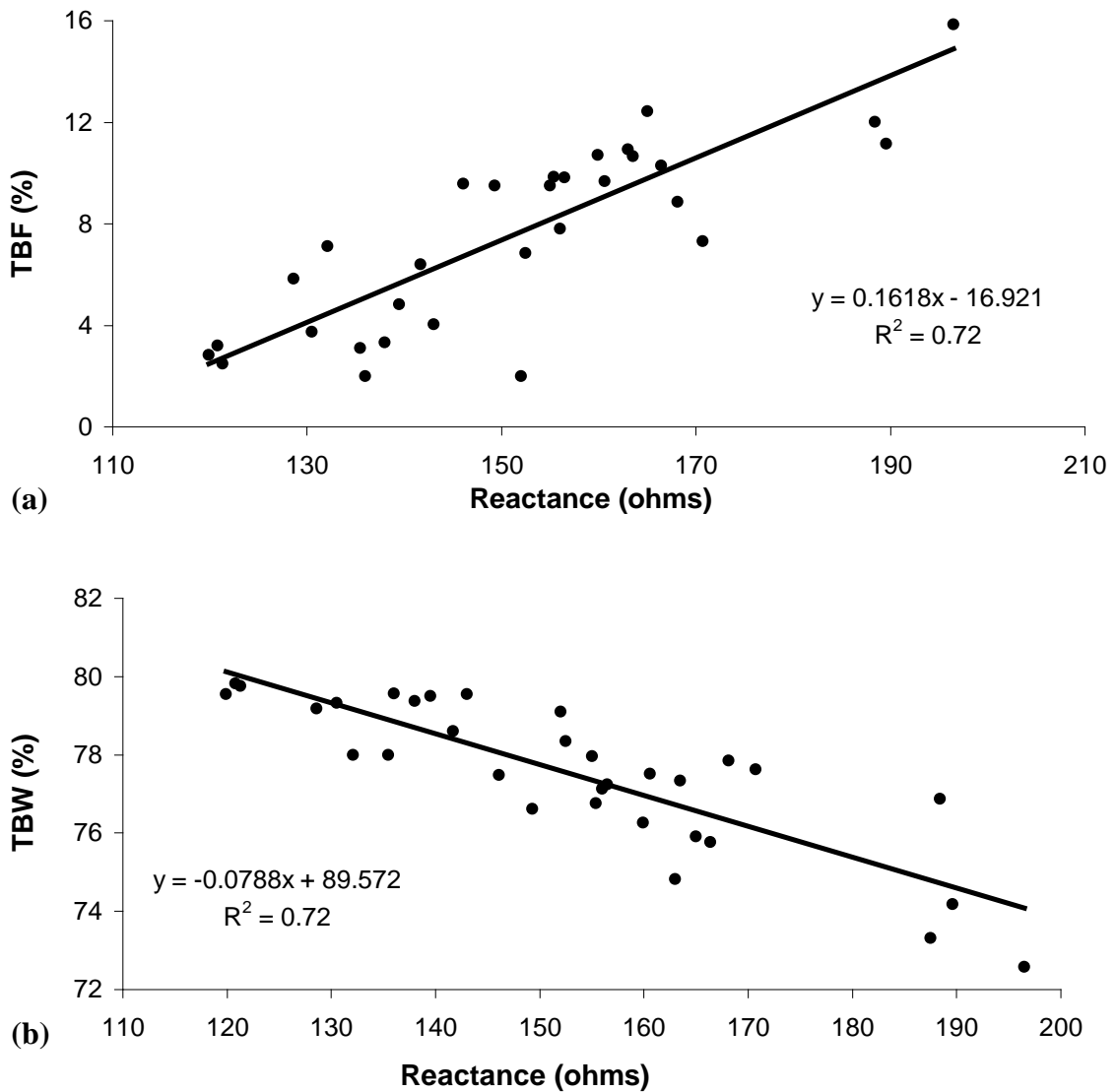
Figure 3-5 Linear relationship between reactance and (a) total body fat (TBF) (b) total body water (TBW) of sampled brook trout. *n=32*

The accuracy of statistically predicting TBW and TBF was improved by the inclusion of length to predict TBF [multiple linear regression, $R^2 = .77$, $F_{(2,29)} = 47.2$, $p = 0.001$; $y = (0.131* Xc) + (-0.048*L) -4.864)$] and Fulton's condition factor to predict TBW [multiple linear regression, $R^2 = .77$, $F_{(2,29)} = 49.8$, $p = .001$; $y = (-0.077* Xc) + (-$

113

5.166*K) +94.239)]. These multiple regression models were applied to predict body composition of experimental and control fish pre and post experiment (Table 3-5).

3.4.2 Changes in Proximate Body Composition

Winter

All sampled brook trout lost weight over the duration of the winter (repeated measures ANOVA: $F_{(1,52)} = 153$, p < .001). Bioelectrical impedance readings indicate that the fish generally gained water content and lost lipid content. There was a significant increase in predicted TBW (repeated measures ANOVA: $F_{(1,52)} = 7.12$, $p = .01$) of 1% and a decrease in predicted TBF (repeated measure ANOVA: $F_{(1, 52)} = 6.24$, $p = .02$) of 21% in the fish, over the duration of the experiment (Table 3-5).

Table 3-5 The changes in physiological condition of all sampled book trout from Jan 16-Mar 11 2008. Change in weight was calculated through actual before and after measures. Change in predicted total body fat (TBF) and predicted total body water (TBW) was calculated using BIA regression models.

		N	Minimum	Maximum	Mean	SE (±)
	pre	54	11.4	78.7	37.3	2.15
Weight (g)	post	54	10.6	71.6	33.5	1.87
	diff		*-0.8*	*-7.1*	*-3.8*	*0.31*
	pre	54	74.6	83.0	80.0	0.22
Predicted TBW (%)	post	54	76.9	85.0	80.7	0.25
	diff		*2.3*	*2.0*	*0.7*	*0.25*
	pre	54	-0.8	15.0	4.8	0.45
Predicted TBF (%)	post	54	-2.0	12.2	3.8	0.48
	diff		*-1.2*	*-2.8*	*-1.0*	*0.41*

Exposed to flow manipulations

There was no significant difference in weight (one-way ANOVA; $F_{(1,52)} < 1$), proximate TBF ($F_{(1,52)} = 1.01$, $p = .32$), or proximate TBW ($F_{(1,52)} < 1$) between the control and experimental fish at the beginning of the experiment. Both control and experimental fish lost a significant amount of weight over the duration of the experiment (repeated measures ANOVA, control; $F_{(1,28)} = 93.5$, $p < .001$, and experimental; $F_{(1,24)} = 62.9$, $p < .001$); however there was no significant difference in weight loss between the two groups (one-way ANOVA; $F_{(1,52)} = 2.07$, $p = .16$), nor was there a significant difference in predicted compositional variables between the control and experimental fish at the end of the experiment (one-way ANOVA; TBW: $F_{(1,52)} < 1$; TBF: $F_{(1,52)} = 1.02$, $p = .32$).

3.5 Discussion

3.5.1 Predicting Body Composition

There is a strong linear relationship between impedance measures of fish and proximate analysis variables: total body water, dry mass, fat-free mass, total body protein, total body ash and total body fat mass (Bosworth & Wolters, 2001; Duncan *et al.,* 2007). Within this study there was a decrease in impedance measures, both resistance and reactance, as TBW content increased and as TBF decreased, coinciding with the results of other studies (Bosworth & Wolters, 2001). Percent TBF was inversely related to both weight and length of individual fish and percent TBF was found to be positively

related to Fulton's condition factor. The larger fish tended to have more TBF; however they had less percent TBF than the smaller fish. There was an inverse relationship between TBW and TBF (R^2 = .82), similar to that found in other studies (Cunjak & Power, 1986a; Peters *et al.*, 2007).

Varying indices have been used in BIA studies to predict body composition. Strong correlations have been found between composition parameters and impedance measurements by using length and resistance measured in series to best predict TBW and total length and reactance in parallel to best predict TBF in brook trout (Cox & Hartman, 2005). BIA has been used to measure relative condition of bluefin tuna by calculating a composition index using length between electrodes and serial reactance (Willis & Hobday, In Press). Willis and Hobday, (In Press) suggest that it is appropriate to use reactance or resistance in parallel or series; furthermore they demonstrated that within their own study, composition determined by an impedance index, using reactance, was a good indicator of nutritional health.

For the purpose of this study, reactance in series provided the strongest single correlate to predict both TBW (R^2 = .72) and TBF (R^2 = .72). Morphometric measures such as length and width were also significant predictors of body composition parameters, albeit not as strong as impedance measures. Total body weight alone explained 23% of the variation in TBW and 41% of the variation in TBF. Total body length explained 24% of the variation of TBW and 46% of TBF. The relationship between Fulton's condition factor and TBF was very low with an R^2 of 0.05, coinciding closely with the relationship between condition factor and TBF (R^2 .03) documented in chinook salmon (*Oncorhynchus tshawytscha*) (Peters *et al.,* 2007). Indices based on

lengths and weights are not as accurate in assessing physiological condition as those based on proximate body composition (Simpkins *et al.*, 2003a), and therefore it has been recommended that fisheries scientist include percent lipid content as an index in determining the physiological status of trout (Simpkins *et al.*, 2003b).

Morphometric measures are not precise predictors of body composition on their own but when included with impedance measures, they play an important role in strengthening BIA regression models. Within this study length was included with reactance to predict TBF increasing the R^2 value to 0.77, and Fulton's condition factor was included with reactance to predict TBW increasing the R^2 value to 0.77. The variance explained in the regression models developed in this study are not as high as in the regression models developed by Cox and Hartman (2005) but are similar to those developed by Bosworth and Wolters (2001) who used reactance as an impedance measure to predict TBF ($R^2 = .75$) and TBW ($R^2 = .65$) in live catfish. The strength of regression models were found to become weakened when applied to catfish that had been filleted (Bosworth & Wolters, 2001).

3.5.2 Changes in Proximate Body Composition

Winter

Salmonid winter feeding is restricted because low water temperatures limit appetite, digestion, and gastric evacuation rates (Cunjak *et al.*, 1987) and consequently they have been shown to lose weight while they adjust to winter (Cunjak *et al.,* 1987; Simpkins & Hubert, 2000). In the winter, trout have to adopt strategies that minimize energy expenditures to economically utilize energy stores (Heggenes *et al.*, 1993);

therefore the energetic cost of a fish to move in the winter may be more pronounced than in the summer. Within the context of this study all of the 54 fish that were recovered at the end of the experiment lost weight over the study period. Because this study did not commence in early winter the preliminary weight measures do not represent pre-winter weight. Late winter weight loss may not be as pronounced as early winter weight loss, because early winter is a stressful period of acclimatization, during which lipid levels have been shown to be rapidly depleted (Cunjak, 1988); therefore the affects of environmental perturbations on fish may be more pronounced in early winter than in late winter.

BIA provided a non-lethal means in which to determine changes in proximate body composition of overwintering trout in this study. There was a small gain in percent TBW and, respectively, a loss in percent TBF of the control and experimental fish over the extent of the experiment. An increase in TBW in overwintering brook trout was also observed by Cunjak and Power (1986a) and lipid levels have been shown to rapidly decrease in early winter for brook and brown trout (*Salmo trutta*) (Cunjak, 1988). Within this study, the mean predicted TBF of the overwintering fish decreased by 21% over the eight weeks. A study evaluating overwinter brook trout lipid loss from October to April, a much longer duration than within this study, found that reproductive males lost 58% TBF, reproductive females lost 42% TBF and non-reproductive trout lost 24% TBF (Hutchings *et al.,* 1999). In addition, it was demonstrated that survival probabilities were negatively associated with average reduction in lipids (Hutchings *et al.,* 1999). Lipid accumulation is the most economical way for fish to store energy for the winter (Ultsch, 1989); therefore during periods of low water temperature salmonids primarily rely upon

stored fat (Pottinger *et al.*, 2003), resulting in a decrease of TBF. This tendency for winter fat loss is not always evident. The regression models built by Cox and Hartman (2005) were applied to measure changes in body composition of wild overwintering brook trout populations as well as to brook trout exposed to winter conditions in a lab and no significant changes in fat composition were detected over a nine week experiment (Webster & Hartman, 2007).

Exposure to flow manipulations

If the experimental fish were unable to habituate to the water withdrawal stressor, and instead had to compensate by adjusting physiologically or behaviourally, it would be expected that there would be an energetic toll affecting their growth, reproduction, and potentially survival (Scruton *et al.,* 2003). Rainbow trout (*Oncorhynchus mykiss)* growth rates have been shown to be significantly lowered when the fish were subjected to reduced discharge, potentially because of reduced drift feeding (Harvey *et al.*, 2006). In a lab setting, fish exposed to fluctuating flows and stable low flows had reduced feeding consumption and reduced growth in comparison to those held at stable high water levels (Flodmark *et al.*, 2004). Within this study, fish subjected to the stress of flow manipulations, did adjust behaviourally by being more active, potentially seeking out more hospitable habitats, but they did not lose significantly more weight or TBF or gain more TBW than the control fish not subjected to the stressor.

Limitations

There are limitations of BIA studies on the basis of their reliance on regression

models (Lukaski, 1999). Within this study there was a strong correlation between

reactance and TBF; however reactance readings may be more strongly related to other

unmeasured body composition parameters that also correlate with TBF. For instance,

reactance readings have been shown to be lower in humans with high extracellular water

(ECW) / intracellular water (ICW) ratio (Segal *et al.*, 1987), and a high ECW/ICW ratio

has been suggested to be an indicator of malnutrition (Kushner, 1992). In humans,

malnutrition is typified by changes in cellular membrane integrity and alterations in fluid

balance; therefore BIA readings can provide insight into nutrition status by indirectly

measuring these changes (Barbosa-Silva *et al.*, 2003). Generally high reactance indicates

better cell membrane integrity and health (Liedtke, 1997). TBF is a general indicator of

overall nutritional health (Peters *et al.*, 2007), consequently the high predicted TBF

readings, indicated by high reactance readings, may only be one of many confounding

but unmeasured biological parameters of fish condition, such as ECW/ICW ratios or cell

membrane integrity.

3.5.3 Conclusion

BIA is a convenient tool because it is safe, inexpensive, rapid and portable means

in which to determine proximate body composition (Kushner, 1992). Changes in percent

TBF and TBW content of overwintering brook trout were predicted using BIA reactance

measures. Reactance measures provided a non-lethal index to access the nutritional state

of fish that was more accurate in predicting body composition parameters than

morphometric measures of length, weight, or condition factor alone. BIA is a convenient tool in determining fish body composition and general health; however results are not always consistent and broad use of regression models may be limited. This study derived body composition regression models using a single fish species from a single geographic area in order to develop a model well suited to the experiment; however the strength of this model may be inappropriate for application in other research that does not examine small stream resident brook trout in the Rocky Mountains during the autumn and winter.

To summarize

1) BIA provides a relatively accurate means in which to measure changes in brook trout body composition accounting for 76.5% of the variability in TBW content and 77.4% of TBF. 2) Mean predicted TBW values increased in the brook trout over the duration of the experiment and mean predicted TBF values and weight decreased. There was no statistically significant difference detected between predicted proximate body composition measures of experimental brook trout subjected to flow manipulations and control brook trout.

Further research

It is important to further investigate the accuracy of using BIA to monitor fish physiology, by examining the relationship between impedance measures and body composition at different times of the year and over a range of life stages. It is also important to further research methods in which to ensure consistent impedance readings

including how readings are affected by the electrodes used, electrode placement, and air temperatures.

Examining the physiological implications of water withdrawal during winter on stream resident fishes is an important area for further study. To further the results of this study it is critical to examine streams that become ice covered, as well as stream reaches that do not provide complex habitat. In addition it is also important to further examine changes in physiology between male and female fishes at different life stages.

3.6 References

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Appendix: External Attachment of NanoTag Transmitters to Small Brook Trout (*Salvelinus fontinalis*)

Radio telemetry provides a means in which to pinpoint individual fish location at any given time providing immediate positional and behavioural data (Alfredsen & Tesaker, 2002; Lucas & Baras, 2000; Murchie *et al.*, 2008). Because of the cost of transmitters, studies using radio telemetry tend to have relatively small sample sizes and consequently, transmitter retention is very important. Transmitters can be attached in three main ways: surgical implantation in the peritoneal cavity, intragastric insertion, or external attachment (Winter, 1996). There is limited information on external attachment of transmitters because this attachment procedure is rarely employed (Bridger & Booth, 2003). The viability of externally attached transmitters can be compromised because of irritation of the dermal layers beneath the transmitter (Herke & Moring, 1999) and because the transmitter can become entangled in aquatic vegetation ultimately increasing mortality (Herke & Moring, 1999; Winter, 1996). Advantages of external attachment include quick attachment (Bridger & Booth, 2003; Cooke, 2003), and rapid recovery (Winter, 1996). The use of external transmitter facilitates studies on small fish or early life stage when the size of the body cavity precludes the use of internal transmitters (Cooke, 2003). In addition this method allows for quick attachment to fish exposed to cold temperatures during winter research.

The objective of this study was to determine if an external attachment method would be a viable means in which to attach a NanoTag transmitter to small stream resident brook trout during the winter.

Brook trout that weighed >20g were preferentially selected for the external attachment of radio transmitters whereby the weight of the transmitter did not exceed 2.5% of the total weight of the fish (Table A-1). Following the advice of (Brown *et al.*, 1999) the suggested 2% tag/body mass ratio (Winter, 1996) was not considered as a strict rule, but instead the smallest possible tag/body weight ratio was used by selecting the smallest available transmitters, with sufficient battery life, in order to minimize any behavioural or physiological responses the fish could have to the transmitters. The coded radio NanoTag transmitters (model NTC-3-M; Lotek Wireless Inc., Newmarket, Ontario) weighed 0.5g in air with dimensions of 7.2mm x 7.0mm x 14.5mm. The transmitters had a 10s burst rate with a 64 day 'calculated operational' longevity and a 51 day guaranteed longevity.

Table A-1 Summary of brook trout length (fork length; mm), weight (g), and tag ratio (transmitter weight to the corresponding fish weight; %) in 2007 (*n=20*) and in 2008 (*n=30*).

	Minimum	Maximum	Mean	SE
Winter 2007				
Length	131.0	189.0	155.1	±3.4
Weight	20.7	60.1	33.1	±2.0
Tag ratio	2.4	0.8	1.6	±0.1

|	Minimum	Maximum	Mean	SE
Winter 2008				
Length	160.0	198.0	180.4	±2.02
Weight	31.1	78.7	49.7	±1.99
Tag ratio	1.6	0.6	1.1	±0.04

The NanoTag transmitters were attached externally to 20 fish in 2007 and 30 fish in 2008. The transmitters were manufactured with a small straw (10mm in length) attached to the side of each transmitter, secured in place by heat shrink wrap. This was done by the manufacturer (Lotek Wireless Inc.) as a trial method to facilitate the external attachment of the NanoTag transmitters that were designed for internal insertion. The straw was threaded with 0.225mm diameter non-absorbable nylon suture thread (Supramid, S.Jackson Inc.; Alexandria, VA). Two small gauged sewing needles were then threaded onto each end of the suture thread in preparation for tagging.

The experimental trout were individually anesthetized using a 40ppm solution of clove oil and placed ventral side down on a wet sponge. The transmitter was attached by simultaneously pushing the threaded needles through the dorsal musculature of the fish and then through the holes in a plastic back plate situated on the opposite side of the dorsal fin (Figure A-1a). The needles were removed and the thread was tightened and tied off with a triple knot (Figure A-1b & A-2). The back plate was 1.5mm x 4mm x 16.5mm and was used in order to prevent tissue abrasion by the thread. Fish were released into two 100m isolated reaches within a small creek. They were monitored for six weeks during the winter of 2007 and eight weeks during the winter of 2008. At the end of each experiment the fish were captured to assess, transmitter retention dermal irritation, and transmitter fouling.
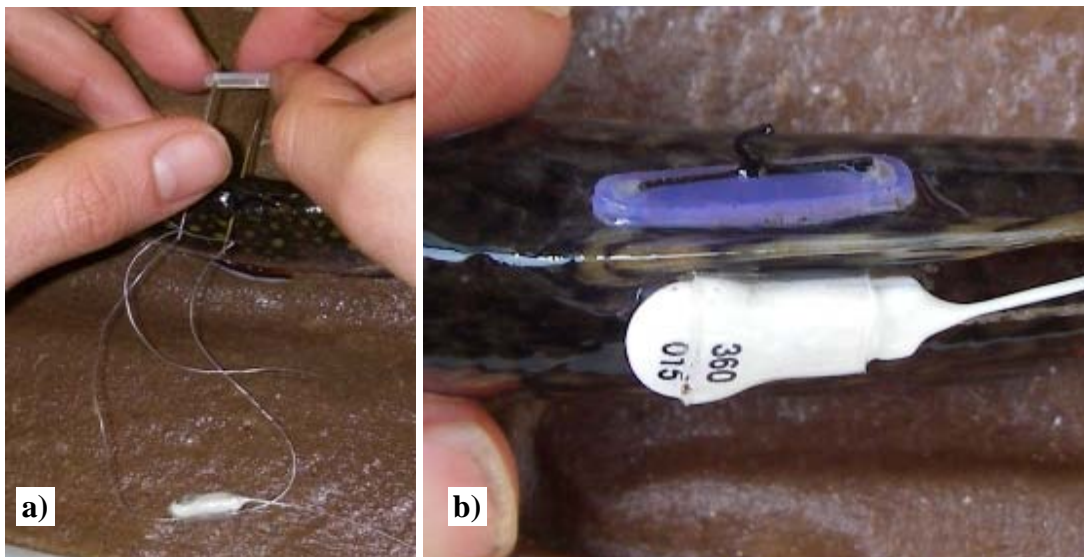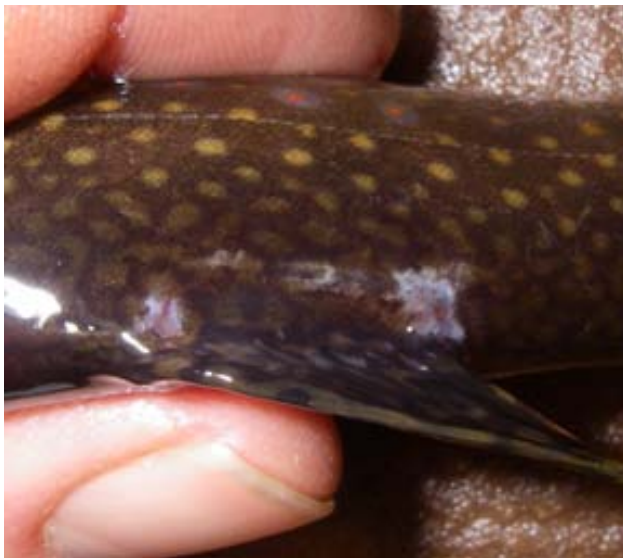

Figure A-1 Photos depicting the process of externally attaching a NanoTag transmitter onto a small brook trout a) during and b) after attachment
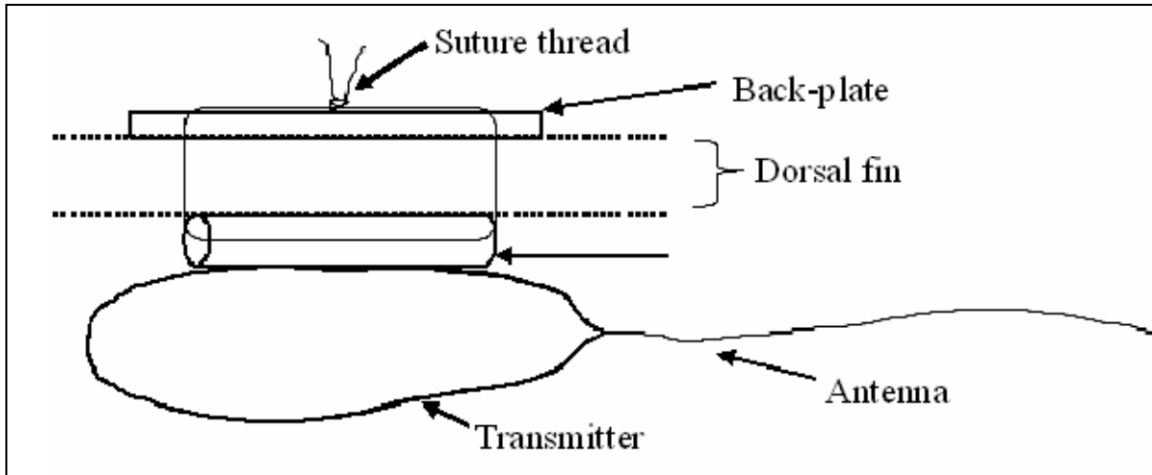
Figure A-2 External attachment diagram used to attach transmitter to small stream resident brook trout during the winter of 2007 and 2008

     One hundred percent of the transmitters stayed attached for the duration of the 2007 and 2008 experiments with limited dermal irritation (Figure A-3). The transmitters showed no signs of fouling resulting from vegetation entanglement. The external attachment method applied in this study was similar to that used in attachment of transmitters in other studies using large fish (Cooke, 2003; Crook, 2004; Mellas & Haynes, 1985); however this study addressed the use of external transmitters on small fish, where there has been limited research (Beaumont *et al.*, 1996). The attachment method used, within this study, provided a viable means in which to quickly attach small transmitters to small riverine trout during winter sampling.

Figure A-3 Dermal irritation on brook trout caused by six weeks of wear from an externally attached radio transmitters during the winter of 2007

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