Characterization of Spawning Habitat, Incubation Environment and Early Growth and Development in Bull Trout (*Salvelinus confluentus*) from Pristine Streams of Northern British Columbia

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Bull trout home to tributary streams in headwater areas for spawning. Site use can be influenced by habitat quality, including physical features such as stream flow, intergravel flow, hyporheic exchange, stream and intergravel-water chemistry, temperature and cover. These variables can influence survival and growth during incubation, however, the effect on bull trout spawning and incubation success is not known. I used three studies to examine the importance of spawning habitat for bull trout spawning in northern B.C. My results show that temperature is likely a key determinant of site use at potential spawning sites as well as incubation success in northern environments. Bull trout growth and yolk utilization were substantially altered by small changes in incubation temperature in both the field and the laboratory. In this context, fisheries and resource managers should take steps to prevent harmful alterations to bull trout spawning areas as the result of human activities.

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Acknowledgements

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CHAPTER 1: INTRODUCTION

Bull trout (*Salvelinus confluentus*) are a large piscivorous char (Family SALMONIDAE) found in cold rivers and lakes in western North America from Northern California to Alaska (Cavender 1978; Haas and McPhail 1991). Several characteristics of this species are thought to make it vulnerable to environmental change and ecological disturbance. Bull trout are known to exhibit aggressive feeding behaviours, have a propensity to hybridize with introduced eastern brook trout, (*Salvelinus fontinalis*) (Markle 1992; Kitano 1994) and show a distinct preference for relatively cold-water habitats (Baxter and McPhail 1996). As a result, bull trout are sensitive to overfishing, competitive exclusion and habitat disruption or loss (Fraley and Shepard 1989). Until recently, bull trout were not managed on the basis of species-specific requirements and as a result many populations are in decline and are considered endangered, at risk or vulnerable (Goetz 1989; Rieman and McIntyre 1995; McCart 1997; Swanberg 1997; Thurow 1997).

Bull trout exhibit homing behaviours and spawning-site fidelity (Pillipow and Williamson 2003; Bahr and Shrimpton 2004). They also appear to select specific microhabitats within particular stream reaches for reproduction (Baxter and McPhail 1999; Baxter and Hauer 2000). It is not clear, however, what advantages and features the selected sites confer in terms of survival and growth for eggs and alevins. Habitat features such as in-stream cover and groundwater influence may be important for spawning-site use. Groundwater and thermal habitat that is stable may be especially important for success of spawning and rearing (Goetz 1989; Baxter and McPhail 1999). In one northern British Columbia (B.C.) stream (Chowade River), Baxter and McPhail

(1999) found that bull trout used a single groundwater upwelling area for spawning despite the presence of other numerous potential spawning sites in an adjacent section of river. Microhabitat factors including stream flow, depth and substrate size were qualitatively similar between the selected and non-selected areas. Groundwater influence was cited as the factor explaining differences between habitats which included the thermal regime, vertical hydraulic gradient and intergravel flow.

The broad use of the term 'groundwater' in many studies has made it unclear, however, which attributes of groundwater are important in spawning areas. Several possibilities exist such as, absolute temperature, temperature differences from surface waters, flow, chemical constituents (including specific ions) and dissolved gases, particularly oxygen. These constituents can vary widely between sources of groundwater, whether it is deep sub-surface water (phreatic) or hyporheic in origin (Freeze and Cherry 1979). Phreatic groundwater is often devoid of oxygen; thus sources of pure phreatic water would be avoided by spawning fish. Hyporheic water, which is the zone of mixing between surface and ground waters, in contrast, can provide a thermally stable as well as an oxygen-rich environment ideal for spawning and incubation and is a zone of substantial biological activity. Mixing of the two sources is normal, with the depth of the hyporheic zone dependant on local sub-surficial stratigraphy (sediment types and sizes), phreatic groundwater flow and surface discharge. For bull trout it is poorly understood at which habitat scale, as well as which chemical and physical attributes of groundwater are important for site use and successful incubation. Baxter and Hauer (2000) analysed the relationship between hyporheic groundwater and redds (spawnng sites) for bull trout at several scales in Montana, USA. They found a strong link with

upwelling and redd distributions among reaches. Reaches are stream sections containing relatively homogenous habitat (e.g. pools and riffles) of similar gradients. In contrast with other studies, however, their data did not support the conclusion that upwelling is important at the habitat scale. Baxter and Hauer (2000) concluded that bull trout in their study area spawn in wide alluvial valleys within stream reaches that have a complex hyporheic interface characterized by extensive zones of upwelling sub-surface water. Specific redds sites were most often located in transitional zones between habitat units (e.g., pool-riffle transitions) with strong localized down-welling of surface water and high intergravel flow. Other features may also be important for spawning site use. Bull trout often spawn in clear, shallow headwater areas adjacent to cover and redds are often found adjacent to stream margins.

This specificity may make bull trout particularly susceptible to anthropogenic alterations in their environment. The decline in populations of bull trout in many jurisdictions appears to be linked to habitat changes brought about by industrial development as well as increased access to watersheds (Fraley and Shepard 1989; Ripley 2004). The threat to fish populations due to changes in the thermal environment caused by resource extraction and global warming, is not however, limited to bull trout. Rombough (1997) reviewed the effects of temperature changes on the development and growth of temperate fishes and concluded that temperate fish are generally sensitive to thermal changes in their environment. In another study, DeMarch (1995) demonstrated that Arctic char (*Salvelinus alpinus*), a coldwater specialist, incubated above 6°C had lower survival and growth compared to char incubated at 3°C. Thus, changes to streams that alter the thermal regime, particularly where fish demonstrate local adaptations and

low plasticity to large changes, have the potential to alter or reduce species distributions.

At present, many B.C. populations of bull trout remain unstudied and management decisions are made based on extrapolation of information collected from other districts. In addition, studies of bull trout habitat have been largely undertaken in the southern portion of their natural geographic range. Thus, the ecology and habitat requirements of northern bull trout populations are virtually unknown. Recent studies from outside B.C. have indicated that bull trout abundance can be linked to habitat variables in a hierarchical scale (e.g., Watershed, Reach, Site, Habitat) (Watson and Hillman 1997; Baxter and Hauer 2000). I suggest that extrapolation of these results be done with caution. Watson and Hillman (1997), for example, found that combinations of habitat variables that predicted bull trout abundance varied substantially between different watersheds over a comparatively small geographic range. Extrapolating knowledge of bull trout life-history and habitat requirements from southern areas of their range may lead to misquided management decisions for northern populations. In some areas of B.C. (particularly remote northern districts) bull trout are relatively abundant and opportunities exist to study their ecology in relatively pristine conditions. Comparatively, bull trout have not been studied as intensively as other salmonids (e.g., Pacific salmon and trout) and there are little data describing the habitat requirements and conditions for successful spawning, incubation and rearing. Furthermore, information describing these life-stages has been identified as a priority for future research (McPhail and Baxter 1996).

My objectives in these studies were to investigate how habitat influences spawning-site use, incubation success as well as, growth and development of embryonic bull trout from tributaries of the Finlay Arm of the Williston Reservoir, B.C. I used a

combination of field and laboratory studies to examine bull trout spawning and incubation habitat requirements. In my first study (Chapter 2), I described bull trout spawning habitat at the scale of individual redds. I focussed on stream and intergravel chemistry, sub-surface flow, temperature and stream features such as large woody debris and channel bed-forms. In my second study (Chapter 3), I examined growth and development of larval bull trout in the context of habitat in the field. I recorded size (length, body weight) and yolk utilization at several sites used and not used for spawning. I also recorded stream and intergravel chemistry, temperature and intergravel flow to describe factors contributing to variation in size within and among sites. Finally, to evaluate the effect of temperature on the growth and development of larval bull trout (Chapter 4), I incubated eggs and alevins under controlled laboratory conditions in a natural and two altered temperature regimes. For this study, I measured survival, size and the timing of development under the three temperature treatments. Conclusions derived from data collected in Chapter 2-4 are presented in Chapter 5 and were used to generate a better understanding of the reproductive and early life history habitat requirements of northern populations of bull trout. This information can support the effective management of these populations.

CHAPTER 2: GROUNDWATER AND THE INCUBATION ENVIRONMENT FOR BULL TROUT IN PRISTINE NORTHERN STREAMS[1].

Abstract

Bull trout are known to home to, and spawn in, specific stream reaches in headwaters. It is not well understood, however, what habitat features are important for successful incubation and survival of incubating bull trout in northern environments. To assess distribution and habitat characteristics of bull trout redds, we employed a synoptic study of the physical habitat of bull trout redds in high-use spawning tributaries within the Finlay reach of the Williston watershed of B.C. We described bull trout spawning sites located in headwater depositional areas influenced by relatively warm groundwater and cold winter temperatures approaching freezing. Individual redds were often associated with at least one type of security cover, high levels of intergravel flow and oxygen, as well as cold thermal regimes intermediate to what was available. Individual redds were often clumped with other bull trout redds. Clumped redds were associated with warmer areas of up-welling, however, in general, redds were located in areas that exhibited both up- and down-welling.

[1] Throughout this chapter I use the first person plural to acknowledge the contribution of others to this work, which will be submitted for publication with the authorship of C.J. Williamson, J.T. Zimmerman and J.M. Shrimpton.

Introduction

Large migratory bull trout home to smaller streams and headwater areas to spawn (Goetz 1989; Baxter and McPhail 1996; Swanberg 1997; McPhail and Baxter 1999; Baxter and Hauer 2000; Pillipow and Williamson 2003; Bahr and Shrimpton 2004; Scholz 2005). Migrations to and from spawning habitat can be extensive and can exceed distances over 500 km (e.g., Pillipow and Williamson 2004). Migrations over long distances to spawning areas in other salmonids are well known and have been particularly well studied in Pacific salmon (*Oncorhynchus sp.*) (e.g., Groot and Margolis 1991). Homing involves behaviours that enable adult salmonids to return to high quality spawning habitats with accuracy (Ditman and Quinn 1996), despite long-distance migration through sometimes complex habitats.

Based on genetic evidence (Thomas et al. 2001; Spruel et al. 2003) and movement studies (Carson 2001; Bahr and Shrimpton 2004; Pillipow and Williamson 2004; and see Scholz et al. 2005 for a thorough review) migratory bull trout appear to home with precision to natal areas to spawn, where spawners show a high degree of fidelity to spawning sites within stream reaches. Collectively, these studies suggest that bull trout are using an adaptive mechanism to seek and select high-quality, reproductive habitats possibly as a means of optimizing juvenile survival or growth. The specific mechanism for homing in bull trout has not been identified; however, in Pacific salmon (*Oncorhynchus sp.*), behaviours involved in homing are dependant on olfactory and visual imprinting at an early life stage. Imprinting can sometimes occur before hatch, but usually takes place during significant phases of development including outmigration from natal to rearing habitats (reviewed by Dittman and Quinn 1996). Environmental and

chemical gradients provide the basis for imprinting and subsequent discrimination between habitats during spawning migrations. Successful imprinting by juveniles and subsequent homing of mature adults to natal habitat for spawning allows salmonids to return to specific rivers and stream reaches where site selection, based on microhabitat variables, can potentially occur. For bull trout, the behaviours that are involved in homing to natal rivers, to tributaries and then finally to spawning sites are likely dependant on gradients in habitat quality at multiple spatial scales from the river or basin scale down to the scale of local habitat (Baxter and Hauer 2000).

In northern latitudes, air and surface-water temperatures below 0°C can limit the distribution of fishes (Power et al. 1999). Groundwater, however, is often warmer in winter than surface water, is less subject to diurnal, seasonal and even annual fluctuations compared with surface water; thus it can provide a stable thermal regime or refuge for different life stages including incubation. Predictability in environmental conditions afforded by stream systems mediated by groundwater (including stable or warm thermal regimes) may provide a basis upon which salmonids have developed adaptive mechanisms to maximize survival and growth through and beyond the embryonic stages. Areas of groundwater may also provide a consistent and distinct chemical signature that aids homing by returning adults.

Groundwater may be especially important in environments where low temperatures result in the extensive formation of ice on the stream-bed (anchor ice) and it has been commonly suggested that upwelling groundwater is important for successful spawning and rearing of larval and juvenile bull trout as well (see reviews by Goetz 1989; Baxter and McPhail 1999). Anchor ice formation in redds results freezing of eggs

and larvae. In one northern B.C. stream (Chowade River), Baxter and McPhail (1999) found that bull trout concentrated at a single groundwater upwelling area for spawning despite the apparent presence of other numerous potential spawning sites in an adjacent section of river. Groundwater influence, particularly upwelling at the site level, may not be the only determinant for spawning site use by bull trout. Several of the studies that have examined groundwater influence on spawning-site use by bull trout have noted the relative contribution of upwelling subsurface water, but have not examined the influence of security cover, hyporheic exchange, vertical hydraulic gradient and local thermal regime on site selection. Each of these features is an important indicator of habitat quality that may dictate site use by spawners.

There are few studies describing the natural incubation environment experienced by salmonids in northern environments during winter. Our objectives for this study were to identify bull trout-spawning sites in tributaries to the Finlay Reach of the Williston Reservoir B.C., characterize stream habitat where redds were located, and describe the physical and chemical environment experienced by incubating eggs and larval bull trout. Streams surveyed for this work are within the northern part of the natural range for bull trout (Haas and McPhail 1991). Information on bull trout spawning habitat is important for the management of spawning areas and will be generally applicable for the protection and management of fish habitats.

Materials and Methods

Study Area

The Finlay reach of the Williston Reservoir in north central B.C. supports several robust bull trout runs that spawn in streams draining the Rocky and Omineca mountains.

Prior to the construction of the W.A.C. Bennett dam on the Peace River, bull trout in this watershed were presumed to have exhibited a primarily fluvial life history where spawning took place in the smaller tributaries with rearing in large rivers, although it is possible that spawning could have taken place in the large rivers as well. At present there does not appear to be spawning habitat in the Williston Reservoir and directed migration followed by spawning has been observed in headwaters of tributaries throughout the watershed (e.g., O'Brien and Zimmerman 2000). Three of these systems, Davis River, Chowika Creek and Swannell River were used in this study (Fig. 1). The Davis River was the primary location for this study and subsequent chapters and has been the subject of ongoing bull trout monitoring and research activities (O'Brien and Zimmerman 2000) since 1998.

Spawning activity by bull trout in the Davis River peaks in early September and spans several weeks (O'Brien and Zimmerman 2000). The Davis River is a third-order stream with a drainage area of approximately 49,000 ha. Chowika Creek is located directly north of the Davis River and has a similar aspect, size and drainage area to the Davis River. Both streams flow westward from the front ranges of the Muskwa Mountains (Northern Rocky Mountains) into the northern end of the Finlay Reach of the Williston Reservoir. The Swannell River drains 104,000 ha from the eastern slopes of the Swannell Ranges of the Omineca Mountains. The Swannell River flows into the Ingenika River, which is the second largest tributary flowing into the Finlay reach of the Williston Reservoir. The study reaches used in all three streams are in pristine condition with no direct industrial development (e.g., forestry, mining) and there are no roads in the headwater spawning areas. All sites were accessed by helicopter.

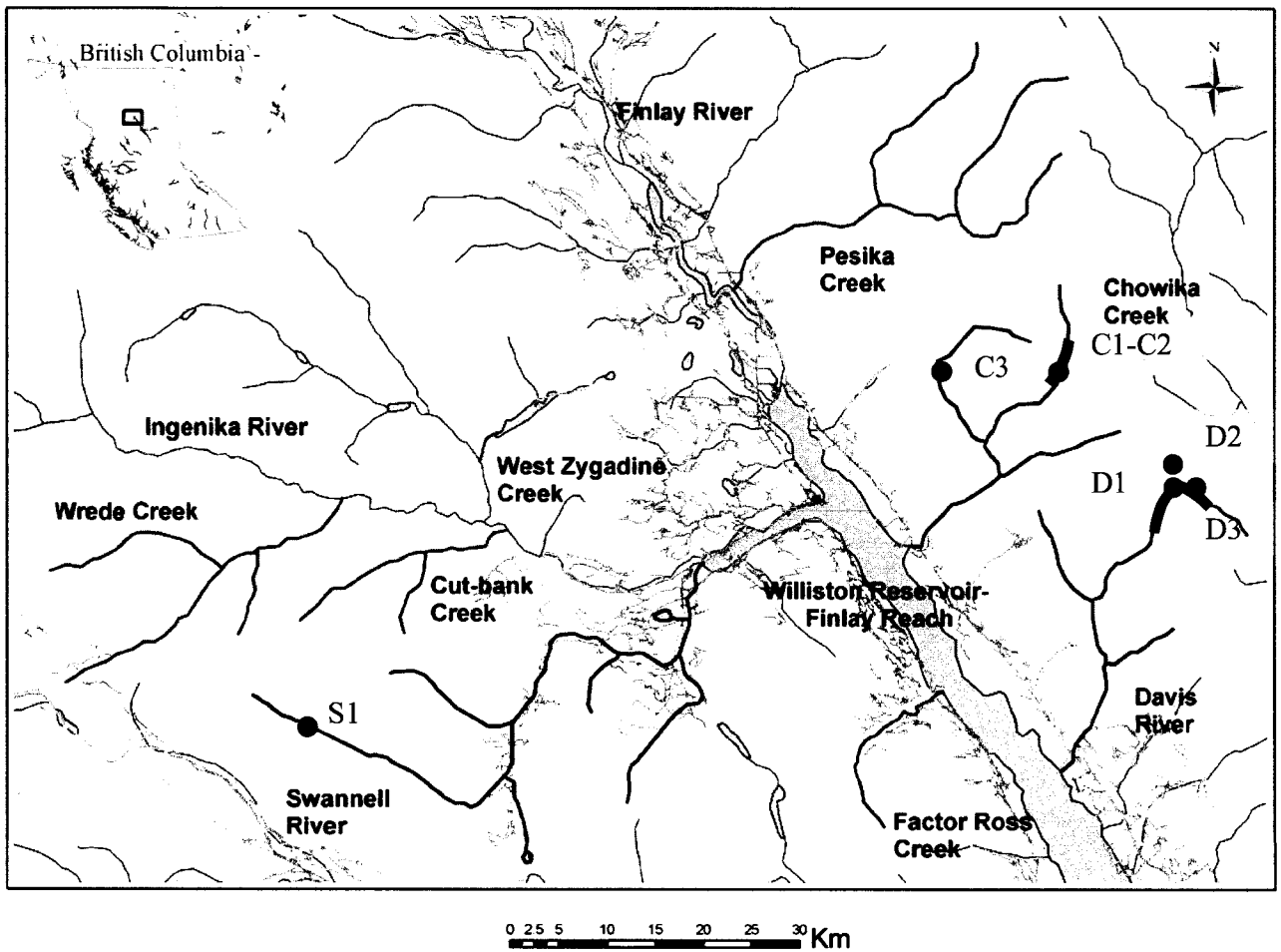


Figure 1. Map of study area showing the three streams used for the redd surveys (dark blue stream lines) and streams surveyed but not used (green line). Artificial incubation sites are plotted as "•" in Chowika Creek, Davis River and Swannell River. High-use spawning areas are shown as a thick dark-blue line. The road network is plotted in orange. The inset map shows the study area outlined inside the red box.

Stream Surveys

To assess distribution and habitat characteristics of bull trout redds, we employed a synoptic approach to find streams that could be considered high-use spawning areas for bull trout. One reach, we selected was located in the upper Davis River watershed (Figs. 1 and 2). The importance of this spawning location was established following the completion of a radio telemetry study that determined that a large proportion of the radio-tagged bull trout returning to the Davis River spawned in 5-km long reach (Zimmerman and O' Brian 2000). Additionally, since 1998, the B.C. Ministry of Environment and the Peace Williston Fish and Wildlife Compensation Program have completed aerial redd counts for bull trout in this section of the upper Davis River on an annual basis to index population trends in the Williston watershed.

In July and August of 2000, we examined seven streams from the vicinity of the Finlay Arm of the Williston Reservoir that were of similar magnitude and order to the upper Davis, including Chowika Creek, Pesika Creek, Factor Ross Creek, Swannell River, West Zygadine Creek, Wrede Creek and Cut-bank Creek for potential as spawning areas. We used several criteria to qualitatively establish whether a site had potential as a bull trout spawning area and could be used in the habitat study. These included: aerial over-flights to determine ease of site access for completing our investigation and to assess spawning-habitat potential; visual ground-based surveys to establish presence of bull trout juveniles in typical juvenile habitat in pools and back-channels; snorkel surveys to locate concentrations of adult and juvenile bull trout; minnow trapping to collect juveniles; and placement of temperature data-loggers to establish whether stream temperatures were within the range used by bull trout.

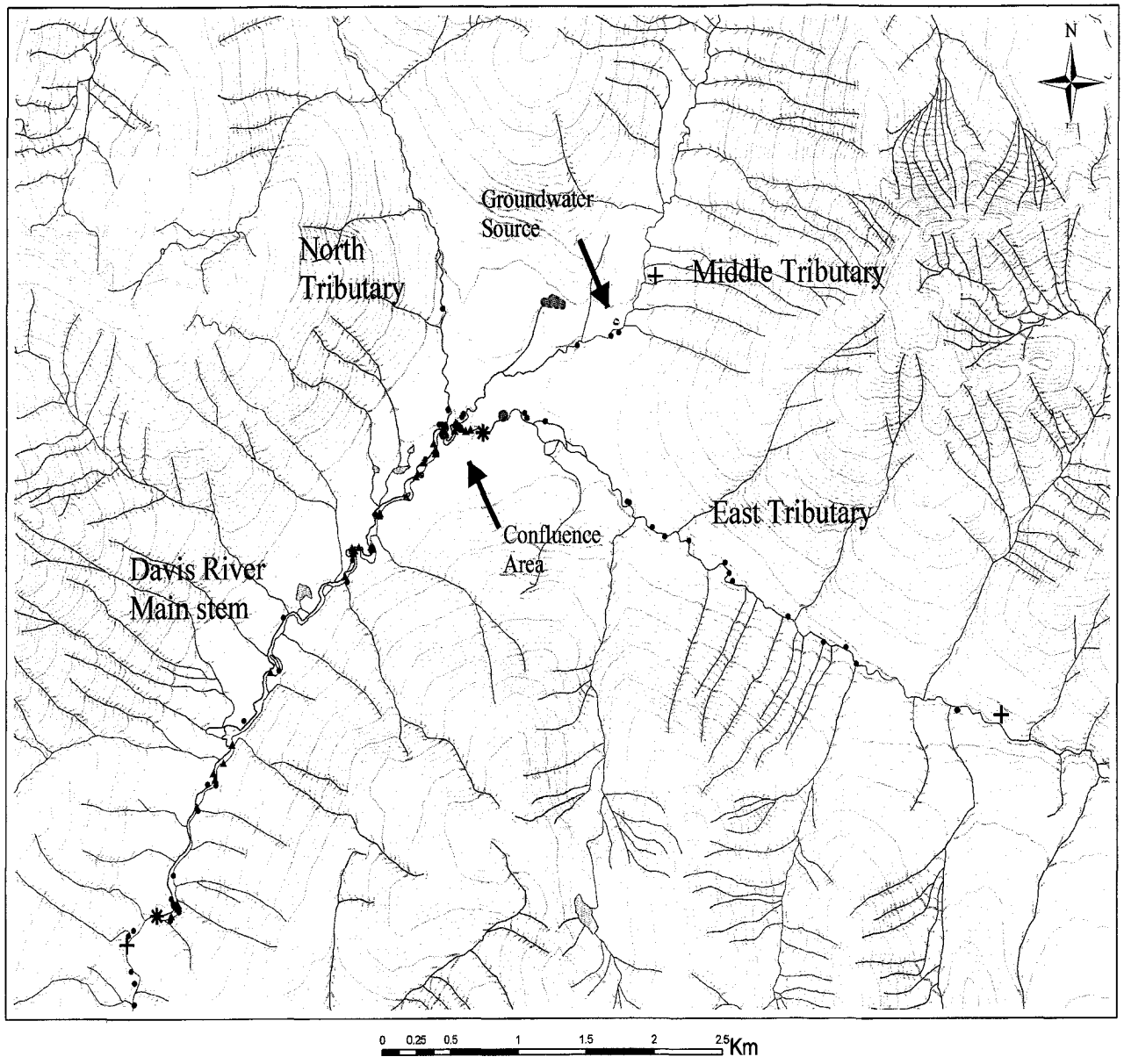


Figure 2. Map of redds surveyed in the Upper Davis River showing the survey limits for aerial and ground-based redd surveys completed in fall 2001 and fall 2002. The "plus" denotes the up and downstream limits of the detailed aerial surveys for 2001 only. The "star" denotes the up and downstream limits for both ground surveys. Circles and triangles represent redds observed in fall 2001 and fall 2002 respectively. Red symbols represent 2001 surveys, Blue symbols represent the 2002 surveys. Surveys completed in 2002 were not analyzed as part of this thesis and are presented for comparison only.

Following these assessments, two additional stream reaches were selected; one in the upper Swannell River (Fig. 1) and one in Chowika Creek (Figs. 1 and 3). We selected both the Swannell River and Chowika Creek as sites for this study because both streams were similar in size, both supported juvenile bull trout and both had areas that were similar to the high-use spawning area in the Davis River. The upper Swannell River, however, was only examined in fall 2000 due to a lack of bull trout redds, in addition to the difficulty in visually identifying potential redds or disturbed areas at this site.

Redd Habitat Characteristics

We completed redd surveys on the ground in fall 2001 in the upper Davis River to assess habitat conditions within redds. The surveys were completed between river km 42 and 47 (Fig. 2). Over a two-day period, a sub-set of the total redds counted on the ground were physically surveyed to document redds through a greater length of the study reach. We assessed habitat within the intergravel environment as well as habitat adjacent to the redds. Bull trout are the only large salmonid known to spawn in fall in the upper Davis, Chowika and Swannell watersheds (Unpublished Data, Ministry of Environment, Prince George, B.C.). Bull trout redds, therefore, were identified by areas of "cleaned" gravel with a distinct depression and tail-spill area. "Cleaned" areas on the streambed were created when periphyton bearing substrate was moved downstream as the result of digging by the female. Redd digging resulted in patches of "clean looking" gravel surrounded by olive- or brown-stained substrate.

We surveyed redds on foot, working downstream from the upper part of the study area. A UTM grid coordinate was recorded with a global-positioning system unit (Garmin Inc. GPS Map 76S) for each spawning site. A series of habitat measures that

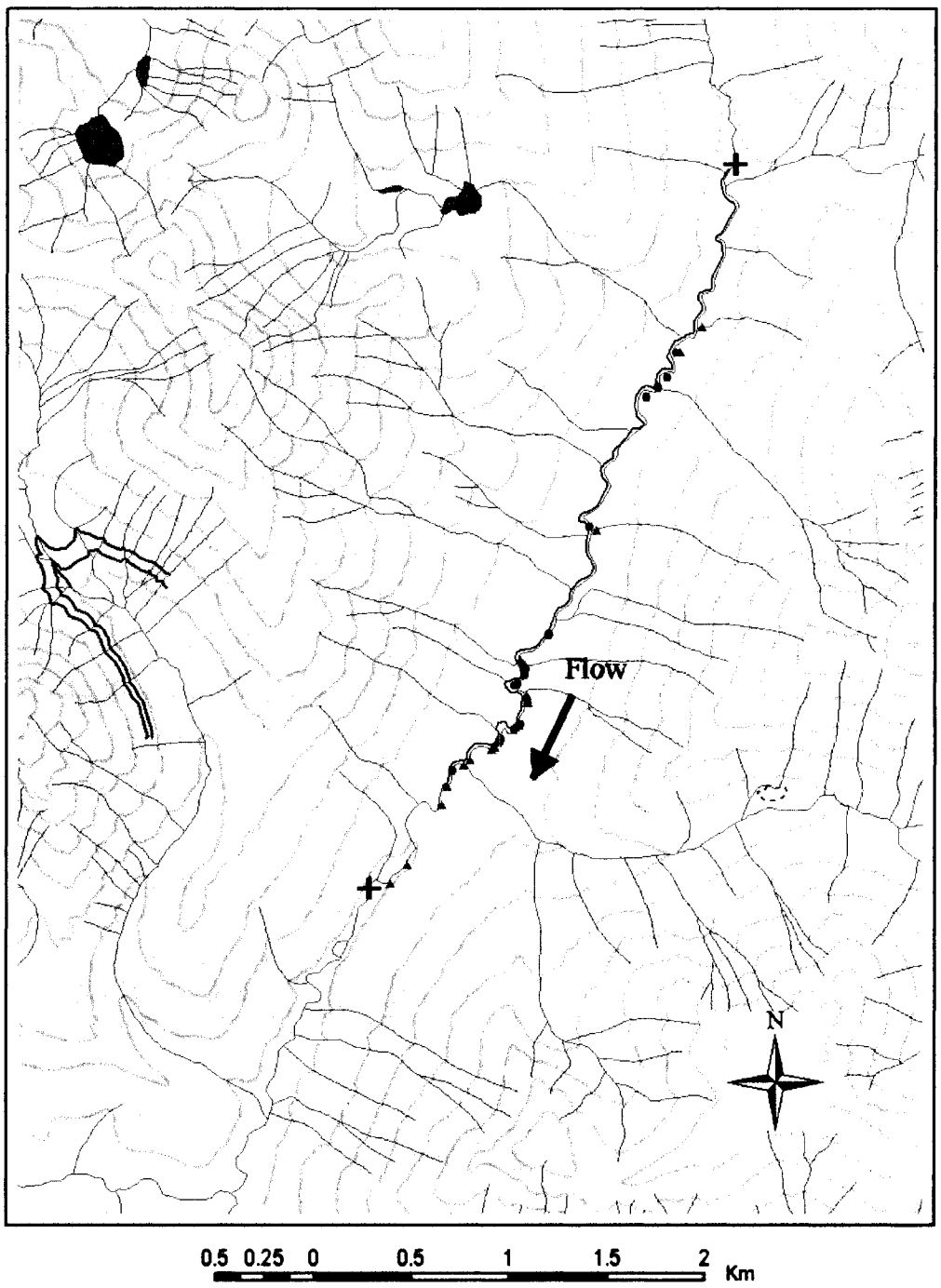


Figure 3. Map of redd distributions in high use spawning area in Chowika Creek for 2001-2002. The "plus" denotes the up and downstream limits of the detailed survey. Circles and triangles represent redds observed from the air in fall 2001 and fall 2002, respectively.

included redd dimensions and area, stream depth, stream velocity, stream temperature, oxygen, pH and conductivity were recorded at each site. To save time during the ground survey and to extend the distance covered through the spawning reach, we sub-sampled redds for physical and chemical measures on the basis of aggregations. Where there were redds separated by only a few metres, we typically sampled only one redd. We noted redd position relative to the stream margin and prominent cover features such as pools, under cut-banks, overhead vegetation, and woody debris. We recorded redd dimensions as the spatial extent of the disturbed area at redd locations. The disturbed area at redd sites was typically elongated along the direction of stream flow forming an ellipse shaped redd. Where spawning sites were superimposed, we attempted to delineate each individual redd. Area was approximated from the formula for calculating the area of an ellipse $AB\Pi \cdot 4^{-1}$, where A is the length of the long axis and B is the width of the redd. We measured stream depth to the nearest mm at the leading edge of each redd with a metre stick. We also recorded stream velocity ($m \cdot s^{-1}$) at the leading edge of each redd. Stream velocity was collected at a depth 15-cm off the stream-bed to approximate the nose velocity of a fish holding in the current over the redd site. We used a current meter counter with a Price AA type current meter head (CMC-20, Hydrological Services Ltd, AU) to measure flow velocities. We recorded temperature and oxygen concentration ($mg \cdot l^{-1}$ and percent saturation) directly from the stream at each site using portable electronic meters (Handy MK II, Oxyguard International A/S, DK.; Multiline P4, WTW GmbH, DE). We calibrated oxygen meters to local elevation and barometric pressure according to the manufacturer's procedures. Water samples were also collected from each site and conductivity as well as pH were measured in the laboratory (Model 744,

Metrohm Ltd, CH). Conductivity and pH meters were calibrated to known standard solutions before the samples were analyzed.

Intergravel Redd Characteristics

We constructed mini-piezometers (Lee and Cherry 1978) to measure intergravel temperature, water chemistry, vertical hydraulic gradient and to calculate hydraulic conductivity at redd sites. Our mini-piezometers followed the design used by Geist et al. (2000), incorporating the following modifications as suggested by D. R. Geist (pers. comm; Battelle, Pacific Northwest National Laboratory, Richland, Wa.). We constructed our mini-piezometers from a 1.5- to 2-m length of 3.1-mm inside diameter, 6.4-mm outside diameter poly-vinyl-chloride (PVC) tubing attached to a 10-cm section of perforated polyethylene tubing (6.4-mm inside diameter, 9.5-mm outside diameter) containing 12, 3.2-mm- diameter holes. The lower end of the perforated well tip was attached to a machined, brass drive-point and then was wrapped with an 11x10-cm section of 240-μm Nitex ® screening that was fastened with hot-melt glue.

To collect water adjacent to egg pockets within redds, we inserted our mini-piezometers into the streambed using the methods described by Lee and Cherry (1978) to a depth of approximately 30 cm with the first well openings situated at a depth of approximately 20 cm. Each piezometer was installed as close to the edge of a redd site as practicable but were not installed directly in a redd tail-spill (location of egg pockets) to minimize direct disturbance to bull trout eggs. We collected water samples from the piezometers with a 125 ml, metal ear syringe with an elongated tip that fit easily inside the PVC tubing. Where possible, we extracted two volumes of the syringe from each piezometer before collecting samples in order to clear water stored in the piezometer, as

well as to clear water from the disturbed area immediately surrounding the piezometer tip. We collected measures of temperature, oxygen, conductivity and pH as described above.

Hyporheic Exchange

We collected two measures of hyporheic flow at each site. First, we used vertical hydraulic gradient (VHG; unit-less measure) as a measure of the extent of upwelling or downwelling water within each redd that was calculated using the following equation:

$$VHG = dh/dl$$

where *dh* is the difference in pieziometric head between the water level in the piezometer and the surface of the stream and *dl* is the difference in height between the stream bed surface and the first well opening in the piezometer tip. We measured head differential with the aid of a manometer (Lee and Cherry 1978) mounted to half (50 cm) of an aluminum metre stick. We mounted a rubber photographic lens-cleaning bulb to one end of the manometer as a suction device for drawing water from the piezometer and stream surface into the manometer.

We used a falling head test (Lee and Cherry 1978) to approximate hydraulic conductivity (K) of the sediment around redds. For the falling-head tests, we used a graduated, 60-ml plastic syringe as a reservoir. The locking tip was bored out of the syringe such that the tubing leading from the piezometer could be inserted inside the outer sheath of the syringe tip without constricting the inside diameter of the piezometer tubing. For each test, a timed 50-ml volume of water was allowed to pass though the through the syringe into the piezometer tube (Lee and Cherry, 1978).

We calculated hydraulic conductivity using the following equation (Hvorslev

1951; from Lee and Cherry 1978):

$$K_h = \frac{q * \ln[(mL \div D) + 1 + (mL \div D)^2]^5}{2\pi L H_C}$$

where

D = diameter of intake
L = length to intake
H_C = pieziometric head (cm)
q = flow of water ($cm^3\ s^{-1}$)
m = transformation ratio (K_h / K_v) assumed to be 1 as suggested by Lee and Cherry (1978).

Estimates of the horizontal hydraulic conductivity K_h were used to calculate the vertical component of water flow (v; specific discharge $cm \cdot s^{-1}$) through the substrate surrounding bull trout redds using Darcy's equation ($v = K_v \Delta h / \Delta l$) where $\Delta h / \Delta l$ is VHG measured as the pieziometric head at each redd site (Freeze and Cherry 1979). For calculations of specific discharge we assumed isotropic flow where the vertical and horizontal components of hydraulic conductivity are equal.

Temperature

Following aerial redd surveys in 2000 and aerial and ground surveys in fall 2001, we installed data loggers that automatically recorded temperature (Optic Stowaway Temp Logger, Onset Computer, Bourne, MA) in the intergravel environment immediately adjacent to a selection of bull trout redds to monitor incubation temperatures. We placed the data loggers at approximately 25- to 30-cm depth in the gravel to record temperature at the depth of the egg pocket. We installed four data loggers at redd sites in the fall of 2000 in the upper Davis River; three were installed near a high-use spawning area, and one was installed in the Upper Swannell River at the only redd site we located in that stream that year. In fall 2001, we installed six dataloggers adjacent to redds in the upper

Davis River and two in the upper Chowika Creek for comparison. We programmed temperature data loggers to record hourly temperatures. For both years of the study, we installed all loggers at natural redd sites at the conclusion of ground-based surveys in the last week of September and after spawning was complete. The data loggers were left to record data until the following summer.

Data Analysis

To examine the relative distribution of redds in the Davis River, we plotted each spawning location at a scale of 1:20 000, using a B.C. provincial government GIS system (ArcMap 9.1, ESRI, Redlands, CA). After mapping, we grouped redds into sites according to their proximity to other redds, where a site was defined as a collection of redds that were no more than 50-m distant from any redd in the group. We then calculated descriptive statistics for the mean number of redds at each site. We also calculated descriptive statistics for habitat measures at each redd, including means, standard deviation and minimum as well as maximums (SPSS v.11.5, Chicago, IL; Excel 2003, Microsoft Corporation, Redmond, Wa.) for all measured and calculated variables. Stream and intergravel temperatures were collected over the course of two days at different times of the day for each redd site. As a result these measurements may not be directly comparable between sites due to daytime heating of the stream. To account for daytime heating during the surveys, we corrected stream temperatures to a fixed time and day using hourly stream temperatures recorded by a data logger installed by the B.C. Ministry of Environment in the upper portion of the high-use spawning area. We used the corrected measurements for the analysis of redd results. We examined the corrected stream temperatures statistically for normality and compared redd temperatures to

corrected stream temperatures using a paired-sample t-test (SPSS). We also used single-sample t-tests to compare pH, specific conductance with known values in the stream to test for differences between the stream and intergravel waters. We also tested the hypothesis that absolute specific discharge within the redds was significantly different from zero.

To compare thermal regimes at individual redd sites, we tabulated the descriptive statistics for electronically logged temperature data for each site by month and by season. We then summed mean daily temperatures cumulatively for each site to calculate accumulated thermal units (ATU) during the putative incubation period. We used temperatures from October 30 and the estimated date of emergence of June 14 (see Chapter 3) for this comparison. These values potentially underestimate the actual total ATU as the loggers were installed approximately two weeks after the peak spawning period estimated by Zimmerman and O'Brien (2000), and the actual date for egg deposition at each site is unknown.

Results

We observed the highest densities of bull trout redds in the headwaters of both the Davis River and Chowika Creek (Fig. 2 and Fig. 3). Our results are based on formal ground-based surveys as well as visual observation made from the air during flights to and from the study area. We only observed one redd in the Swannell River. The reaches in the Davis River and Chowika Creek are hereafter described as high-use reaches. The reach examined in the Swannell River is hereafter described as a low-use reach. Thirty-nine bull trout redds were observed on the ground in the high-use spawning area in the upper Davis River in 2001 (Fig. 2), however, redds were clumped together in a number of

sites. Sixteen sites were identified containing from one to six redds ($\bar{x}$ = 2.4; s = 1.9). Within these sites, redds were typically aggregated, where 79% (30) of redds were located at only 50% of the sites. The mean number of redds at sites with more than one redd was ($\bar{x}$ =3.8; s = 1.8). At two sites super-imposition of redds was observed; each of these locations contained six redds.

Habitat information, including cover type and location relative to the stream margins was recorded for 37 redds. Redds were categorized as stream edge (< 3 m from the stream edge) or stream center (> 3 m from the stream margin). All but three of 37 redds (91.7 %) were located near the stream margin. Of the habitat cover types, woody debris and stable under-cut banks were numerically the most common security cover types (Table 1). Only four redds (11%) were not associated with obvious cover and all four were found in open, relatively shallow areas. In contrast, almost 90% of redds were associated with at least one of three in-stream cover types and 43% of these redds were associated with two or more cover types.

Depth and stream velocity were collected at 27 redds. All redds were located in < 1 m of water and the mean depth of water was 0.43 m (Table 2). Redd area averaged 0.23 m^2 (Table 2). Eighteen (47%) redds were sampled for chemical and physical attributes; descriptive statistics, including sample sizes for each measure are provided in Table 3. Within each redd, corrected stream and intergravel temperatures were significantly different (t_{17} = 9.51, P < 0.0001). Mean redd temperature (8.13 °C) was on average 2.95°C warmer than the corrected mean stream temperature (Tables 2 and 3). Specific conductance of intergravel water in redds averaged 271.0 $\mu S \cdot cm^{-1}$ and was significantly different from a stream water sample (241.1 $\mu S \cdot cm^{-1}$) collected the previous

Table 1. Cover types associated with redd sites surveyed in the upper Davis River in 2001. Columns are tabulated by the percentage of redds with a score for each category.

Redd Number	Stable Undercut-bank	Over-head Vegetation	Woody Debris	Pool	Instream Cover	No Obvious Cover
1	1	1			1	
2				1	1	
3			1		1	
4				1	1	
5	1		1		1	
6	1		1		1	
7	1		1		1	
8	1	1	1	1	1	
9						1
10			1		1	
11	1				1	
12	1				1	
13	1				1	
14	1				1	
15	1				1	
16	1		1		1	
17						1
18						1
19				1	1	
20	1	1	1		1	
21				1	1	
22	1	1	1		1	
23	1	1	1		1	
24	1	1	1		1	
25		1	1		1	
26	1	1	1		1	
27			1		1	
28	1		1		1	
31		1	1		1	
32						1
33			1		1	
34			1		1	
35			1		1	
36			1		1	
37	1	1	1		1	
38	1	1	1		1	
39			1		1	
Total %	51.4	29.7	62.2	13.5	89.2	10.8

Table 2. Habitat and chemical data collected in the stream environment at redd sites in the Upper Davis River in September 2001.

	Stream Environment				
Site	Depth (m)	Velocity ($m \cdot s^{-1}$)	Area (m^2)	Stream temp. (°C)	Stream temp. (°C)
$\bar{x}$	0.43	0.40	0.23	5.9	5.2
s	0.15	0.15	0.11	0.7	0.6
Min.	0.25	0.04	0.08	4.5	4.4
Max.	0.90	0.70	0.59	7.2	6.6
n	27	27	23	18	18

Table 3. Habitat and chemical data collected in the intergravel environment at redd sites in the Upper Davis River in September 2001.

Site	Redd Temp. (°C)	Redd-Stream Temp. Difference	Oxygen % Saturation	Oxygen $mg \cdot l^{-1}$	pH	Conductivity (µS/cm at 25 °C)	Vertical hydraulic gradient VHG	Absolute Vertical hydraulic gradient |VHG|	Specific Discharge v ($cm \cdot s^{-1}$)	Absolute Specific Discharge |v| ($cm \cdot s^{-1}$)
$\bar{x}$	8.1	2.3	80	9.4	8.1	271.0	-0.17	0.25	-0.12	0.30
s	1.4	1.2	9.5	1.1	0.2	18.9	0.45	0.41	0.30	0.10
Min.	4.9	0.0	62	7.4	7.6	247.9	-1.51	0.01	-0.47	0.12
Max.	10.8	5.3	95	11.1	8.4	319.6	0.24	1.51	0.36	0.47
n	18	18	18	18	18	18	18	18	18	18

day at the upper end of the high use spawning area ($t_{17} = 6.70$; $P < 0.001$). The average pH of the intergravel environment was less basic (pH = 8.1) than that of the stream (pH = 8.4) ($t_{17} = -6.22$, $P < 0.001$). The mean concentration of intergravel oxygen was also significantly less than the stream sample, at 9.4 $mg \cdot l^{-1}$ ($t_{17} = -3.156$; $P = 0.006$), but all redds had concentrations greater than 7.4 $mg \cdot l^{-1}$. In contrast the mean oxygen saturation (80%) in the gravel was not significantly different from that of the stream sample ($t_{17,} = -0.873$; $P = 0.395$). The vertical hydraulic gradient (VHG) was indicative of down welling at 12 out of 18 measured redds, with a range of 0.24 to -1.5. Extrapolating to redds adjacent to the sites where intergravel water was sampled ($n = 30$) and assuming similar VHG at redds that were immediately adjacent to measured redds, 13 redds exhibited upwelling compared with 17 redds with down-welling. In addition, the mean absolute specific discharge was high and was significantly greater than zero ($t_{17} = 12.32$, $P < 0.0001$) (Table 3).

Over the course of the two-year study, we collected incubation temperatures successfully from 11 natural redds (Fig. 4). Mean daily incubation temperatures for all natural redds during the incubation period (end date June 14) ranged from almost 0°C to a maximum of 8.3°C (Table 4). Hourly-temperature data are presented as monthly means (smoothed data) to simplify presentation and comparison of trends (Figs. 4 and 5). Mean-monthly-incubation temperatures for all natural redd sites during the incubation period (end date June 14) ranged from almost 0°C to a maximum of 4.9°C (Fig. 4). In mid September in both years, redd temperatures ranged from 3 to 5°C. By early December the temperature of most redds had declined to near 0°C with the exception of

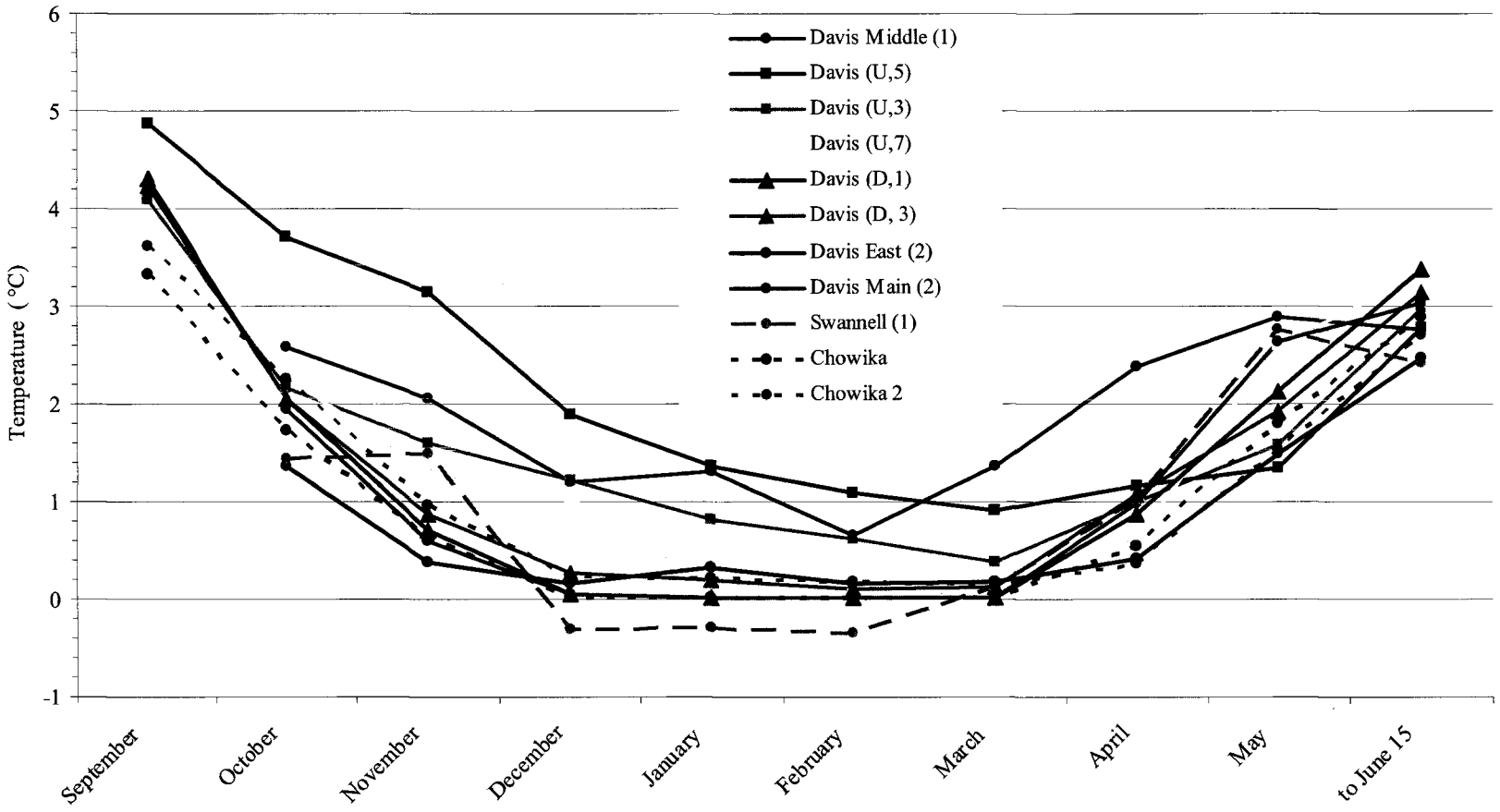


Figure 4. Plot of mean monthly temperatures for selected natural redds collected in the winter 2000-2001 and 2001-2002. Where hydraulic gradient data were collected, U = upwelling; D = downwelling; The number in brackets for each site indicates the number of redds present at a site.

Table 4. Mean temperatures for selected natural redds from the Davis River Chowika Creek and Swannell River collected in 2000-2002. Temperatures were calculated over times to the eyed stage, hatch and button-up as determined in Chapter 4. Where hydraulic gradient data were collected: U = upwelling; D = downwelling. (*n*) indicated the number of redds present at a site. For the periods: Pre-winter, *n* =1464 and 61 days; Pre-hatch, *n* = 2568 and 107 days; Pre-emergence, *n* = 2135 and 89 days.

	Redd Locations										
to Nov 30 (Pre-Winter)	Chowika (1)	Chowika (2)	Davis (D,1)	Davis (D, 3)	Davis (U,7)	Davis (U,3)	Davis (U,5)	Davis East (2)	Davis Main (2)	Davis Middle	Swannell (1)
$\bar{x}$	1.19	1.62	1.39	1.48	1.79	1.89	3.44	0.88	1.28	2.32	1.46
SE	0.02	0.02	0.03	0.02	0.03	0.02	0.01	0.02	0.02	0.02	0.03
CV	78.18	52.00	75.24	63.16	54.73	31.99	14.74	80.73	76.40	25.13	43.80
Min.	0.01	-0.05	-0.01	0.06	0.06	0.59	2.1	0.14	0.01	0.5	-0.01
Max.	4.47	3.76	5.16	4.78	5.12	4.34	5.36	3.32	4.28	3.98	4.57
To March 17 (Pre-Hatch)											
$\bar{x}$	0.01	0.19	0.03	0.18	0.36	0.79	1.36	0.22	0.02	1.15	-0.28
SE	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.01	0.03	0.00
CV	184.44	49.45	209.96	116.66	142.73	52.31	36.13	61.52	377.16	65.00	-474.64
Min.	0.01	-0.05	-0.01	0.06	0.06	-0.36	0.68	-0.01	0.01	0.01	-4.64
Max.	0.17	0.42	0.31	1.17	2.14	1.53	2.73	0.47	1.13	2.56	2.69
To June 14 (Pre-Emergence)											
$\bar{x}$	1.22	1.07	1.54	1.50	1.64	1.41	1.35	1.08	1.73	2.42	1.74
SE	0.03	0.02	0.03	0.03	0.03	0.02	0.01	0.02	0.02	0.03	0.03
CV	100.92	88.70	100.00	80.81	88.63	63.93	37.88	92.50	92.66	44.67	88.71
Min.	0.01	0.1	-0.01	0.06	0.06	0.11	0.68	0.14	-0.14	0.01	-0.01
Max.	5.25	4.22	7.01	5.71	5.89	5.11	5.67	5.04	7.39	6.01	8.3
ATU (Oct 1 to Emergence)	182	215	225	242	294	326	476	174	235	480	215

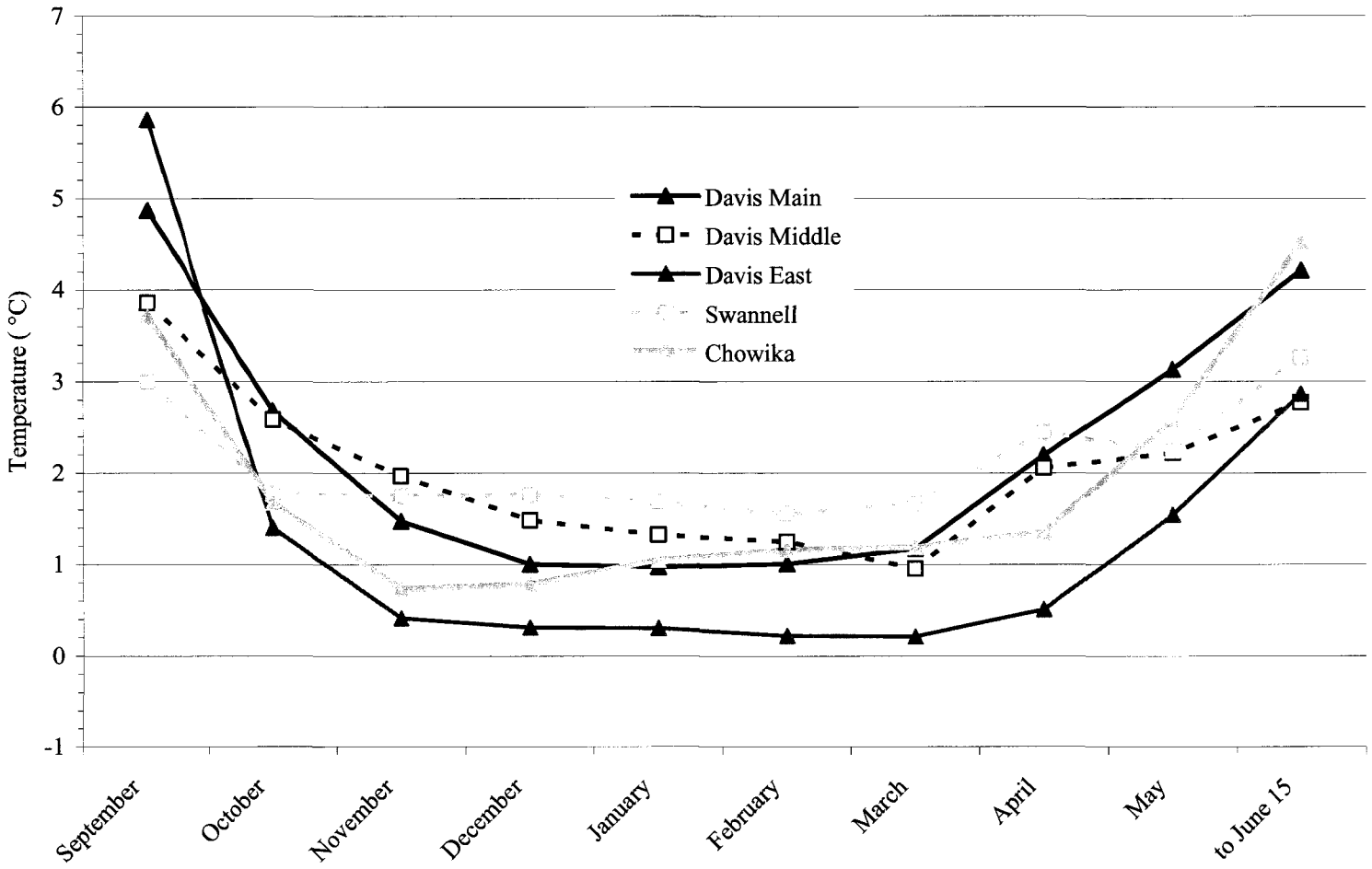


Figure 5. Plot of mean monthly temperatures for selected stream flow sites collected in the winter 2001-2002. Open and dashed lines represent non-selected spawning areas.

three sites in the Davis River main stem that also exhibited superimposed or multiple redds, and one site in the middle tributary of the Davis River, that contained one redd (Fig. 4). By early March temperatures began to increase and were in the range of 2.5-3.5°C by mid June. Where we measured vertical hydraulic gradient in 2001, the warmest sites (n = 2) (i.e., those above 1.0°C) exhibited up-welling, an indicator of groundwater influence; whereas the coldest sites were associated with down-welling (n = 2), an indicator of stream water influence. The warmest sites were also associated with the highest number of redds at a given site (Figs. 4 and 5). In addition to upwelling, each of the warm sites influenced by groundwater, demonstrated more gradual cooling during the incubation period and exhibited a more gradual warming trend in spring compared to the cool sites (Fig. 4). One redd site located in the Swannell River exhibited a variable temperature regime where hourly temperatures well below 0°C were recorded and where ice formation would have been lethal to incubating embryos for long periods during winter. Of four other sites with minimum recorded temperatures below 0°C (Table 4) three recorded temperatures within 0.05°C below 0°C for long periods of time (days to weeks) and one recorded temperatures less than −0.05°C to as low as −0.36°C for 15 h. The warmest average monthly temperatures were encountered in the fall towards the end of November and in spring (approximately mid April) after hatch would have been complete (see Chapter 4). Average redd temperature for the period, winter to putative hatch, ranged from 0.01°C to 1.36°C with two sites exhibiting average temperatures over this period of up to 1.15°C and 1.35°C (Table 4). Estimates for total accumulated thermal units demonstrated a relatively wide range of values from 174 to 480 ATU (Fig. 5). Four sites associated with groundwater discharge demonstrated thermal regimes with

higher total ATU ($\bar{x}$ = 394) than did the remaining sites ($\bar{x}$ = 212) (t_8 = 4.40; P = 0.0023). The Swannell River site was excluded from this comparison due to prolonged periods of below freezing temperatures. With the exception of one high use redd site in the Davis River (D U,5) the other redd locations had substantially lower total ATU's than stream bed temperature, indicating an important difference between stream and intergravel temperatures within sites (Fig 6).

Discussion

Bull trout home to specific areas to spawn that are often found in headwater reaches (Fraley and Shepard 1989; Rieman and McIntyre 1995; McPhail and Baxter 1996; Baxter and McPhail 1999; Bahr and Shrimpton 2004) and our results are consistent with these observations from other watersheds. The determinants of site use at the reach scale and site scale are, however, less clear. For the purpose of this discussion we differentiate between habitat use versus habitat selection as it is unclear whether bull trout are returning to specific sites as the result of precise homing to a site where spawning was previously successful or whether bull trout are using specific indicators of habitat quality to select good nest sites.

In other studies, groundwater has often been described as a driver of spawning selection (e.g., McPhail and Baxter 1996; Baxter and McPhail 1999) and this has been observed for other char species and salmonids in general. These species include: chinook (*Oncorhynchus tschawytcha*), (Geist 2000); chum (*O. keta*) (Leman 1993); brown trout (*Salmo trutta*) (Hansen 1975); eastern brook char (*Salvelinus fontinalis*); (Webster and Eriksdottir 1976; Curry et al. 1995; Blanchfield and Ridgeway 1997; Curry et al. 1995); Arctic char (*S. alpinus*) (Cunjak 1986); and bull trout (Baxter and McPhail 1999; Baxter

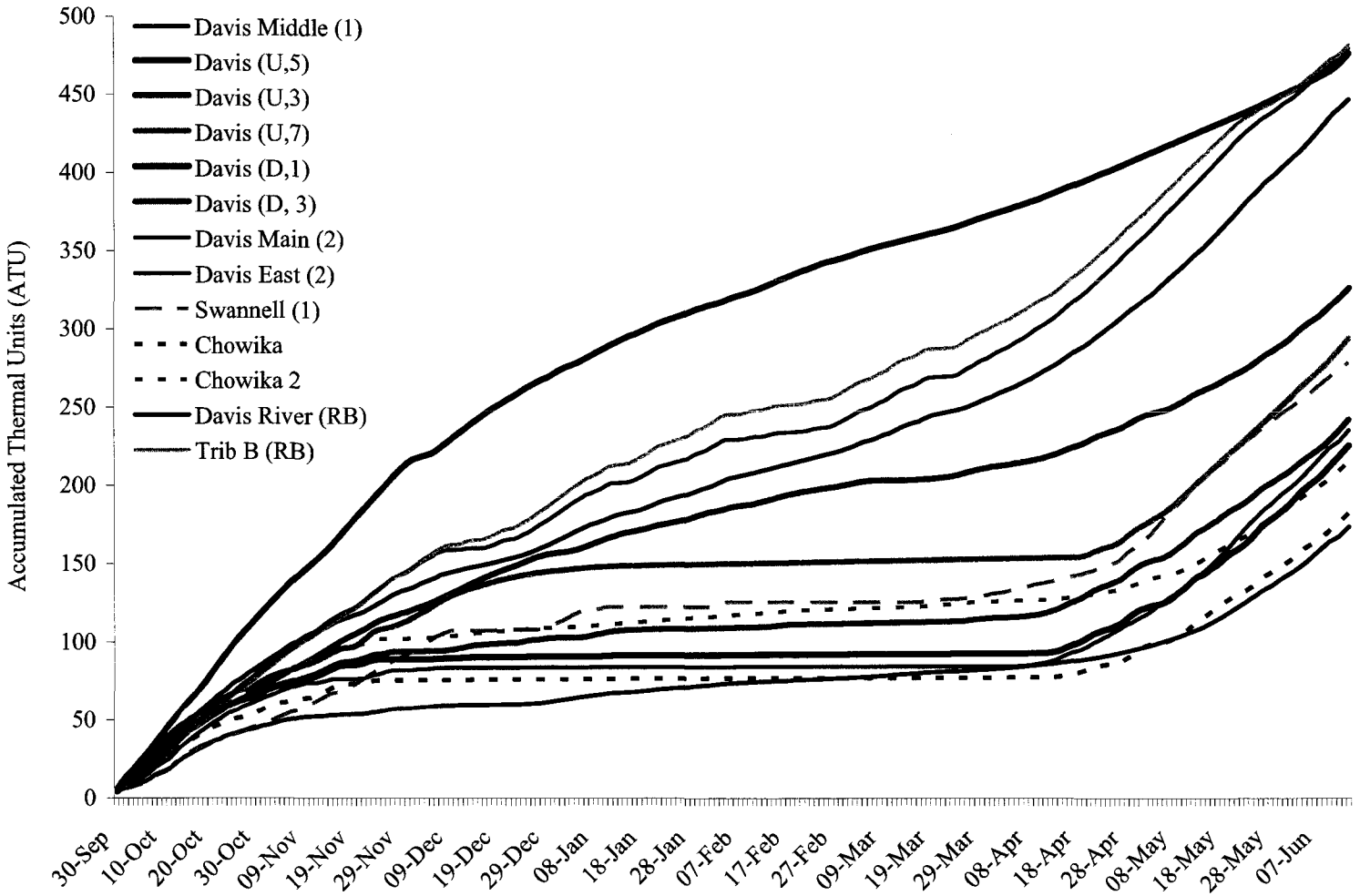


Figure 6. Accumulated centigrade thermal unit (degree day) profiles calculated for selected natural redds and stream flow sites collected in the winter 2000-2001 and 2001-2002. Where hydraulic gradient data were collected: U = upwelling; D = downwelling. (*n*) indicated the number of redds present at a site; RB = river bottom.

and Hauer 2000). Groundwater influence is, however, only one component of habitat and many other physical factors such as substrate, oxygen, and gravel permeability are also important to salmonid incubation success (Chapman 1988). It is important, therefore, to understand at which scale habitat features are important and at what level they affect site selection and use (Baxter and Hauer 2000).

We began our study by assessing the distribution of bull trout within several large watersheds of the Williston Reservoir. Our results confirm previous work (O'Brien and Zimmerman 2000) that large migratory bull trout move to and spawn in smaller tributary streams. At the sub-basin or reach scale we found that bull trout were using headwater depositional areas influenced by relatively warm groundwater during the incubation period. Within reaches, several micro habitat features including high hyphorheic flow rates, relatively warm temperatures and security cover appeared to be important features that were often associated with spawning sites.

Habitat Use at the Reach Level

In the Davis River and Chowika Creek, redds were only observed at high densities in two specific reaches near headwater areas (Figs. 3 and 4). Habitat features at the reach-scale in both high-use areas are consistent with high-quality spawning habitat that has high levels of hyporheic exchange and thermal signatures moderated by groundwater (McPhail and Baxter 1996; Baxter and McPhail 1999; Baxter and Hauer 2000). The lack of spawning in a wider range of habitats in our study area suggests that bull trout were preferentially using specific reaches with specific characteristics such as warm thermal profiles and high rates of hyporheic exchange. Considering the limitations of our investigation, it is possible that some bull trout were using other portions of both

watersheds as we did not examine in detail all small tributaries and many lower reaches using ground-based surveys. Based on aerial and ground-based redd counts, results from a previous telemetry study (O'Brien and Zimmerman 2000), and redd surveys in the Davis River subsequent to this project (Ministry of Environment and Peace Williston Fish and Wildlife Compensation Program, unpublished data, Prince George, B.C.), we are confident, however, that the majority of spawners were using the reaches examined.

Within the Davis River, spawning sites were located mainly in a 5-km long valley segment downstream of the confluence of three similar sized tributaries (confluence area) (Fig. 3). The valley bottom in this mainstem reach is up to 1200-m wide, the channel is sinuous and low gradient (Fig. 3), and the substrate is composed primarily of gravel and cobble with little fine sediment present. Stream temperatures in this reach were relatively warm (Figs. 4 and 5) during the winter incubation period suggesting a strong groundwater influence. Another spawning area with a high density of redds, in the Chowika Creek drainage, also had similar geomorphic characteristics to that of the high-use area in the Davis River (Fig. 2). Stream temperatures in the Chowika Creek reach were on average cooler than the main spawning area in Davis River (Fig. 2) and were similar to temperature measurements collected in the east tributary reach in the Davis River (Figs. 5 and 6). This observation is consistent with the idea that absolute temperature is not the only factor influencing habitat use at the reach scale.

In the Davis River east tributary, aerial redd counts were intermediate to counts in the middle tributary and the main-stem reach (Fig. 3). Differences in habitat use within spawning areas in the Davis River and between spawning areas in the Davis River and Chowika Creek may be explained by differences in habitat quality and habitat

availability. Habitat quality could limit habitat use where variables such as temperature are adequate for incubation but not optimal for survival or growth. For example, the east tributary within the Davis River is much more laterally confined and is higher in gradient than the main-stem Davis River and both of these features together could limit hyporheic exchange with the effect of reducing potential groundwater influence on spawning sites (Baxter and Hauer 2000). Reduced groundwater influence could explain differences in the level of spawning between reaches in the Davis River if bull trout are indeed selecting warmer groundwater-fed areas. Thus, differences in use between reaches in different stream systems may be explained by the availability of quality habitats within a given watershed. The high-use reach in Chowika Creek is to our knowledge the only high-quality site used for spawning in that system. Chowika Creek is generally colder than the Davis River, however, and the Chowika Creek spawning site is higher in elevation (1120 m vs. 940 m). If warmer incubation habitat were available elsewhere in the Chowika Creek drainage as is the case in the Davis River system, lower levels of use may otherwise have been observed at the site we studied.

Other authors have indicated that bull trout appear to select groundwater-fed areas for spawning that are relatively warm (e.g., Baxter and McPhail 1999; Baxter and Hauer 2000; and for a review see McPhail and Baxter 1996) and one account noted selection for a spring-fed site (Boag and Hvenegaard 1997). Based on patterns of use, our results are generally consistent with these observations, however, two of our study reaches were not well used despite both having relatively warm thermal profiles indicative of groundwater influence. In the Davis River middle tributary (Fig. 3), we observed an area with a direct groundwater input to the stream that remained ice-free even at very low winter

temperatures (-25°C). The lack of spawning within this reach seems counterintuitive; however, our observations may be explained by higher levels of fine sediments, compared with the other nearby reaches. The deleterious effects of fine sediments on salmonid eggs and incubation are well known (reviewed in Chapman 1988). Accumulations of fine sediment in redds can reduce the amount of interstitial space available for incubating eggs and can result in lower egg and embryo survival through reduced oxygen uptake and reduced rates of metabolite transport.

Habitat characteristics at the reach scale, including stream sinuosity, gradient and bed characteristics in both the Davis River and Chowika Creek were qualitatively similar to other spawning areas used by bull trout in Northern B.C. (e.g., Pillipow and Williamson 2004, C. Williamson 2006, unpublished observations, Ministry of Environment, Prince George, B.C.). Baxter and Hauer (2000) also recorded similar spawning areas in Montana, USA, where bull trout typically used bounded alluvial valley segments constrained by downstream geomorphic knick points. These areas can provide zones of high hyporheic exchange (Baxter and Hauer 2000) that in turn moderate the thermal regimes of incubation habitat. Warmer intergravel temperatures during winter could also increase embryo survival by preventing the formation of anchor ice, which leads to high embryo mortality rates (Baxter and McPhail 1999; Chapter 3). In our study, we observed that areas of alluvial gravel deposits (including both spawning and non-spawning areas) characteristically had free flowing water beneath surface ice, during cold periods when air temperature approached -25°C, whereas laterally confined reaches in downstream areas often had extensive formations of anchor ice and little or no surface ice.

Redd Habitat Characteristics

As described above, many populations of salmonids apparently seek specific habitats for spawning. Consequently, redd distributions are often clumped and limited to specific sections of available stream habitat. For bull trout the selection of spawning habitat has been linked to groundwater inputs at or near the spawning sites (Boag and Hvenegaard 1997) and differences in habitat use may be categorized and explained by factors related to habitat abundance, habitat quality and behavioural traits related to habitat selection (Essington 1998). In the Davis River, redds were typically aggregated within reaches despite apparently high levels of alternate available habitat (Figs. 3 and 4) and we observed two sites in the Davis River where redds were superimposed. Gradients in habitat quality could explain differences in habitat use where quality has an effect on survival or growth of either adults or their progeny.

In the Davis River strong hyporheic exchange was evident and intergravel flow and oxygen all appeared uniformly high at all redd sites. Intergravel flow rates (measured as absolute specific discharge) ranged from 0.12 to 0.47 $cm \cdot s^{-1}$ (Table 3). These observations are within the ranges provided by Freeze and Cherry (1979) for clean sand and gravel substrates. In the Davis River oxygen levels within redds were not always at saturation, but all sites had concentrations above 7.4 $g \cdot l^{-1}$ ($\bar{x}$ = 9.4 $mg \cdot l^{-1}$) (Table 3), which is within the range of oxygen levels considered optimal for salmonids (Barton and Taylor 1996). Oxygen levels remained high in a related field incubation experiment (Chapter 3) at nearby sites where we were able to collect additional oxygen measurements during winter.

Based on thermal and chemical measures, groundwater influence was evident at

redds examined in this study (Table 3). Oxygen levels, pH and conductivity all indicated that surface waters were mixing with groundwater in the hyporheic zone (Freeze and Cherry 1979). Interstitial oxygen levels were lower and pH and conductivity were on average higher than surface waters adjacent to each redd. Similarly, intergravel temperatures were warmer than those of the stream for each redd measured. Measures of vertical hydraulic gradient, however, did not always indicate upwelling. In fact, only 13 redds exhibited upwelling compared with 17 redds where down-welling was measured. Three of the warmest upwelling sites (Figs. 5 and 6) in the Davis River also had the highest number of redds which suggests that some bull trout may be more successful in habitats with these characteristics. In contrast to Baxter and McPhail (1999) who observed exclusive use of an upwelling site, our results suggest that bull trout can use a wider range of habitats for spawning where localized upwelling may not be the most important factor for site use. This hypothesis is supported by the work of Baxter and Hauer (2000) who found that bull trout in Montana generally spawn in areas of extensive upwelling at the reach scale. At the habitat scale spawning sites were predominantly located in transitional bedforms that exhibited localized downwelling. Both our results and those of Baxter and Hauer (2000) support the hypothesis that groundwater influence at the reach scale scale is a key factor influencing the use of bull trout spawning habitats.

Factors Moderating Site Use

In addition to site suitability, predation risk is likely an important factor influencing use of spawning microhabitats, particularly in northern environments that lack anadromous sources of protein for large carnivores. In the Davis River and Chowika Creek, redds were most often situated near stream margins and the majority of redds in

the Davis River (89%) were associated with at least one type of in-stream security cover (Table 1).

Bull trout that reproduce in clear headwater streams in northern B.C. and particularly spawners that use stream margin habitat can be highly visible to predators. The use of sites associated with cover such as woody debris or undercut banks may help to reduce predation risk. Considering the relatively shallow average redd depth (0.43-cm) in the Davis River and the relative proximity to the stream margins for most redds, predation risk in the absence of cover may be considerable. Although we did not observe any direct predation in our study, grizzly bears (*Ursus arctos horribilus*) were observed near the spawning areas on several occasions and several adult bull trout captured for a related incubation study (Chapter 3 and 4) had large dorsal wounds and scars consistent with bites from large predators. Predation on bull trout during spawning is not unique to our study. Bahr (2002) also observed mortality during spawning in a bull trout population in the Morice River, B.C. watershed during a telemetry study. It was unclear, however, whether these fish died as the result of spawning and were consumed dead or were captured live by predators. In another example, Chandler et al. (2001) reported 80% mortality of small bull trout that were radio-tagged in two streams in Oregon during the spawning period where mammalian predators were an important factor. Predation intensity can be high for other salmonids during spawning and it is thought to influence habitat use and body morphology in these circumstances. Quinn et al. (2001) examined the relationship between age at maturity, body size, water depth in spawning streams and predation intensity for several populations of sockeye salmon. Their results show a positive relationship between mean water depth among streams and body depth where

predation by bears was substantial.

Lack of Habitat Use

It is notable that bull trout spawning activity was observed at very low levels (< 1 redd per km of stream) in two of the warmest groundwater influenced reaches we studied. This result was unexpected considering the potential benefits of warmer temperatures associated with upwelling groundwater which includes accelerated growth and enhanced survival for incubating fish (e.g., Garett et al. 1998). The seasonal temperature profile of the Davis River middle tributary is indicative of a stream that is heavily influenced by a relatively stable source of groundwater (Fig. 6). In winter, stream-flow in this reach (Fig. 2) can be more than 2°C warmer and in summer is typically cooler than adjacent tributaries as the main-stem section of the Davis River. It is important to note that this reach also had higher levels of coarse woody debris than other reaches which resulted in more potential cover. It is not clear why fish are not spawning at higher densities in this reach. One explanation for the lack of use may be that bull trout are avoiding the higher winter temperatures associated with sites in this reach. At warmer sites, embryonic development may proceed at too rapid a rate for successful incubation. Brannon (1987) postulated that spawning date and incubation period for several species of Pacific salmonids is timed to take advantage of increases in spring primary production. The range of suitable incubation temperature may be limited for northern populations of bull trout. Relatively high incubation temperatures may result in premature emergence from the gravel or premature depletion of yolk. Depletion of yolk combined with reduced food availability prior to spring could reduce juvenile survival thus reducing the fitness of parents that spawned in the warmer habitat.

Specific habitat features, such as low intergravel flow rates and lower oxygen availability due to higher levels of fine sediments, may also be important factors affecting the level of use. One of the few redds observed in this reach was located on top of a disturbed area that was used for a field incubation experiment in the previous year (Chapter 3). It is interesting to note that during a walking survey of this reach in the fall of 2000, we did not locate any redds, but many juveniles were plainly visible which suggests that this type of reach and access to it may be important for other life stages.

Similarly, we observed low levels of spawning activity in the upper Swannell River where the lack of use may be related to the influence of groundwater, however, this is more difficult to explain than for the Davis River and use may be influenced by factors other than habitat quality. The upper Swannell River (Fig. 1) has many similar characteristic to that of the high-use spawning area in the Davis River including: stream size and width, high sinuosity, low gradient, clean gravel, substrates and groundwater influence, however, only one redd was observed. Unlike the other study reaches used for spawning, we observed few mature adults during our reconnaissance surveys in the Swannell River and we found these fish holding at the downstream end of the study reach (J. T. Zimmerman and C. J. Williamson 2006 unpublished observations, Ministry of Environment, Prince George, B.C.). Both groundwater and stream temperatures in the Upper Swannell were cooler than the Davis River during spawning season and winter temperatures were nearly as warm as the most selected sites in the Davis River. Thus, cool temperature during spawning may also be a factor in the lack of use.

It is also possible that the upper Swannell has been used more extensively for spawning than we observed. First, we noted that redd identification was more difficult in

the Swannell drainage due the substrate colour as well and the lack of periphyton growth. To confirm this general observation we disturbed areas of gravel and found these patches very difficult to see compared with the other watersheds where redds and disturbed areas were highly visible. It is, therefore, possible that we did not observe some redds that were actually present. Second, as part of our initial work to identify study sites, we electro-shocked a number of areas in the upper Swannell reach while looking for juvenile bull trout. We were able to find small numbers of bull trout fry at one site, suggesting spawning had taken place in that reach, however, we failed to find fry at other locations. Lack of spawning at sites influenced by groundwater in this reach may also be linked to a potentially smaller population with fewer spawners. Unlike the Davis River and Chowika Creek, there is substantially more development related to forestry in the valley bottom of lower Swannell River which includes a relatively high density of roads (Fig. 1). This development could have resulted in better access to bull trout staging areas for anglers. Bull trout are aggressive-piscivores that are highly vulnerable to capture with fishing gear; thus road access, due to forest development as well as proximity to forestry camps and a small community, may have led to impacts on the population of spawners resulting in fewer redds. For example, Ripley et al. (2005) have linked access to declines in bull trout abundance in the Kakwa River drainage in Alberta, Canada. In the Swannell drainage, bull trout could have been using other sites, with more suitable habitat characteristics within the drainage. During our initial examination of the Upper Swannell many of the smallest tributaries were not surveyed.

Summary

The use of specific areas influenced by groundwater with specific thermal signatures can help to maximize reproductive success of fish by limiting the effects of stochastic environmental process that act to lower survival during incubation. At the reach scale, our results are consistent with previous work that indicates bull trout spawn in valley segments influenced by groundwater that have zones that exhibit high rates of hyporheic exchange. We found evidence of localized groundwater influence at the habitat scale, where bull trout were spawning in a variety of habitats with localized warm upwelling groundwater or downwelling streamflow. Finally we observed several habitat features common to site used for spawning, including high intergravel-flow rates, high levels of interstitial oxygen and proximity to security cover.

CHAPTER 3: FACTORS AFFECTING GROWTH AND SURVIVAL OF LARVAL BULL TROUT IN PRISTINE NORTHERN STREAMS.[2]

Abstract

The importance of the yearly temperature regime as well as other habitat variables for incubation and development of bull trout is not well known. It is also not known how small deviations from the thermal and chemical regime characteristic of bull trout spawning habitat may affect incubation success and ultimately juvenile survival. To understand these relationships, we examined the influence of intergravel temperature, pH, conductivity, intergravel flow, and oxygen on bull trout survival, development, embryo size and yolk utilization. We assessed incubation success and growth using fertilized bull trout eggs contained within perforated plastic capsules. The capsules were buried at typical redd depths in three tributaries draining into the Williston Reservoir, B.C., where bull trout are known to spawn. Survival during incubation was independent of all physical and chemical measures we examined at both used and non-used sites and habitat quality was relatively high at all sites. In contrast, thermal regime was an important determinant of the rate of bull trout development and size. Warmer temperatures associated with groundwater influenced sites that were not used for spawning significantly advanced development, size and yolk utilization relative to used sites. Thus, differences in thermal regime between sites are a likely explanation for observed patterns of use.

[2] Throughout this chapter I use the first person plural to acknowledge the contribution of others to this work, which will be submitted for publication with the authorship of C.J. Williamson, J.T. Zimmerman, and J.M. Shrimpton.

Introduction

Salmonids typically reproduce in tributary streams with clean gravel substrates, often after a substantial migration from feeding or rearing areas. During migration, adults can encounter a wide variety of potentially suitable spawning habitats. Site selection tends to be precise with year-after-year fidelity for specific areas or habitats within watersheds, either by individuals, successive migrants or successive generations (Fleming and Reynolds 2004). Behaviours involved in site selection have likely evolved as an adaptive response to maximize reproductive fitness in areas with variable habitat conditions (Brannon 1987; Hendry et al. 1998; Kinnison et al. 1998; Fleming and Reynolds 2004). Site selection can involve homing to specific locations within a watershed (e.g., Dittman and Quinn 1996), selection of specific channel locations (Chapter 2), or selection of specific habitat conditions such as intergravel flow and temperature (Baxter and McPhail 1999). Additionally, localized site selection may be influenced by environmental or chemical cues such as oxygen levels, water temperature or groundwater inputs (Chapter 2). Habitat and other factors influencing survival and growth during incubation have been particularly well studied in salmonids (reviewed in Chapman1988). Investigations of habitat quality for incubation have historically focused on stream sediments, stream flows and related land management practices (Reiser and Bjornn 1979; Chapman 1988). More recent research has integrated the role of fluvial processes and geomorphic structure as mediators of biological processes (Baxter and McPhail 1999; Baxter and Hauer 2000; Geist 2000) and has highlighted the importance of sub-surface groundwater, including surface water chemistry and hyporheic flow, and hyporheic exchange for incubating salmonids.

The quality of incubation habitat can affect the survival and growth of juvenile fish (see Chapman 1988). Due to the difficulty of winter field research, however, few studies have investigated *in situ* the effects of habitat on the survival and growth of incubating salmonids in cold environments. To better understand the role of subsurface fluvial processes on the incubation of salmonids in a cold northern environment, we investigated the role of groundwater and five related habitat parameters on the incubation success of bull trout in three northern British Columbia streams. Bull trout appear to select specific microhabitats within particular stream reaches for reproduction (Baxter and McPhail 1999; Baxter and Hauer 2000); however, it is not clear what advantages and features the selected sites confer in terms of survival and growth of eggs and alevins. In this study, survival, somatic growth and yolk utilisation of bull trout eggs and alevins were examined in two field incubation experiments during the fall and winter incubation periods of 2000-2001 and 2001-2002. We examined differences in survival and embryo size in reaches that were used frequently and sites not used or infrequently used by bull trout for spawning to better understand the importance of habitats selected. We measured habitat parameters including intergravel flow (specific discharge and vertical hydraulic gradient), temperature, oxygen, pH and conductivity as each of these factors could influence survival, embryo size and yolk utilization, and may be used as cues by spawners for redd site selection. Comparison of size and survival among reaches exhibiting different habitat characteristics may help to explain patterns of site use or site selection. Identification of these features could help in the management and protection of spawning habitat.

Materials and Methods

Study Area

Over the two-year study, experimental sites were located at six reaches of three tributary streams to the Finlay Reach of the Williston Reservoir (Fig. 1). For this experiment, sites were located in the Davis River, Chowika Creek and Swannell River. A more detailed description of the study location is provided in Chapter 2.

Gamete Collection

We collected eggs and milt from adult bull trout we captured by angling in the Davis River. None of the fish captured showed any interest in, or aggression to lures or bait, therefore all the fish were caught in shallow water, by "snagging" of a pectoral fin. With the exception of one male, all spawners were from the upper portion of the Davis River watershed near a spawning area with relatively high levels of use. In 2001, we captured one male in the middle reaches of the Davis River. In 2000, we captured two females and two males and in 2001, we captured three males and three females. To test for local adaptation including differences in size and survival relative to specific environmental conditions in spawning streams, we attempted to collect additional spawners from Chowika Creek and the Swannell River for the second experiment in 2001. Logistical constraints associated with flight bans following the events of September 11, 2001, however, prevented us from collecting additional gametes. We collected several fish in Chowika Creek, but the females were near the end of spawning and had few eggs.

Prior to artificial spawning, we anaesthetized each fish with a 10-ppm solution of clove oil and ethanol (1:9 clove oil to ethanol) until they exhibited a loss of equilibrium

and slow opercular movement (Anderson et al. 1997; Prince and Powell 2000). To prevent activation of sperm or eggs, we removed excess water on the outside of each fish with a dry towel, before gametes were removed. The gametes were gently massaged by hand from the fish into cool, clean plastic containers. Following gamete collection, we allowed each fish to fully recover in river water before release. We mixed eggs and milt for each family cross separately in clean plastic buckets, without water. After approximately 2 min, we added river water to the eggs, and then left them for 5 min. We then washed excess milt from the eggs and then left them to water harden for 30 min.

Egg Capsules

We placed the fertilized eggs into plastic incubation capsules, and then deployed the capsules by helicopter to the incubation sites within 24 h after fertilization. Our capsule design (including size) was similar to that used by Baxter and McPhail (1999) and Cope and Macdonald (1998). We used plastic, a less thermally conductive material, instead of stainless steel to inhibit and arrest the unnatural formation of anchor ice in redds. We constructed the capsules (13-cm long by 5.5-cm diameter) from perforated, extruded food-grade polyethylene tubing (1-mm by 3-mm slots) that were covered at each end by a perforated, polyethylene-plastic cap (T-series CAPLUG®). We manually perforated each cap with at least 60, 1.5-mm diameter holes. We added clean, sieved gravel < 3-cm diameter to each capsule to provide an internal substrate consistent with natural redds. In 2000, we placed approximately 30 eggs (counted by weight) into each capsule. In 2001, we placed 40 or 45 eggs, counted in batches with a counting paddle, into each capsule where sample size was dependant on the number of eggs available within each family group.

Incubation Sites

We used a nested experimental design for the incubation sites. In experiment one (fall-winter 2000-2001), we used three experimental reaches, two in the Davis River and one in Chowika Creek for incubating eggs (Fig. 1). In the second experiment (fall-winter 2001-2002), we used six reaches, three in the Davis River, two in Chowika Creek and one in the Swannell River (Fig. 1). We selected reaches based on two broad categories of use by bull trout-spawners for both years: high-use and low-use. High-use sites were located among redds in the Davis River and Chowika Creek at sites that were determined to be important spawning areas following reconnaissance work for our synoptic study of redd habitat (see Chapter 2). Low-use sites were selected in areas not used for spawning where the habitat appeared qualitatively similar to highly-used sites. For example, one low-use site in the Davis River (Davis River middle tributary) was selected as it was located in the vicinity of the main spawning area. Three sites were located in reaches that were used in year one or were adjacent to sites used in year one.

In each reach, we excavated three artificial redds that were within 3 m of each other (i.e., redds nested within reaches). We excavated the artificial redds using a shovel so that fine sediments were carried downstream with the current and each redd had an upstream depression followed by a tail-spill mound, similar to a natural redd. We buried the capsules within the tail-spill mound so that a minimum of four capsules containing the eggs from each of up to five families (i.e., up to 20 capsules) were buried in clusters at approximately 25-cm depth in each artificial redd (i.e., capsules nested within redds). We selected this depth to be representative of eggs within a natural egg pocket within a redd and this depth is within the range of redd depths observed for large-bodied (65 to 85

cm) salmonids (Devries 1997; Steen and Quinn 1999). We collected two incubation capsules for each family cross at each of two sampling intervals. In experimental year one, we selected the first sampling period (December) to correspond with the period shortly after hatch. We selected the second date to roughly correspond to the end of the pre-emergence period, based on information from Gould (1987). The sampling dates were adjusted in year two to better correspond with the goals to sample alevins just post-hatch and well past post hatch as outlined for year one. Following collection, we transported the capsules by helicopter in water filled tubs (to prevent freezing) to a field laboratory where we removed the eggs and alevins from the capsules and recorded survival. Once counted, we rapidly froze the live eggs and alevins on dry ice to simultaneously euthanize and preserve them for growth analysis in the laboratory.

Alevin Survival, Size and Yolk Utilization

We enumerated live and dead eggs or alevins in each capsule on each collection date. To compare embryo sizes among stream sites under a range of physical conditions adjacent to natural spawning sites and areas that appeared suitable, but where bull trout were not found to spawn, we collected measurements of body size and yolk utilization from all alevin and embryo samples. Before collecting each measurement, we dissected embryos that had not hatched from the eggs. We recorded embryo and alevin standard lengths to the nearest 0.01 mm under a dissecting microscope using Vernier callipers. We used an analytical balance that measured to 0.0001 g to determine wet and dry weights. Embryos and alevins were re-frozen after we collected lengths and weights to keep the alevin and the yolk intact, while the yolk material was dissected away from the alevin. We dried tissue samples individually in aluminum weighing boats for a minimum

of 24 h at 60°C. After drying, we stored the tissue in a sealed dessicator containing $CaSO_4$ until dry weights could be collected.

Temperature, Hyporheic Flow and Artificial Redd Chemistry

In the field, we installed data loggers to record temperature (Optic Stowaway temp logger, Onset Computer, Bourne, Ma) within the hyporheic zone at each artificial redd at a depth of approximately 25 to 30 cm (capsule depth) to continuously monitor incubation temperatures. We also placed a single data logger on the river bottom at each site to monitor stream temperature. We programmed each data logger to record temperature on an hourly basis for the duration of the incubation period.

In 2000, we attempted to measure intergravel chemistry using rigid standpipes with screened tips installed in the center of each redd, however, this method proved unsatisfactory due to cold winter conditions (-20 to -25°C in December, 2000) and shifting river ice. Several of the standpipes were knocked down by ice while many others were difficult or impossible to sample as ice-plugs had formed within the tubes at the level of the stream surface. Following the first egg collection period in 2000, we removed the remaining standpipes to prevent uncontrolled disturbances to the incubating eggs. For the second egg collection period in 2000, we attempted to install mini-piezometers using Lee and Cherry's (1978) design. This design, however, also proved problematic under cold conditions and data collection was again limited.

In 2001, we successfully installed two mini-piezometers(Geist 2000) (see Chapter 2 methods) within each artificial redd, one at 30 cm, and one at approximately 50 cm below the redd. The use of flexible vinyl mini-piezometers facilitated storage of the piezometers below the water surface near the stream bed between field trips; this

prevented the piezometer tube from freezing into surface ice during extended periods of cold weather. To monitor intergravel temperatures below redds and to aid the identification of strong groundwater influence in the vicinity of the artificial redds, we installed one additional temperature logger at a depth of 50 cm within each artificial redd cluster.

At each artificial redd, we recorded intergravel water temperature, pH, specific conductance, oxygen, vertical hydraulic gradient and hydraulic conductivity (see Chapter 2) on three occasions: two weeks after egg capsules were buried, when egg capsules were retrieved in mid-winter, and when egg capsules were retrieved in late winter. We also recorded stream water chemistry for the same chemical parameters during each site visit to allow comparison with hyporheic water.

Data Analysis

Survival and Size

To examine estimates of survival and measures of size for each field experiment (i.e., each year), we completed separate ANOVA's for each experimental year, and for each dependant variable (i.e., survival, length, dry-yolk weight, dry-body weight and total dry weight) (Sokal and Rolf 1995). Factors in the models included sample date, family or parent, reach, redd and capsule. Capsule was nested within redd and redd was nested within reach. Within this group of analyses, to understand the effect of parent or family on each dependant variable, we completed separate ANOVA's with family or with parent using the nested designs described above. In year one each family was used in each reach (not nested), in year two family was nested within reaches due to limited number of egg relative to the number of sites. To understand the effect of parent on size, we tested the interaction between male and female for year one of our experiment in ANOVA's that

included both male and female as factors in the tests. This test was not possible for year two as parent was nested within reach (i.e., not all combinations of parent and reach were possible). For analysis of survival and size, we completed all ANOVAs using the general linear model (GLM) procedure in Minitab (v.14.1, State College, PA) and each factor was treated as a fixed variable, as each alevin was sampled destructively. We used the adjusted sum of squares to test significance. To examine the differences between dependant variables (survival, length, yolk and tissues weights) at each experimental level (date, reach, redd, capsule and family) for each test, *post hoc* comparisons were completed when significance was found for any given factor, using the Tukey-Kramer multiple comparison procedure in Minitab. We used the Tukey-Kramer procedure as this method for multiple comparisons is suitable for un-balanced experimental designs and is more conservative than other similar procedures (Sokal and Rolf 1995). We screened all data before attempting any analysis. Screening involved examination of outliers, erroneous values and transcription errors. As a result of the low sample sizes for some experimental factors, following screening only data that were obviously erroneous were deleted from the datasets. Thus, we only excluded data that were transcription errors. As part of each procedure, we visually checked all ANOVA models for normality and heterogeneity using the output provided by Minitab, which included: normal probability plots, histograms and scatter plots of the residuals for each model. Percent survival data were $\arcsin\sqrt{x}$ transformed for all tests (Tabachnick and Fidell 2001). All ANOVA tests met the assumptions of normality and heteroscedasticity.

Habitat

Chemical parameters (specific conductivity, oxygen and pH) and specific

discharge were compared among sample units using nested ANOVA including use, reach and piezometer as fixed factors within each ANOVA model. Use was used as a categorical factor based on either sites used or not used for spawning. Reach was nested within use and piezometer was nested within reach. Piezometer measurements were collected at two different substrate depths below one-of-three redds within each reach. Piezometer measurements collected on any given date were therefore treated as replicates within a reach. Our sample sizes for measurement of our dependant factors was too low to support a repeated measures ANOVA design; therefore, to better understand groundwater influence and chemistry within the intergravel environment in each study reach, we assessed differences between surface waters and piezometers at two intergravel depths using a constructed variable. Thus the factor "piezometer" was calculated as the difference between each piezometer measure and the surface which allowed us to better understand within reach variation as the differences between hyporheic and surface waters. For each dependant variable we completed separate ANOVAs for each of the three sample dates to better understand how chemistry and specific discharge varied within reaches and between sites used and not used for spawning. Next, we used a similar approach and design described above to examine differences among factors, including site use, reaches and piezometer depth through time (September and April). Thus, for comparisons through time we subtracted the last measurement collected for each site (April) from the first measurements collected (September). For both within reach and between time tests, this approach resulted in non-normal data that were successfully transformed (except pH) using natural log-skew transformations that we completed using Stata 9.2 (Stata Corp., College Station, TX). We used Kolmogorov-

Smirnov goodness of fit tests on ungrouped data to test for normality following transformation (Sokal and Rolf 1995). For simplicity of presentation, we used data means to report our results for comparisons between reaches and through time. To understand temperature differences between redds and among reaches, we plotted temperature data for each redd as a time-series and analysis was restricted to qualitative description and qualitative contrasts of the profiles. All ANOVA tests met the assumptions of normality and heteroscedasticity. We used the adjusted sum of squares to test significance. All data were screened for erroneous values, outliers and as part of each procedure, we visually checked all ANOVA models for normality and heterogeneity using the output provide by Minitab, which included: normal probability plots, histograms and scatter plots of the residuals for each model.

Results

Hatch and Survival

In both years, hatch occurred during the month of March for reaches that were used for spawning or had similar temperature regimes to the sites used for spawning (Table 5). At two warmer sites that were not used for spawning (Swannell River- S1 and Davis River - D2), hatch occurred earlier than all other sites (Table 5). Due to experiment-wide differences in survival between year one and year two, we first present descriptive statistics for survival for both years. In year one survival was generally high (Fig. 7) and individual egg capsule survival ranged from 0 to 100% with a mean survival rate of 64.0% (n = 122, s = 23.7). In year two survival was low (Fig. 7). Individual capsule survival rates ranged from 0% to a maximum of 52.5% and the mean survival rate was 9.6% (n = 193 s = 11.5).

Table 5. Percent hatch and accumulated thermal units (ATU) for all capsule sites in both experimental years. Sites are categorized by level of use and thermal profile.

Reach		Year One				Year Two			
		Dec 12, 2000		Mar 22, 2001		Mar 01, 2002		Apr 29, 2002	
	Days Post Fertilization	95		195		169		228	
		% Hatch	ATU	% Hatch	ATU	% Hatch	ATU	% Hatch	ATU
Davis River 1 (High Use)		0	186.3	100.0	225.8	4.4	198.0	100.0	230.5
Davis River 2 (Low Use-Warm)		0	239.5	100.0	356.8	100.0	343.9	100.0	433.0
Davis River 3 (High Use)		-	-	n/a	-	0.9	176.9	100.0	189.5
Chowika 1 (High Use)		0	152.3	80.0	147.6	-	-	-	-
Chowika Creek 2 (High Use)		-	-	n/a	-	0.0	149.3	100.0	167.1
Chowika Creek 3 (Low Use- Cold)		-	-	n/a	-	0.0	151.9	100.0	155.1
Swannell River 1 (Low Use-Warm)		-	-	n/a	-	100.0	470.3	100.0	601.7

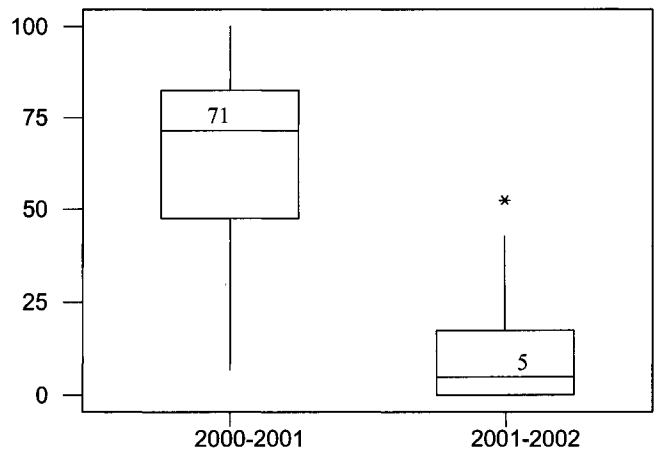


Figure 7. Boxplots of percent survival both experimental years. For each experimental year the medians, interquartile ranges (box) , limits (whiskers) and outliers (*) are presented.

We observed highly significant differences (F's > 6.51; P's $\leq$ 0.001) in survival between collection dates, among reaches and among families in both years with the exception of 'reach' in year one where there was no difference ($F_{2,99}$ =1.21; P = 0.303) in survival (Appendix A, Tables 10 and 11). In both years, survival was significantly lower on the second collection date (Tables 6 and 7) (Y1 $F_{1,99}$ =12.61; P = 0.001 and Y2 $F_{1,148}$ = 11.17; P < 0.0005) (Appendix A Tables 10 and 11). Pairwise comparisons between families for year one revealed significant differences between families one and two, and families one and three (Tukey-Kramer P < 0.001 and P < 0.005). Family one demonstrated the lowest mean survival (47.74%) next to family four (62.14%, Table 6). Individual parental effects on survival in year one were not significant for males (Y1 $F_{1,124}$ = 2.75; P = 0.100) or females (Y1 $F_{1,124}$ = 1.07; P = 0.303) (Appendix A, Table 12). In year two, family two typically had the highest mean value for survival, which was more than double that of all the other families, however *post hoc* tests indicated no significant difference for many paired comparisons (Table 7). We were unable to test individual parental effects simultaneously due to limitations of the experimental design related to the nesting of families within reach. When tested separately, however, male effects on survival were significant, however females were not (Y2 Males, $F_{5,197}$ = 15.53; P < 0.0005 and Y2 Females, $F_{5,197}$ = 2.06; P = 0.073) (Appendix A Tables 13 and 14). Pair-wise comparisons among all males were highly significant (Tukey-Kramer; P's < 0.0005). Survival for reaches is presented for comparison with survival at the similar reaches in year one (Table 7), however, no additional statistical treatment of survival data for year two was considered due to the low and variable survival that was likely the result of poor fertilization success rather than an experimental effect (see discussion).

Table 6. Least squares means (ANOVA) for percent survival in experimental year 1 (2000-2001). All means are back transformed, thus standard errors are not provided. A "*" signifies significant results (ANOVA; $P's < 0.0005$). Letters within groups show overlap in Tukey's simultaneous 95% confidence intervals. Least squares means for parental effects are from a different ANOVA (Appendix A Table 12) than results for the rest of the table (Appendix A Table 10).

	$\bar{x}$	*post hoc*
Collection Date*		
December 1, 2000	74.80	
March 22, 2001	53.34	
Family*		
1	47.74	A
2	74.29	B
3	72.40	B
4	62.14	B
Reach		
Davis River 1 (High Use)	70.66	
Davis River 2 (Warm)	60.77	
Chowika Creek 1 (High Use)	61.68	
Parent		
Male 1 (Families 2 &4)	67.98	
Male 2 (Families 1 &3)	59.29	
Female 1 (Families 1 &2)	60.94	
Female 2 (Families 3 &4)	66.39	

Table 7. Least squares means (ANOVA) for percent survival in experimental year 2 (2001-2002). All means are back transformed. A "*" signifies significant results (ANOVA, P's < 0.0005). Letters within groups show overlap in Tukey's simultaneous 95% confidence intervals. Least squares means for male and female effects are from a different ANOVA (Appendix A Tables 13 and 14 respectively) than results for the rest of the table (Appendix A Table 11).

	$\bar{x}$	*post hoc* Groups
Collection Date*		
March 1, 2002	10.69	
April 29, 2002	3.68	
Reach*		
Chowika Creek 2 (High Use)	6.48	A
Chowika Creek 3 (Low Use)	11.44	B
Davis River 1 (High Use)	5.69	B C
Davis River 2 (Low Use Warm	2.37	B C D
Davis River 3 (High Use)	3.04	B C D
Swannell River 1 (Low Use W:	24.20	B
Family(Reach)*		
Chowika Creek2-Family-1	6.48	A
Chowika Creek3-Family-1	11.44	A B
Davis River1-Family-1	9.74	A B C
Davis River1-Family-2	16.34	A B C D
Davis River1-Family-3	5.16	A B C D E
Davis River1-Family-4	2.28	A B C E F
Davis River1-Family-5	0.82	A B E F G
Davis River2-Family-1	2.22	A B C E F G H
Davis River2-Family-2	12.48	A B C D E F I
Davis River2-Family-4	0.30	A E F G H J
Davis River2-Family-5	0.28	A E F G H J K
Davis River3-Family-1	4.89	A B C D E F G H I J K L
Davis River3-Family-2	14.21	A B C D E F I L M
Davis River3-Family-3	3.73	A B C D E G H I J K L M N
Davis River3-Family-4	0.69	A E F G H J K L N O
Davis River3-Family-5	0.01	A E F G H J K L N O P
Swanell River1-Family-1	24.20	B C D E I M
Male (Reach)*		
Male 2 (Family 3)	4.73	A
Male 3 (Families 4/5)	1.11	B
Male 5 (Families 1/2)	11.46	C
Female (Reach)		
Female 1 (Family 3)	9.50	
Female 2 (Families 1/5)	6.52	
Female 3 (Families 2/4)	12.54	

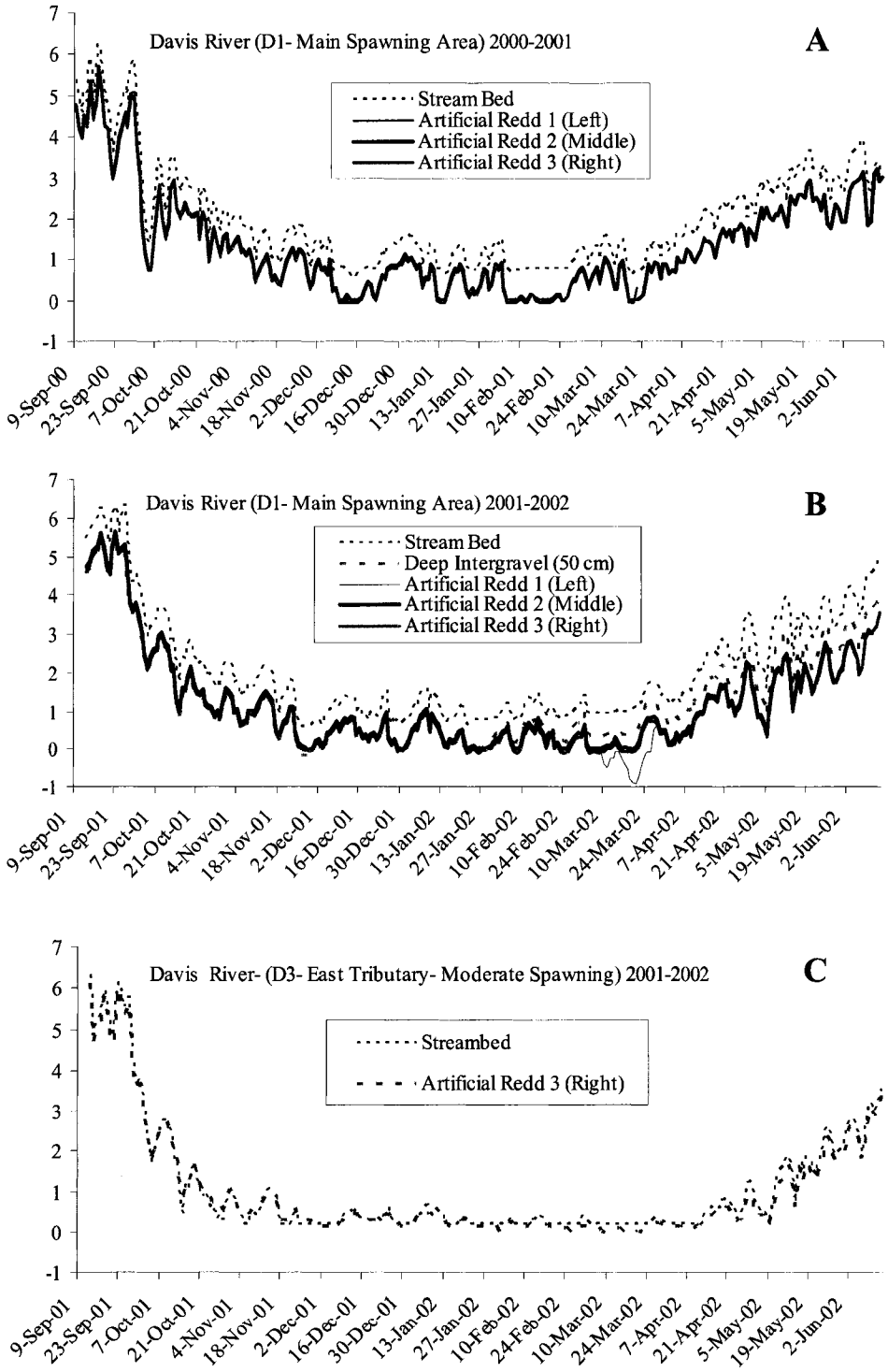
Additional analysis of survival in the context of physical habitat was also not attempted due to the lack of physical differences (except temperature) between sites in year one and the low survival in year two.

Intergravel Environment

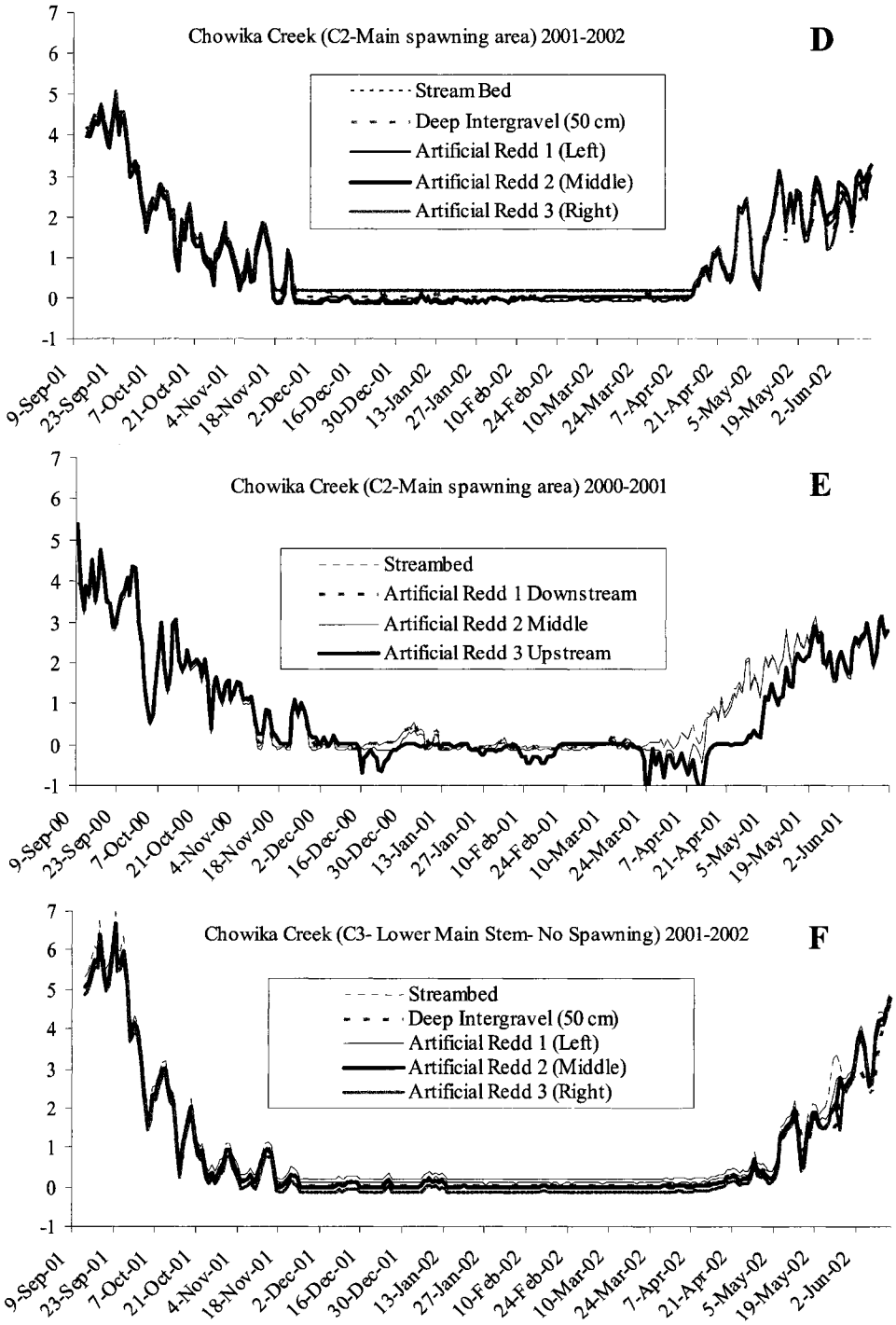
Temperature

Intergravel temperatures ranged from a maximum of 7.0°C to minimum of -1.7°C across all artificial incubation sites. All but three artificial redds remained above freezing during the incubation period in both years (Figs. 8a-8i). Temperatures at sites naturally used for spawning (Davis mainstem, Davis east tributary and Chowika 2; Figs. 8a-8e) were intermediate to sites not used for spawning (Swannell, Chowika 3 and Davis (middle tributary); Figs. 8f-8i). Thermographs measured from all but two incubation reaches followed a predictable pattern for northern streams where temperatures declined to near 0°C at the onset of winter, remained low and stable for several months with a warming trend again in spring at the onset of snowmelt and freshet (Figs. 8a-8i). Two exceptions to general trends were found: the Davis River middle tributary (Figs. 8g and 8h) and the upper Swannell (Fig. 8i). The Davis River, middle tributary, site exhibited a relatively variable thermograph in both years, with comparatively sharp temperature changes of up to 2.5°C over days; a relatively short period of time (Figs. 8g and 8h). Temperature loggers in both of these reaches exhibited temperatures that were above 1°C throughout much of the winter.

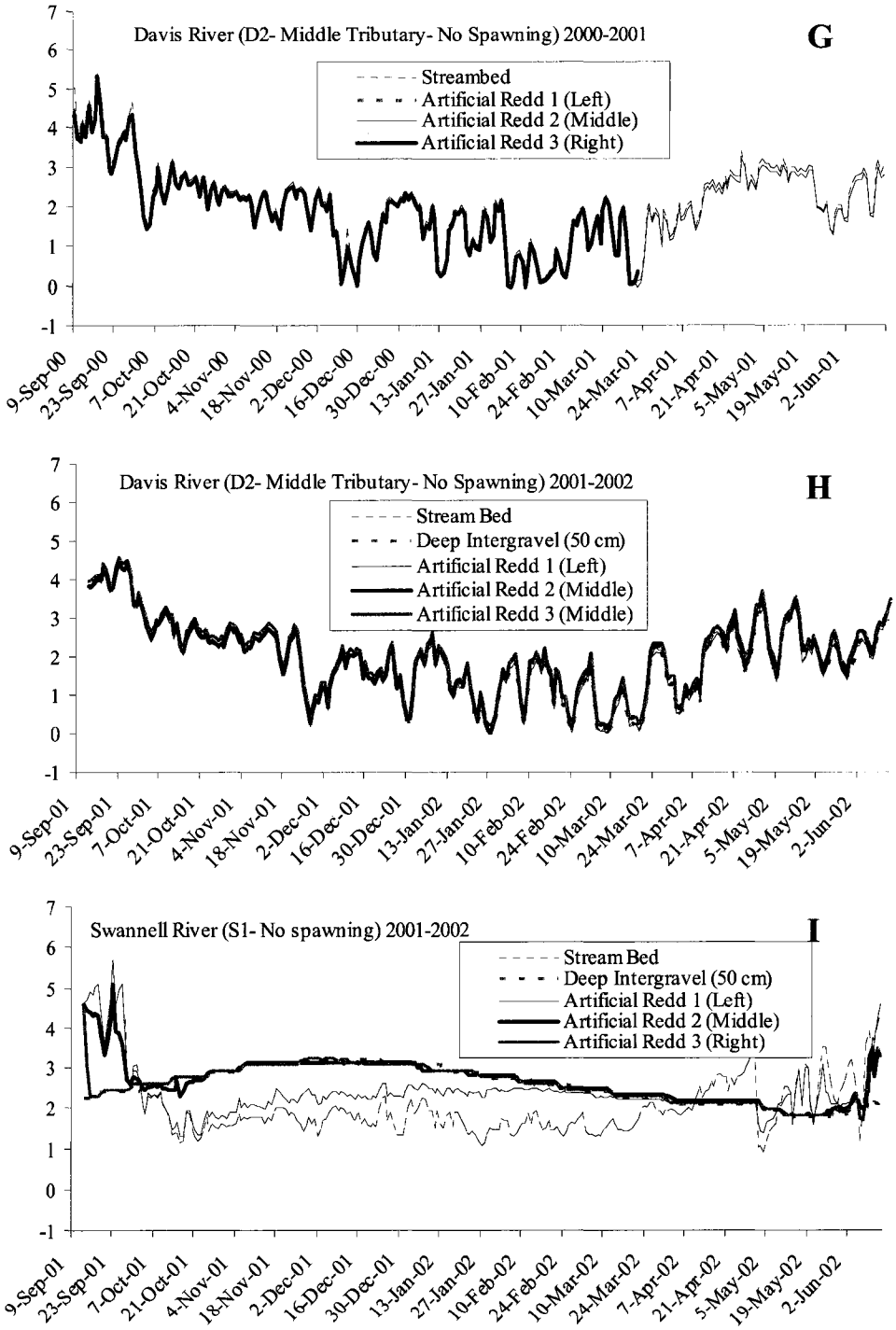
Within reaches, stream and intergravel temperatures were nearly identical for most sites except for the Davis River mainstem (Figs. 8a and 8b), and the upper Swannell River (Fig. 8i). Daily mean stream temperatures from September 12 to March 28, in the Davis River mainstem reach in 2001 were on average 0.62°C (range 0.37 - 0.89°C)



Figures 8a-8c. Temperature profiles recorded for artificial egg incubation site for both years. Each figure presents temperature data for up to three artificial redds (30-cm intergravel), one deep (50-cm Intergravel) and the stream-bed for each site.



Figures 8d-8f. continued.



Figures 8g-8i. continued.

warmer than daily mean intergravel temperatures. In year 2, the mean daily difference from September 11 to April 29 was 0.85°C (range 0.26-1.99). Similarly, deep intergravel temperatures (50-cm depth) were within the range of temperatures collected at 30-cm depth at all sites except the Davis River main-stem and the upper Swannell River site. At the Davis River main-stem site, the deep intergravel logger was warmer than the shallow loggers and had accumulated more thermal units by the end of the experiment (Table 5).

The Swannell River site thermograph was relatively unique in comparison to other sites. Differences in temperature between the stream surface, the shallow and the deep buried loggers were not constant through time and appeared to vary as groundwater had an increasingly dominant influence as winter progressed (Fig. 8i). Following late September, a relatively warm ground water influence was evident, starting with the deep buried logger, which then progressed from the deepest part of the channel (right logger) to the shallowest part of the channel (left logger) over the winter. The Swannell River site was the warmest overall during the incubation period and had accumulated the most thermal units by the conclusion of the experiment (Table 5; Fig. 8i).

Hyporheic Flow and Intergravel Chemistry:

ANOVA tables for analysis of hyporheic flow and chemistry are provided in Appendix B. Three sites used naturally for spawning and one site not used (Swannell River) had the highest mean values for specific discharge which often differed from piezometers at 50-cm depth (Fig. 9a). In September absolute specific discharge was highest at sites used for spawning ($F_{1,22} = 6.32$; $P = 0.020$) but there were no significant differences between used and un-used sites in March and April, ($F_{1,22} = 6.32$; $P = 0.343$; $F_{1,22} = 2.84$; $P = 0.109$) (Appendix B Table 15). Differences between reaches were not significant (F's < 2.65; P's > 0.061) during all dates, however, intergravel flow was

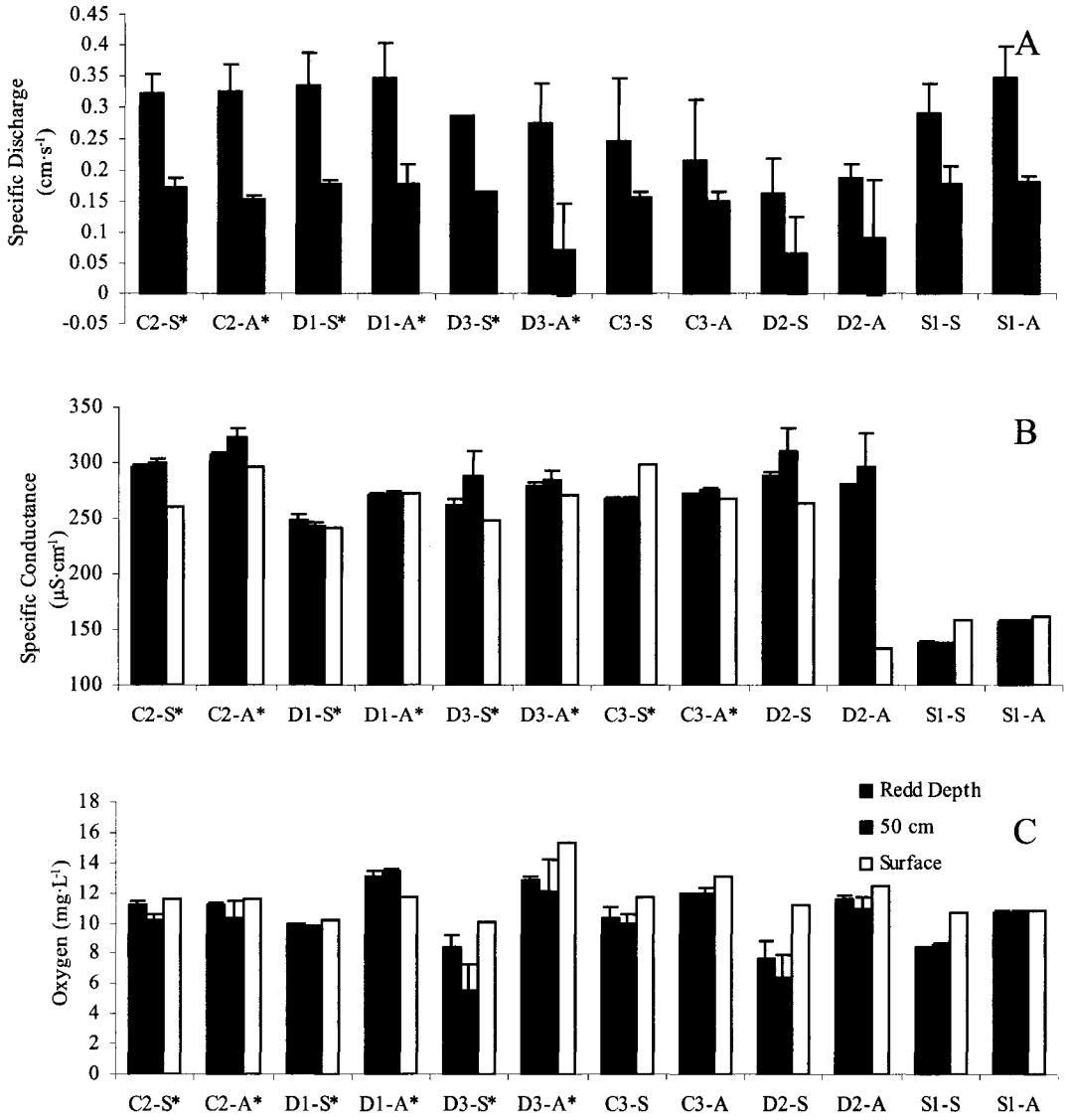


Figure 9. Specific discharge and intergravel chemistry for artificial redds in year two during the September and April collection periods. Data presented are data means and standard errors for samples collected in the stream (chemistry only) and at redd depth (30 cm. Letters following each reach name signify collection dates where S = September and A = April. A "*" following reach name shows sites where bull trout spawned.

significantly higher in the shallow piezometers within reaches over each of the three sample periods (Fig 9a) (F's > 4.28; P's < 0.01) (Appendix B Table 15a - 15c). There were also differences between sites used and not used and between reaches from September to April (F's > 12.26; P's < 0.0005) (Appendix B Table 17). Specific conductance was high at all sites in the Muskwa Mountains (Northern Rocky Mountains) and differences between reaches were small but highly significant (F's > 22.16; P < 0.0005) (Appendix B Table 16) for all three time periods. The upper Swannell River (Omineca Mountains) site had the lowest conductivity, which was almost half that of the streams on the opposite site of the Rocky Mountain Trench (Fig. 9b). Comparison of specific conductance between surface waters and the shallow and deep piezometers showed highly significant differences among sites (F's > 22.16; P's < 0.0005) for September and March, but not April at the level of site use ($F_{1,29} = 0.83$; $P = 0.374$) (Appendix B Tables 16a-16c). Similarly, differences between the surface and piezometers at depth within reaches were not significant (F's < 1.51; P's > 2.18) (Appendix B Tables 16a-16c). We did however observe significant differences in conductivity from September to April between sites used and not used ($F_{1,22} = 26.52$; P < 0.0005) but not among reaches ($F_{1,22} = 1.03$; $P = 0.459$) (Appendix B Table 18b). These results indicate differences in hyporheic mixing through time for sites used and not used for spawning but not among reaches.

Oxygen concentrations were high at almost all sites at the redd level, however, deep piezometers from two sites (Site D3 and D2) had relatively low concentrations < 6.5 $mg \cdot l^{-1}$ (Fig. 9c). Differences in oxygen concentration between piezometers and surface waters at sites used for spawning were significantly less than at sites not used for

samples September and March ($F_{1,22} = 27.27$; $P < 0.0005$ and $F_{1,23} = 22.32$, $P < 0.0005$) but not for April (Fig. 9) ($F_{1,18} = 0.84$, $P = 0.553$) (Appendix B Table 17) and F's >2.67, $P's < 0.0005$) (Appendix B Table 18c), indicating less hyporheic mixing at sites not used for spawning. We observed the lowest oxygen levels in September at the two warmest, groundwater-influenced sites; Davis (middle tributary) and Upper Swannell River (Fig. 9c) at the 50-cm depth. We were unable to complete the analysis of pH among sites and between collection dates to non-normal distribution of the data, however, pH at all sites was within a narrow range (7.14-8.44) and differences are unlikely to be of biological significance. Numerically, Swannell River had the highest pH levels compared to the rest of the sites.

Embryo Size and Yolk Utilization

In both years measurements of growth and yolk utilization varied across experimental units and differences in measures of embryo size and yolk utilization were highly significant for most experimental factors including; date, reach (Fig. 10a-d), redd, capsules, family (Fig. 11a-d), and parent (Fig. 12a-d; Table 8). However, *post hoc* comparisons between redds within reaches and between capsules within redds were generally not significant. Thus, growth differences were largely explained by variation occurring at the level of the reach and our presentation of results is confined to factors at the level of the reach and above.

In general, the longest and heaviest alevins were found within the warmest reaches (D2 and S1) (Figs. 10a, b, d) that also had the highest ATU's (Figs. 13 and 14). For example, by the end of the experiment in year 2 alevins at site S1 were on average 23.6 mm whereas alevins collected on the same date from site D1 were 20.1 mm.

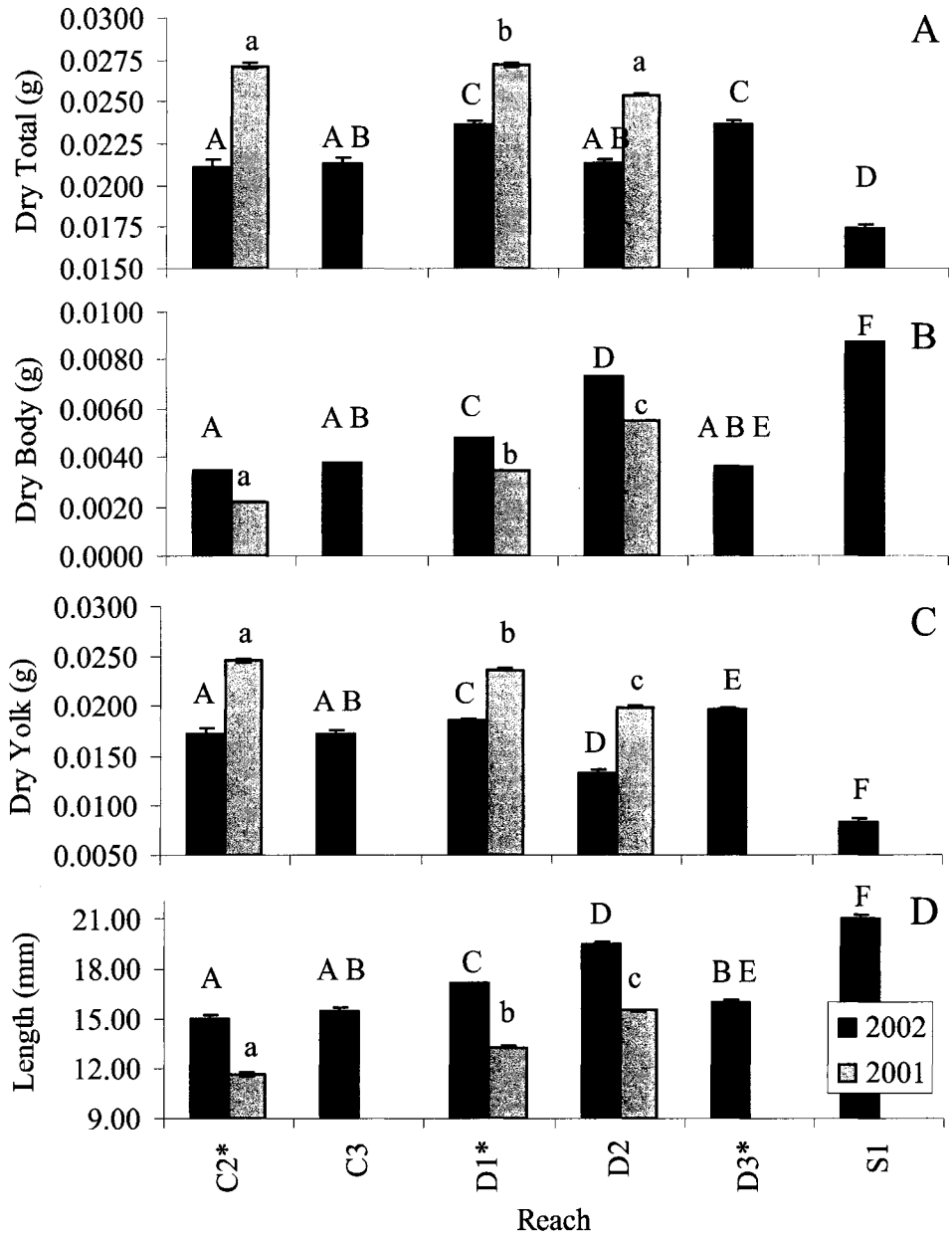


Figure 10. Effect of reach on growth and yolk utilization for alevins collected in both field experiments. Marginal means with standard errors are presented for total dry weight, dry-body weight, dry-yolk weight and wet length except for dry-body weight where only back transformed means are provided. Shared letters represent overlap in Tukey's simultaneous 95% confidence intervals. Lower case letters are for year one and upper case letters are for year two. A "*" indicates sites where bull trout spawned.

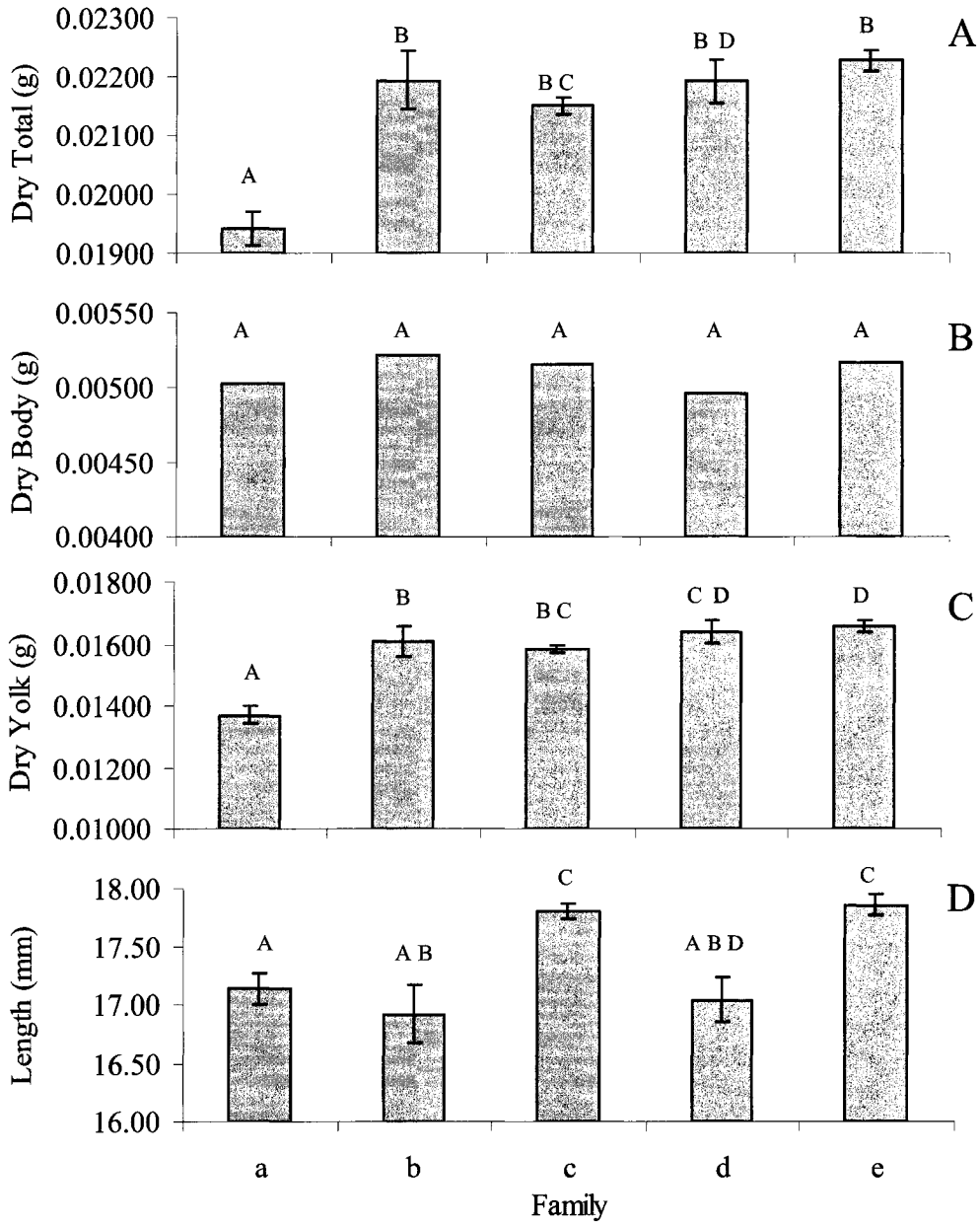


Figure 11. Effect of parent on growth and yolk utilization for alevins collected in both field experiments. Marginal means with standard errors are presented for total dry weight, dry-body weight, dry-yolk weight and wet length, except for dry-body weight where only back transformed means are provided, thus standard errors are not provided for dry-body weight. Shared letters represent overlap in Tukey's simultaneous 95% confidence intervals. Uppercase and lowercase letters differentiate males from females.

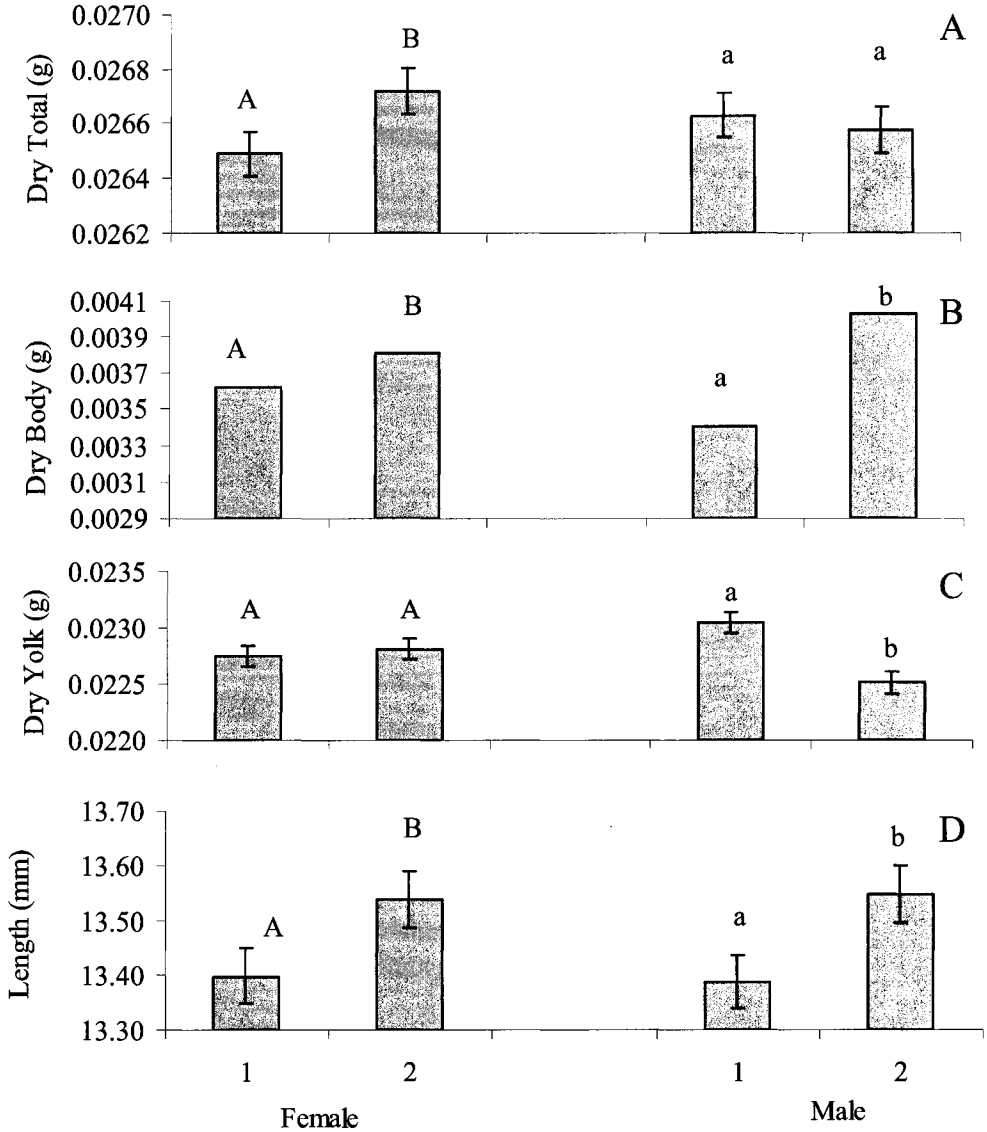


Figure 12. Effect of family (year 2) on growth and yolk utilization for alevins collected in both field experiments. Marginal means with standard errors are presented for total dry weight, dry-body weight, dry-yolk weight and wet length except for dry-body weight where only back transformed means are provided, thus standard errors are not provided for dry-body weight. Shared letters represent overlap in Tukey's simultaneous 95% confidence intervals.

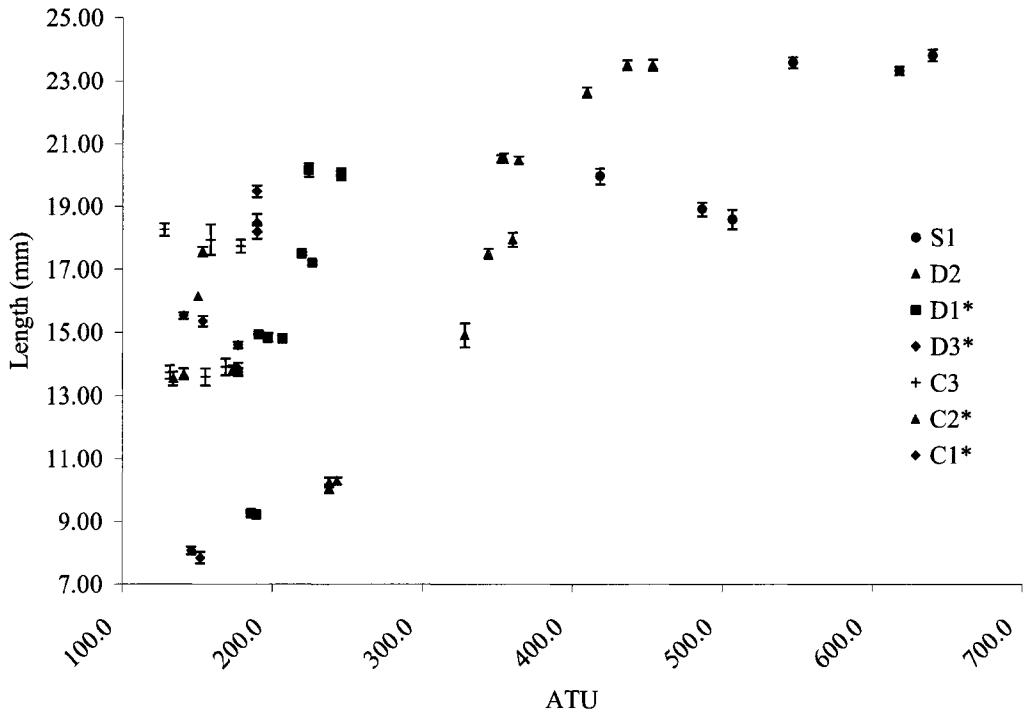


Figure 13. Means and standard errors of lengths for alevins from artificial redd sites collected over both years plotted against accumulated thermal units (ATU). Each point is an arithmetic mean for all alevins from one redd on a single date. Individual reaches are differentiated in the legend by letters for each stream and by numbers for each reach. ("S" for the Swannel River, "D" for the Davis River and "C" for Chowika Creek). A "*" indicates sites where bull trout spawned.

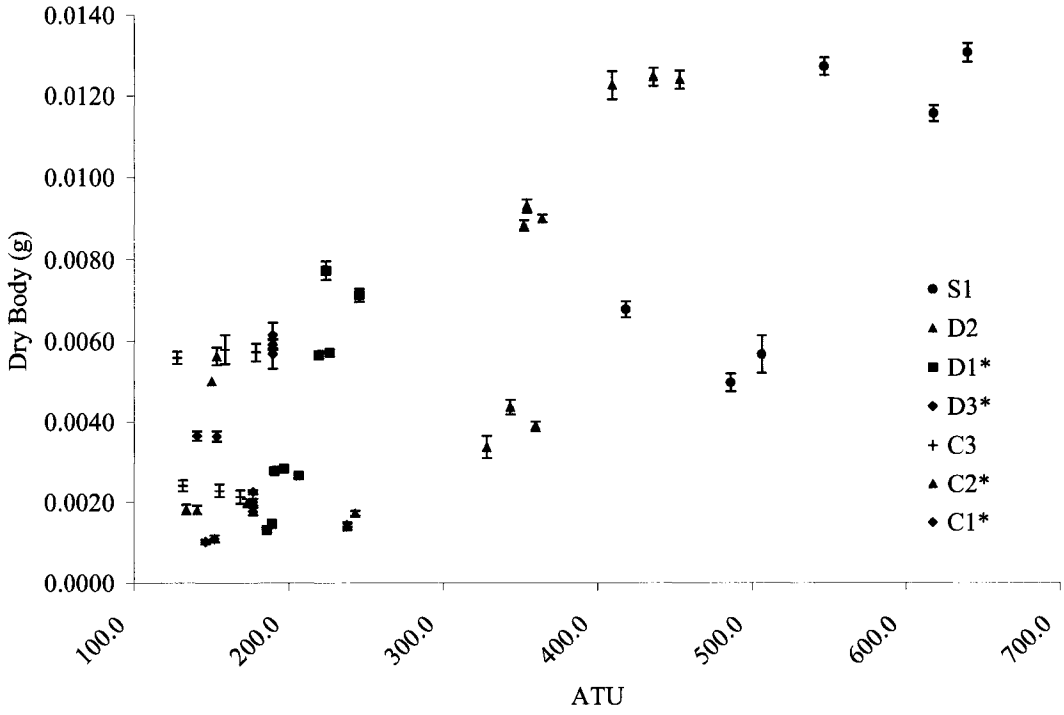


Figure 14. Means and standard errors of dry-body weight for alevins from artificial redd sites collected over both years plotted against accumulated thermal units (ATU). Each point is an arithmetic mean for all alevins from one redd on a single date. Individual reaches are differentiated in the legend by letters for each stream and by numbers for each reach. ("S" for the Swannel River, "D" for the Davis River and "C" for Chowika Creek). A "*" indicates sites where bull trout spawned.

Alevins from the coldest site (C2) were 16.9 mm. Dry-body weight increased significantly over time at the warmer sites (F's > 3158.99; P's <0.0005) (Appendix C Table 21 and Table 25) and the heaviest alevins were found at the warmest sites (Fig. 10b). Dry-body weight was similar among the coolest sites (C1-C3, D3) in year 2 (Fig.10b). Yolk utilization was also highest at the warmest locations (Fig. 10c) and alevins collected in April 2002 at site S1 had nearly exhausted their yolk reserves and only had on average 0.0027 mg of dry yolk remaining, whereas alevins from site C2 had 0.014 mg of yolk. Total dry weight (combined dry yolk and dry-body weight) was lowest at the two warmest sites (Fig. 10a and 15). Over the course of the experiment, there was little difference in ATU's between collection dates for low temperature sites (C1-C3, D3) (Table 5) and growth and yolk metabolism proceeded despite the low temperatures (Figs. 13, 14 and 15).

The magnitude of differences for dry weights and length due to individual parents was generally small (Figs. 11a-11d), however, female parental effects on length, total dry weight and dry-body weight were significant in year one (F's > 4.08; P's < 0.05) (Appendix C Tables 19, 20 and 21) (Table 8; Figs. 11a, 11b and 11d). In all cases female 2 was linked to higher growth and a larger total weight, but not higher yolk reserves (Figs. 11a-11d). In contrast, ANOVA showed male parental effects for length, dry-body and dry-yolk weight (Appendix C Table 19-21) (Figs. 11d, 11b and 11c). In year two we were unable to test individual parental effects on growth factors due to limitations imposed by low survival rates and the nested study design; therefore we substituted family as a combined factor in place of individual parents. In year two, there were highly significant differences in length, total dry weight and dry yolk that were apparent

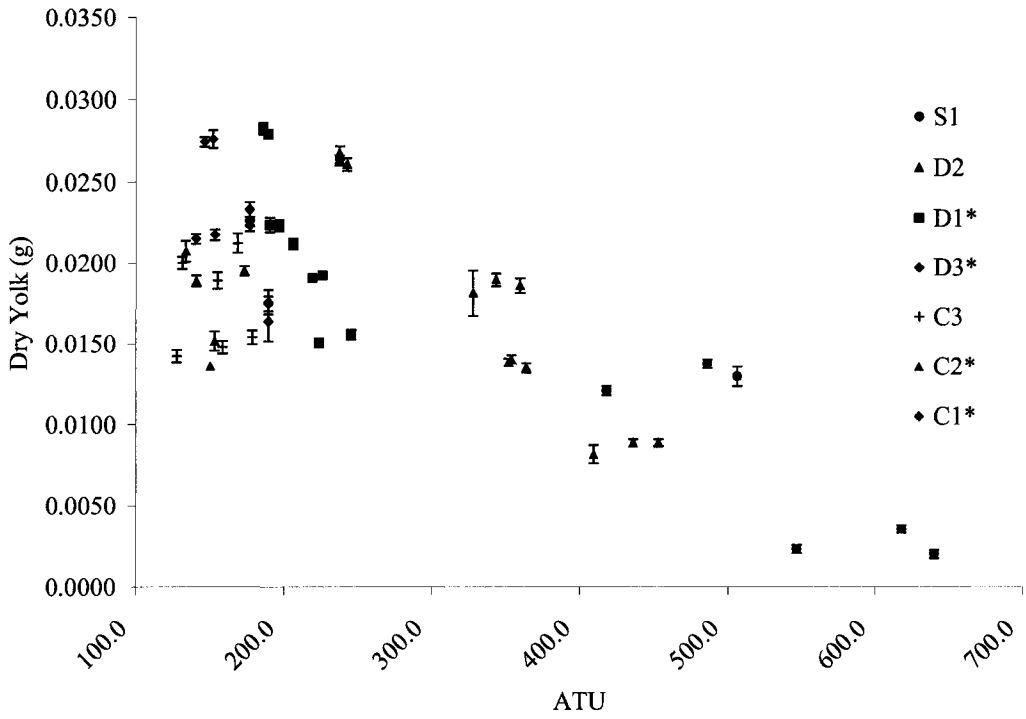


Figure 15. Means and standard errors of dry yolk weights for alevins from artificial redd sites collected over both years plotted against accumulated thermal units (ATU). Each point is an arithmetic mean for all alevins from one redd on a single date. Individual reaches are differentiated in the legend by letters for each stream and by numbers for each reach. ("S" for the Swannel River, "D" for the Davis River and "C" for Chowika Creek). A "*" indicates sites where bull trout spawned.

between families (Table 8, Fig. 12 a, 12c and 12d) (F's > 8.97; P's < 0.0005) (Appendix C Tables 23, 24, 26), however a family effect was not apparent for dry-body weight (Fig. 12b) ($F_{4,718} = 1.28$; $P = 0.342$) (Appendix C Tables 25). In general, the magnitude of differences in yolk reserves between families was small where the differences were on average less than 0.005 mg. Differences in length between families were more striking where families C and E (linked by a common male) were almost 0.8 mm longer on average than the other three families (Fig. 12d).

Discussion

Many studies have emphasized the importance of the incubation environment, particularly temperature, for salmonids and it has been suggested that Pacific salmon have adapted their migration timing and patterns to exploit different habitats across species ranges to maximize egg-to-fry survival (Brannon 1987). Groundwater and hyporheic exchange have frequently been cited as important factors influencing spawning site selection and potentially incubation success in several salmonid families, including chinook salmon (*Oncorhynchus tshawytscha*) (Geist 2000), chum salmon (*O. keta*), (Leman 1993), brown trout (*Salmo trutta*) (Hansen 1975), eastern brook char (*Salvelinus fontinalis*), (Curry et al. 1995; Blanchfield and Ridgeway 1997; Curry et al. 1995), Arctic char (*S. alpinus*) (Cunjak et al. 1986), and bull trout (Baxter and McPhail 1999; Baxter and Hauer 2000; Chapter 2). Patterns of use vary between species, for example brook trout (Webster and Eriksdottir 1976) and Arctic char (Cunjak et al. 1986) in some locations, appear to have strong preferences for upwelling water. This preference is not as clear with other species, such as chum salmon (Leman 1993) and bull trout (Baxter and Hauer 2000, Chapter 2) and there appears to be intra-specific variation between

populations. Leman (1993) observed that two populations of chum salmon occupying the same river system appeared to select spawning sites differently where one had a preference for sites with groundwater up-welling into the hyporheic zone and the other preferred sites with strong hyporheic exchange (mixture of both up- and downwelling). Differences in preferences within and between species are likely representative of a range of adaptive responses to a range of available habitats that are suitable. The adaptive response to this variation (i.e., site selection and its various mechanisms) is likely driven by combinations of habitat features that affect juvenile growth and survival and ultimately incubation success at a population level. Few studies however, have attempted to link attributes of groundwater to incubation success within a population of fish.

Our aim was to understand how variation in the incubation environment of bull trout affected size and survival of eggs and alevins in reaches selected and not selected for spawning. Our results have shown that all reaches examined were of relatively high quality based on intergravel flow (specific discharge) and measures of chemistry and did not affect survival and size, despite instances of significant differences between sites. Temperature regimes differed substantially between some sites, but did not appear to affect survival during incubation. Temperature, however, had a strong effect on size and development and the temperatures we observed at sites used for spawning were intermediate to sites not used. In particular, we found that small changes in temperatures had relatively large effects on the size of embryos and alevins as well as the timing of hatch within and among the stream reaches examined. Considering that the habitats we examined appeared to be of high quality, our results suggest that temperature is a key habitat attribute for incubation success and that bull trout are selecting spawning sites

with stable, intermediate incubation temperatures relative to what is available.

Site Selection Habitat and Groundwater Influences

Chemical and physical measures (i.e., oxygen, pH, intergravel flow, conductivity and temperature) at all reaches appeared to be within a suitable range for bull trout survival. Within our study areas, we observed temporal and spatial differences among sites and between deep and shallow piezometers for some locations but not all (Fig. 9a-9c). Spawning site selection did not appear to be influenced by any one factor measured and no clear patterns of use, based on measurements collected at the six sites were observed. Several factors in combination may determine site selection, but for ease of presentation the factors are described individually below. Temperature may be a key determinant of site use, as sites intermediate to the range of available temperature regimes were used for spawning to a greater extent. Gravel permeability and interstitial oxygen levels may also partially explain spawning site use in the context of site quality; lower intergravel flow and lower oxygen concentrations were linked to sites that were less used, however these effects did not appear to be strong. Finally, we found evidence that water chemistry (including pH, conductivity and oxygen concentration) varied among and within sites and combined with temperature, these features may be important indicators of groundwater that could act to influence spawning site selection.

We used specific discharge as a measure of gravel permeability, an indicator of site quality and a potential indicator of groundwater discharge (Freeze and Cherry 1978; Baxter and McPhail 1999; Baxter and Hauer 2000). Values of specific discharge were highest at high-use reaches in the Davis River and Chowika Creek (D1 and C2) and at one non-selected reach in the Swannell River (S1) (Fig. 9a). Specific discharge was

lowest at two of the non-selected reaches in the Davis River and Chowika Creek (sites D2 and C3; Fig. 9). Oxygen concentrations, another indicator of habitat quality, were well within the ranges suitable for incubating salmonids (Barton and Taylor 1996) (Fig. 9c). Oxygen concentrations were, however, lowest in two sites not used for spawning (D2 and S1) and were more variable at these sites compared to the other sites used for spawning. We also recorded measurable differences within reaches, between redds and between deep and shallow piezometers, and surface waters, however, these differences were not statistically significant (Fig. 9c). Given our relatively small sample size (three measures per redd, 12 per reach spread over three dates), it is possible that differences that would have been of biological significance were masked by low statistical power. Our observations for lower individual measurements of oxygen concentration and lower specific discharge correspond well with visual observations of fine sediment levels within each of the sites we used for the incubation experiment and may be indicators of site quality. During construction of the artificial redds in reaches C3 and D2, we noted (but did not specifically measure) higher levels of fine sediments relative to the other reaches with reach D2 having the highest levels. High levels of fine sediments are known to reduce interstitial spaces within the gravel nests of salmonids resulting in lower oxygen levels and suffocation of incubating salmonid embryos leading to reduced emergence success (Chapman 1988; Bjornn and Reiser 1991).

Measurements of pH and specific conductance (Fig. 9b) were relatively homogenous within and betweens reaches on the eastern side of the Rocky Mountain trench where relatively high conductivity and alkaline pH reflected the prevalence of calcareous sedimentary rock in the Muskwa Mountains. Differences between Swannell

River and the remaining sites were likely the result of differences in geology, where the Swannell Ranges are primarily igneous rock of either volcanic or deep origin which would have less propensity to leach minerals into stream and groundwater compared to rock containing calcite. For both pH and conductivity, we did not observe differences between surface waters and, deep and shallow piezometers that would likely be biologically significant, with the exception of site D2 (Fig. 9b). Site D2 had significantly lower conductivity in surface waters which could be related to a strong groundwater influence at this site. Higher conductivity and lower relative pH due to increased levels of dissolved carbon dioxide and increased levels of dissolved organic carbon along with increased dissolved solids are characteristics of subsurface or groundwater (Kaplan and Baker et al. 2000; Newbold 2000). We also found further evidence of a strong groundwater influence at this site. In winter we noted a nearby seepage area that remained relatively warm and ice free even when air temperature was below -25 ^{0}C. In some circumstances, localized discharge of deep origin groundwater devoid of oxygen may have negative influence on site selection (Dent et al. 2000; Geist 2000) and this may partially explain lack of use at site D2.

Temperature patterns within and between stream reaches in our study area varied substantially during the winter incubation period and the influence of relatively warm groundwater intrusions was evident (Fig. 8). The two warmest sites (S1 and D2), remained well above 0°C even during the coldest months, accumulated the most thermal units (Table 5) and were also the least used sites for spawning. Site S1 also demonstrated a large-degree of spatial heterogeneity within the site between temperature loggers during any given time period. As base-flows declined during winter a thermally-stable

groundwater intrusion was evident at depth within the stream-bed, where the intrusion progressed from the logger nearest the thalwag to the logger near the stream margin during winter (Fig. 8i). Based on these observations, including the lack of use at sites S1 and D2, we suggest that in thermally heterogeneous environments, bull trout may be avoiding sites with relatively high or temporally variable intergravel temperatures. Temperatures above an optimum range may produce rapid growth and advanced size, but may induce pre-mature exhaustion of yolk reserves leading to excessive mortality in the natural environment. Thus, predictable thermal habitat, both spatially and temporally, in cold northern environments could form the basis upon which northern stocks of bull trout use sites for spawning. Brannon (1987) observed that several species of Pacific salmonids have adapted their spawning timing and probably spawning site selection on the basis of thermal regimes in an effort to enhance success of their progeny. The timing of spawning and duration of incubation in these populations was thought to optimize the timing of emergence to precisely coincide with an increase in stream productivity in spring. In our study area, relatively high or variable temperatures may therefore have a significant negative influence on the rate of development of incubating bull trout if the timing of emergence was suboptimal. Variable or low intergravel flow rates and oxygen concentrations or inappropriate temperatures at potential spawning sites may, therefore, partially explain patterns of use in our study area. Although, site selection could be dependant on several factors, including groundwater influence, water chemistry, substrate and temperature regime, temperature is likely the strongest link to site selection.

Habitat and Survival

Habitat parameters, including incubation temperature, specific discharge and

chemical measures collected within reaches were all sufficient to maintain life at all sites and likely had little effect on survival during incubation at the sites we used in year one of our study. Year one mean survival was relatively high (Fig. 7) at all sites and was within the range reported for experiments using similar artificial incubation vessels in stream gravels: for bull trout 76.1-88.6 % (Baxter and McPhail 1999); for sockeye, 27-51% (Cope and Macdonald 1998) and for chinook 22-58% and steelhead 61%; (Merz et al. 2004). We did not make similar comparisons for year two due to low and variable survival likely due to poor fertilization success (see below), although one groundwater fed site in the Swannell drainage demonstrated a relatively high survival possibly indicative of suitable site conditions during incubation. Further study at this site would be required, however, to establish this relationship.

Differences in survival between two reaches naturally selected for spawning and one low-use site for year one were not apparent (Tables 7 and 8) despite some significant differences in measure of intergravel flow rates, intergravel chemistry and incubation temperatures (Figs. 8-9). Habitat quality was therefore sufficiently high to support relatively high levels of survival at all sites. In fact, virtually all of the habitat measures we collected for our incubation experiment were within the ranges that we recorded in a survey of natural redds for a related field study (Chapter 2). Our observations, therefore, suggest that all the habitats we measured, including those chosen and not chosen by bull trout, appeared to have been suitable for adequate survival during incubation. In other words, at a minimum threshold habitat quality, survival is unaffected and site selection may be beyond that threshold may be influenced by factors unrelated to survival during incubation.

Although not supported by our study, small differences in habitat quality have been observed to affect habitat preferences or survival in other studies. Geist et al. (2000) observed that chinook spawning in the Hanford reach of the Columbia River selected sites with groundwater influence that also had oxygen levels above 7 ± 0.9 mg·l^{-1} and selection for this attribute was presumed to enhance survival. Baxter and McPhail (1999) compared two field sites, one used for spawning and one that was not used and they found that survival was enhanced and less variable for bull trout that were incubated in slightly-warmer areas with upwelling groundwater compared to an adjacent area that was not used for spawning. Although the differences in survival were small, survival at the selected spawning area was 88.6 percent compared to 76.1 percent for the non-selected site. Cope and Macdonald (1998) compared *in situ* survival of sockeye salmon embryos among incubation sites of different apparent habitat qualities, where quality was based on observations of relative use and measures of physical habitat, including gravel permeability. As with our results, they did not find differences in survival among sites and they argued that their initial assumptions of habitat quality, based upon high and low site use by spawners, were not valid and that truly marginal areas were not used for spawning at all.

Similar to the approach of Cope and Macdonald (1998), we selected our field sites on the basis of relative levels of use by spawners to test for the effects of habitat quality on survival and growth. In our experiment, the middle tributary (low-use) reach in the Davis River was not used for spawning and we assumed this site to be marginal due to the lack of use; likewise the two other sites in the Davis River and Chowika Creek that were used in year one were selected on the basis of relatively high use by spawners

(Chapter Two) and habitat quality was assumed to be high. Given that survival was similar at all sites (Table 8) the high intergravel flow (Fig. 9a) and high levels of oxygen (Fig. 9c), it is apparent that the Davis River low-use site was not marginal based on these measures. It is notable that different temperature regimes did not affect survival despite temperature differences between sites within the Davis River of more than 2°C during the winter incubation period (Figs. 8g and 8h). These differences resulted in values for accumulated thermal units (ATU) for the warm site that were more than 200 ATU greater than the cooler site by the second collection date in year one(Table 5). Temperature differences were even more pronounced for other sites used in year 2 (Table 5) where difference in ATU's between the Swannell River site and the high-use site in Chowika Creek approached 440 ATU over approximately the same time period.

In both the Davis River and Chowika Creek, natural incubation sites were generally less than 1°C once winter set in (late Nov - Chapter 2). Thus, it is possible that temperature differences much greater than 1°C or 2°C could have stronger negative effects on survival during incubation on populations adapted to spawning in cold environments. In more southern stocks, cold temperatures may have the opposite effect for fish adapted to spawn in warmer locations. For a southern population of bull trout, McPhail (1979) noted slightly lower survival for fish incubated at 2°C compared with 4°C, however, at both temperatures survival was greater than 80 percent. Adaptation to local temperature conditions appears to be a common feature among stocks of different salmonids (Brannon 1987; Murray and McPhail 1988; Beacham and Murray 1989; Baxter and McPhail 1996).

One limitation of our study is that we recorded survival well before the normal

emergence period from the gravel, thus survival may have lower after emergence 1.5 months later (near the middle of June; Chapter 4). Survival at these sites could also have been influenced in part by the methods we used, as artificial egg capsules may have had a positive bias on survival. The capsule may act to reduce infiltration of fine sediments to the eggs during incubation, thus promoting survival. Second, emergence from the gravel is normally a period of high natural mortality and our methodology did not test for survival post-emergence, which could have been more than a month before natural emergence. Considering the low levels of use by spawners at sites C3 and D2 and S1 (Chapter 2) and our methods for measuring survival, it is possible that bull trout may have been avoiding these areas due to the fine sediment loads and lower oxygen levels despite our observations that the these sites were adequate for survival. Further examination of survival at a wider range of sites and with a variety of incubation methods could be used to test these effects.

Parental Effects on Survival

For both of our experiments, variation in survival for both years was partially explained by family or parental effects. Inadequate fertilization or poor gamete quality may be largely responsible for the observed effects in year two, including low overall survival. Family differences in survival in our experiment were apparent for both years (Tables 6 and 7), however, individual parental effects were not significant in year one when survival was generally high. Parental effects on year two survival were strong for males but not for females (Table 6). Male three for example was linked to the lowest mean survival across all families (Table 7). In contrast, male five sired families one and two which had the highest mean survival. It is possible that the observed effects reflected

natural variation in survival among family crosses as the result of a paternal effect on early development. Withler (1987) investigated differences in a paternal effect on the survival and development of a hatchery stock of chinook salmon. She noted that the effect was heritable and affected early embryonic development. Patternal effects, both heritable and environmental are generally uncommon (Heath et al. 1999). In our study, we strongly suspect that the male effect for year two was related to gamete quality and subsequent fertilization success. Stress due to an extended holding time for adults captured on September 9 and 10, 2001 or our egg handling methods are likely mechanisms for poor fertilization success or poor gamete quality. Prior to spawning in 2001, adults were retained in holding tubes in the river for several days following the events of September 11, 2001 as flight bans prevented us from returning to the field sites to spawn these fish until September 13, 2001. It was further observed that during spawning in year two, the volume of milt obtained appeared to be less than the previous year, although we did not measure this directly. An assessment of fertilization success would have enabled us to determine whether or not the poor survival was an artefact of the experiment or a natural occurrence. Unfortunately, we were unable to determine this in the lab with confidence and confirmation was therefore not possible. Eggs that were examined a few days after fertilization were very light in colour and we found it difficult to identify any evidence of cell division.

Temperature, Size and Development

Small temperature differences between sites over the course of incubation had profound effects on the size and development of bull trout at different sites within our study area. Higher incubation temperatures advanced the timing of hatch, increased yolk

utilization (Fig. 15) and resulted in heavier and longer alevins at warmer sites by the conclusion of our experiment (Tables 6, Figs. 13 and 14) despite relatively small temperature differences between sites (Table 5, Fig. 8). Large sizes and advanced development at higher temperatures are not un-expected for salmonids (Combs 1965; Peterson 1976; Heming 1982; Humpesch 1985; Tang et al. 1987; Garrett 1988; Murray and McPhail 1988; Beacham and Murray 1989; Baxter and McPhail 1999; Huuskonen 2003), however, considering the relatively low range of incubation temperatures we observed across sites used for our experiment (Figs. 8a-f), the magnitude of differences in sizes and decreases in the time to hatch at the warmest sites were surprising. For example hatch likely took place in late January through the month of February for the warmest sites based on the results of a related laboratory study (Chapter 4), whereas hatch occurred in March or later for the cooler sites.

Many other incubation studies for salmonids have noted substantial increases in mortality and relatively slow or decreased size for temperatures similar to those characteristic of our study. Few of these experiments, however, have explored low temperature conditions that would be encountered naturally by any given stock and thus offer limited inference about adaptation (or lack thereof) to cold conditions. Much of this experimentation has employed constant temperature regimes to test temperature effects on southern or coastal stocks that are likely adapted for more temperate conditions. Very low temperatures (i.e., < 2°C) during early incubation are unrealistic for most species and have been observed to increase mortality for many stocks, including those adapted to cold temperatures (e.g., Murray and McPhail 1987; Tang et al.1987). Cold temperatures during early incubation can disrupt development prior to organogenesis, (e.g., Peterson

1976) and few salmonids (including northern stocks) could be expected to spawn at these temperatures. In our study area for example spawning was largely complete before temperature declined below 4-5°C.

Summary

We found relatively large significant differences in size (lengths and weights), yolk utilization, as well as differences in the timing of developmental stages across habitats (reaches) that were used and not used for spawning. Significant differences in size were also noted at lower factor levels than the reach, as we also observed differences between artificial redds and between families and individual sires and dams. We found that physical and chemical habitat quality appeared to be high at all sites, and with the exception of temperature all features we measured appeared to be poor predictors of survival and size during incubation. Bull trout in our study area appeared to be well adapted to temperatures below 1°C after the onset of winter and for majority of the incubation period. Finally, we found relatively high survival at all sites, and size was relatively constant among the sites naturally selected for spawning. Small increases in incubation temperature, however, resulted in large sizes and higher yolk utilization; thus, based on the observations in this study and a related synoptic study of habitat at redd sites (Chapter Two) it appears that the range of preferred temperatures is relatively narrow. Temperatures outside this range may result in pre-mature yolk exhaustion and advanced developmental timing that could lower survival post emergence.

CHAPTER 4 : THE EFFECT OF AN INCREASE IN INCUBATION TEMPERATURE ON A COLD-WATER ADAPTED SALMONID, THE BULL TROUT.[3]

Abstract

Fish are most sensitive to changes in temperature during early life-stages, including incubation. As fish grow and develop, the thermal tolerance of most species becomes greater. We examined the effect of small increases in temperature above a natural temperature regime on stages of development for larval bull trout until button-up; the stage when all yolk has been utilized and the end of the larval phase. We incubated eggs in a laboratory environment where temperature was controlled. Our objective was to assess body size (length and weights), yolk utilization and the timing of developmental stages under natural and two elevated thermal regimes (+1.5°C and +3°C). We observed substantial differences in alevin length and yolk utilization rates among temperature replicates within both the field (Chapter 3) and laboratory experiments. The timing of hatch, button-up as well as size and yolk utilization were all advanced relative to the control temperature for both of the experimental temperature replicates. We also observed significant increases in mean white muscle fibres in the warmest temperature treatment.

[3] Throughout this chapter I use the first person plural to acknowledge the contribution of others to this work, which will be submitted for publication with the authorship of C.J. Williamson and J.M. Shrimpton.

Introduction

Temperature has a critical role in determining behaviour, growth, development and ultimately survival in fish. Reproductive behaviours in many fish, including salmonids, are adapted to take advantage of a wide variety of thermal regimes in lakes, rivers and streams (e.g., Brannon 1987; Leman 1993; Power et al. 1999). Once eggs are deposited by females into gravel nests and are covered, parental care is essentially complete and the development of eggs to emergence proceeds largely as a function of the temperature of the incubation habitat (Fleming and Reynolds 2004). In northern and interior environments, there is evidence that salmonids can be highly selective when choosing spawning locations, and the locations of redds are often linked to areas of groundwater influence with distinct temperature regimes (Hansen 1975; Leman 1993; Cunjak et al. 1986; Curry et al. 1995; Blanchfield and Ridgeway 1997; Geist and Dauble 1998; Ridgeway and Blanchfield 1998; Power et al. 1999; Baxter and McPhail 1999; Baxter and Hauer 2000; Geist 2000). Groundwater moderates both warm summer temperatures and cold winter temperatures where water can often decline to temperatures near 0°C in winter (Power et al. 1999).

In general, the zone of thermal tolerance during embryonic development is considerably more narrow than it is later in life (Rombough 1997) and the rate of embryonic and larval development is highly dependent on temperature. Optimum temperatures for incubation vary inter- and intra-specifically (Brannon 1987; Beacham and Murray 1988) and temperatures outside of the optimum can alter growth and development rates, influencing both the timing of emergence and first feeding in juveniles as yolk reserves are finite (Heming et al. 1982). Above thermal optima the

conversion efficiency of yolk is inversely related to temperature and yolk reserves are less efficiently converted at warmer temperatures (Heming 1982; Ojanguren et al. 1999). Increases in temperature may, therefore, lead to precocial emergence of smaller fry with a lower potential for survival for salmon trout and char; this is particularly important for interior (versus coastal) populations which tend to experience relatively cool incubation temperatures. Sublethal effects can also include changes in muscle fibre density and cellularity, which have implications for ultimate growth potential (Johnston 2001). Thus, changes to the temperature regime can disrupt the timing and rate of development during incubation and can ultimately lead to changes in reproductive success or output (Markevich and Bilenskaya 1992).

A number of factors have been shown to alter temperature of aquatic systems. Timber harvest can increase stream temperature approximately 1°C (Holtby 1988; Shrimpton et al. 1998). Additionally, some scenarios of global warming predict increases in air temperatures of 2-4°C (Hengeveld 1990; Wood and Macdonald 1997) which may lead to changes in stream hydrology and changes in temperatures in north temperate streams. A stable incubation environment may be particularly important for embryos that are immobile for long periods after spawning until hatch. Eggs and alevins that over-winter in the hyporheic zone in stream reaches benefit from the high oxygen content of the surface water and thermal stability of the groundwater (Baxter and McPhail 1999; Power et al. 1999). Changes in surface water temperature resulting from land-use practices or global warming, therefore, have the potential to impact rate of development, growth, and yolk conversion efficiency of incubating salmonid eggs and alevins.

In B.C., bull trout are a species of concern and are blue listed by the B.C.

Conservation Data Centre (B.C. CDC, 2006). This species has received considerable interest as it has been shown to have a low tolerance to habitat disturbances and has a preference for cooler water temperatures (Goetz 1997). Currently, quantitative descriptions of the thermal tolerance or temperature optima for developing bull trout from northern streams are not available. Our objectives for this study were to measure development and changes in body size (length and weight) and yolk utilization during incubation for bull trout from the Davis River, B.C., under a natural temperature regime and two elevated temperature regimes. The results of this study will provide fisheries resource managers a better understanding of potential impacts to bull trout where land-use practices or global warming could disrupt incubation habitats.

Methods

Experimental Setup

To determine the effect of temperature on incubation of bull trout we used three thermal regimes; one similar to a site heavily used by bull trout for spawning in the Davis River, B.C. (Fig. 1), the second approximately 1.5°C warmer than the Davis River temperature, and similar to that of non-spawning streams in the Davis watershed, and the third approximately 3°C warmer than the Davis River temperature (Fig. 16). We modeled the temperature regimes on intergravel temperatures recorded from a natural redd in the upper Davis River during the incubation period in 2000-2001. To reduce noise in the natural stream data and to provide a guide for controlling temperatures in the laboratory, we fit a second order polynomial regression curve (MS Excel 1997, Microsoft Corporation, Redmond, Wa.) to a plot depicting mean daily temperature for this site (Fig. 16). We used this curve as guide to control the Davis River regime, however this curve

only approximates the actual temperatures achieved. The other two regimes were elevated by 1.5°C and 3°C from the Davis regime in the laboratory.

Our experiments were conducted at the Pacific Western Brewery located in Prince George, B.C. Inlet water for each temperature regime was unchlorinated groundwater. The well water was chilled down from 9°C using water chillers (1 Hp and 1/3 Hp Models, Frigid Units, Toledo, Ohio). Each water-chilling unit was placed in a continuously aerated and insulated 170 l plastic reservoir tank. Chilled water from each reservoir flowed to a stack of vertical incubation trays. Outflow from the incubation trays was pumped back into the reservoir from a sump with an overflow outlet. The water reservoir continuously received fresh water at a flow rate of approximately 1 $l \cdot min^{-1}$ and the same volume was allowed to drain from the overflow in the sump. To achieve temperatures close to 0°C in the Davis River temperature replicate, we modified a 1 Hp chiller with 110-v timer, so that the compressor cycled on and off (2 min on and 5 min off), while the circulation impeller ran continuously. This modification prevented the build up of ice on the chilling coils that would have otherwise reduced the chilling efficiency of the unit. Without modification, the coldest temperature we could achieve was approximately 1.5°C. The actual temperature regimes that were achieved for the laboratory experiment were an approximation of Davis River thermograph and were subject to the design limitations of the water chillers and equipment used. For example, diurnal and weather related fluctuations in stream temperature were not reproduced in the laboratory experiment. To record temperature we used three submersible temperature data loggers (Optic Stowaway Temp, Onset Corporation, Bourne, MA) within each temperature replicate. We programmed the data-loggers to recorded hourly temperatures

for the duration of the experiment.

Fish

We captured four adult bull trout (two males and two females) from the upper Davis River during the spawning period in September 2001. We collected gametes from the Davis River fish in the field and placed them in storage containers that were kept cool in ice-chilled coolers. We retained approximately 1200 eggs from both females which were transported unfertilized to the laboratory by plane within 6 h. We fertilized the eggs immediately upon arrival at the laboratory facilities. The remaining eggs from these fish were used for our field experiment (Chapter 3).

At the brewery, we created four family groups by dividing the gametes from the two females and two males and subsequently recombining them to form a two by two breeding design. We mixed eggs and milt for each cross separately in clean plastic buckets, without water. After approximately 2 min, we added water to the eggs, which were then left to sit for 5 min. We then washed excess milt from the eggs which were then left to water harden for 30 min. After fertilization, we divided each family group into 12 portions and placed them in incubation trays (MariSource, Milton, Wa.) under three temperature regimes. We replicated each family group within each temperature regime; four families, three temperatures and two family replicates per temperature. Each tray was divided into four lanes, and one to three family group replicates were placed in each tray. To reduce incident light levels we wrapped each stack in black polyethylene plastic sheeting.

Survival, Embryo Size and Yolk Utilization

Starting at approximately two months after fertilization, we monitored trays for

survival on a weekly basis and we removed all dead eggs. Once the eggs had reached the eyed stage, we mechanically shocked each tray to aid in the identification and removal of unfertilized dead eggs. Thereafter, on at least a weekly basis for the duration of the experiment, we removed and counted dead eggs and alevins, counted live alevins and counted and recorded hatched eggs. We cleaned individual trays periodically for the duration of the experiment. Starting at 50% hatch and then at approximately one-month intervals we collected samples of five to 20 live alevins from each family replicate for analysis of size (length and weight) and yolk utilization. Individual sample sizes were dependent on total family survival to the eyed stage. We simultaneously euthanized and preserved eggs and alevins for later analysis by freezing them instantly at -80°C. Using the methods of Chapter 3, we dissected eggs and alevins into yolk and tissue portions. For each embryo, we recorded standard length to the nearest 0.01 mm using Vernier callipers under a dissecting microscope and then collected weights on an analytical balance (0.0001 g).

Muscle Fibre Density

For each family within each temperature replicate, samples of three to six alevins were collected for analysis of muscle fibre density. Samples for the muscle analysis were collected at 50 percent hatch and at button-up. Fish were preserved in formalin for 48 h and then stored in phosphate buffered saline. To quantify muscle fibre density, alevin samples were mounted in paraffin, sectioned, mounted on microscope slides and then stained. Briefly, after storage each alevin was sectioned with a scalpel just anterior to the dorsal fin insertion and just posterior to the trailing edge of the anal fin. The mid-sections of each alevin for each treatment replicate were transferred in lots to distilled

water and then dehydrated through a graded ethanol series from 50% ethanol to 100% ethanol using six different solutions over a 31-h period, after which they were washed in three, 7-min baths of fresh toluene. Each lot was then bathed in 60°C paraffin for 24 h before being mounted for sectioning with a microtome. Individual fish were then sectioned transversely to the longitudinal body axis in 5- to 6-μm increments to a point at least 15 to 20 μm anterior to the external opening of the anus. Sections were floated on a warm (approximately 45°C) water bath and mounted on gelatin coated microscope slides. Using the procedure outlined by Kiernan (1999) slides for each fish containing the appropriate landmark (opening of the anus) were stained with haematoxylin-eosin. Digital photographs of one section nearest to the external opening of the anus were collected with a Powershot G5 digital camera (Canon Canada Inc.) mounted to an Axio Star Plus microscope (Carl Zeiss MicroImaging, Inc.Thornwood, NY). For our analysis we used one half of each myotome section. Muscle myofibres and myomere cross-sectional areas from myotome sections were then counted and measured using ImageJ image analysis software (Rasband 1997-2006).

Data Analysis

Measures of alevin size (dry-body weight, dry-yolk weight, length and total body weight) as well as muscle fibre counts for each myomere were examined using nested ANOVA where factors in the model included, temperature treatments, parents, and tray and the interaction of parent (male crossed with female). Tray was nested within temperature treatments. All treatment levels were treated as fixed factors. All ANOVA's were completed using the general linear model (GLM) procedure in Minitab (v.14.1, State College, PA). We used the adjusted sum of squares to test significance. *Post-hoc*

comparisons were completed, using the Tukey-Kramer multiple comparison procedure in Minitab. The Tukey-Kramer method was used as this method for multiple comparisons was suitable for un-balanced experimental designs and is more conservative than other similar procedures.

All data were screened for erroneous values, outliers and as part of each procedure, we visually checked all ANOVA models for normality and heterogeneity using the output provide by Minitab, which included: normal probability plots, histograms and scatter plots of the residuals for each model. All ANOVA procedures met the assumptions of for normality and homogeneity of variance. To better understand growth trajectories within each temperature replicate, von Bertalanffy growth curves were fit to the length data using a non-linear least squares procedure (Levenberg-Marquardt) (Statistica v.6.1, Statsoft Inc. Tulsa, OK). Estimates of the asymptotic length were computed to compare growth trajectories.

Results

We were able to achieve an approximation of the three temperature treatments for our experiments as described in our methods (Fig. 16). The temperature of the Davis River was 5.6°C in September when the bull trout were spawned. The temperature of the cold-water treatment simulated the natural decline in temperature seen in the Davis system. The mid-temperature group targeted approximately 1.5°C above the Davis River temperature, and the warm water group target temperature was approximately 3°C above the Davis River temperature. At the end of November, the lowest temperatures for each group were achieved; 4°C, 2°C, and 0.6°C for the warm, mid, and cold temperature groups, respectively. We kept temperatures approximately constant until mid-March

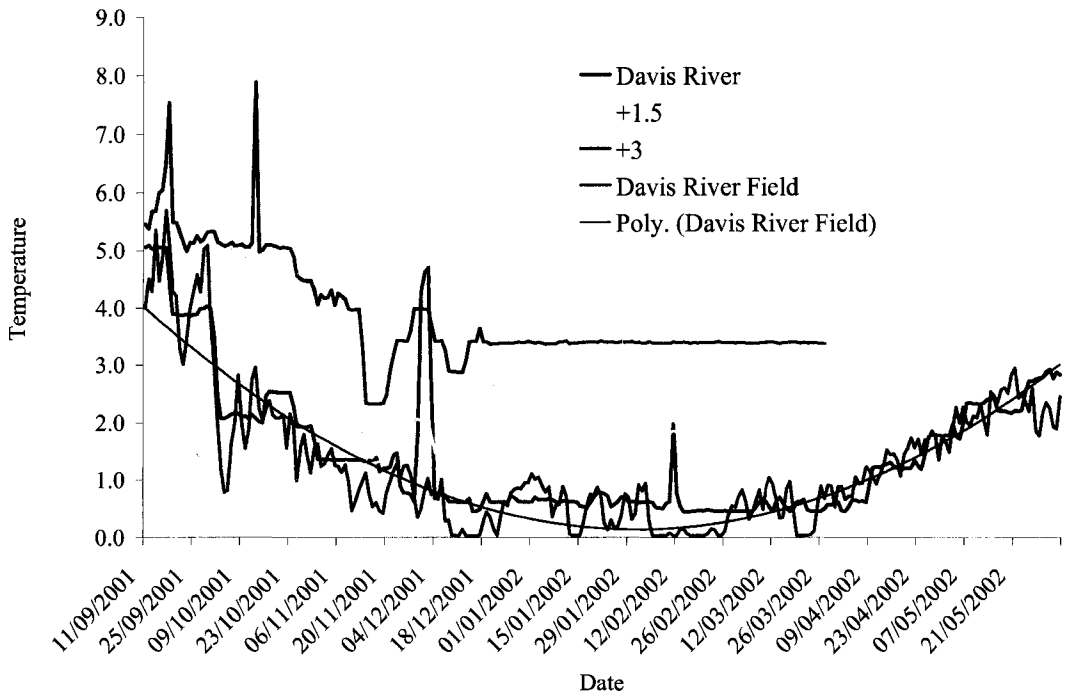


Figure 16. Temperature achieved for three incubation replicates in the laboratory plotted with natural temperatures for a redd from the Davis River (Davis River Field). Natural redd temperatures were collected in 2000-2001. A second order polynomial was fit to this data to provide a general template to guide temperature control of the Davis River treatment regime.

2002. At this time of the year, water temperatures in the Davis River begin to warm (Fig. 16). Temperature spikes (Fig. 16) reflect some of challenges in conducting the experiment including power and equipment failures or the need to clean the incubation apparatus.

On November 8, 56-d after fertilization, eggs for each experimental regime had reached the eyed-stage. Mortality estimates before 100% hatch were not calculated due to poor and variable fertilization success for some families. Poor survival was likely in part the result of poor gamete quality due to an extended holding time for adults after capture (see Chapter 3). Survival was, therefore, not examined in detail for this study. Overall, survival to 100% hatch ranged from 12 to 95% (Table 8). The first samples were collected at hatch for each family group (Table 9). The timing of hatch, button-up as well as size and yolk utilization were all significantly advanced relative to the control temperature for both of the experimental temperature replicates (Table 9, Fig. 17). Compared to control fish, 50% hatch was advanced by 56 and 76 days for the experimental mid temperature and warm temperature groups, respectively (Table 9). Differences between hatch and time-to-button-up increased as temperature increased. Button-up occurred at approximately 77, 86 and 93 days following hatch for the warmest to the coldest group respectively (Fig. 17). These differences show that the effect of warmer temperatures on hatch was stronger than it was on time to button-up.

Differences in length, dry-body and dry-yolk weights among dates, temperatures, females and trays were all highly significant (F's >3.32; P's < 0.001) except for dry-body weights where there was no significant female effect ($F_{1,618} = 2.39$; $P = 0.123$) (Appendix D Tables 27-29). The interaction of male on female was not

Table 8. Survival (%) at 100% hatch for each temperature regime by incubation tray, family, female and male.

Davis River	+ 1.5°C	+ 3°C	Tray	Family	Female	Male
51.5	79.0	77.5	1	A	1	2
11.5	24.0	29.0	1	B	1	3
23.8	94.7	84.6	2	C	2	2
75.0	93.9	87.9	2	A	1	2
16.8	23.1	23.1	3	B	1	3
90.5	88.1	81.0	3	C	2	2
75.0	62.0	51.0	3	D	2	3
52.6	56.1	57.9	4	D	2	3

Table 9. Date and number of days to 50 % hatch and button-up for three laboratory temperature regimes and one natural redd from the Davis River (Davis River Field). "Date" designates the estimated date for each stage; "Days" designates the number of days from fertilization; and "ATU" represents the summed average daily temperature for each period specified. The "Davis River Field" column is presented for comparison only and does not represent a specific spawning or fertilization time in the field.

Stage		+ 3°C	+ 1.5°C	Davis River	Davis River Field
50 % Hatch	Date	31-Dec-01	20-Jan-02	17-Mar-02	17-Mar-02
	Days	110	130	187	187
	ATU	493.3	434.1	285.2	218.3
Button-up	Date	27-Mar-02	23-Apr-02	02-Jun-02	02-Jun-02
	Days	196	223	263	263
	ATU	767.9	604.4	390.3	334.8

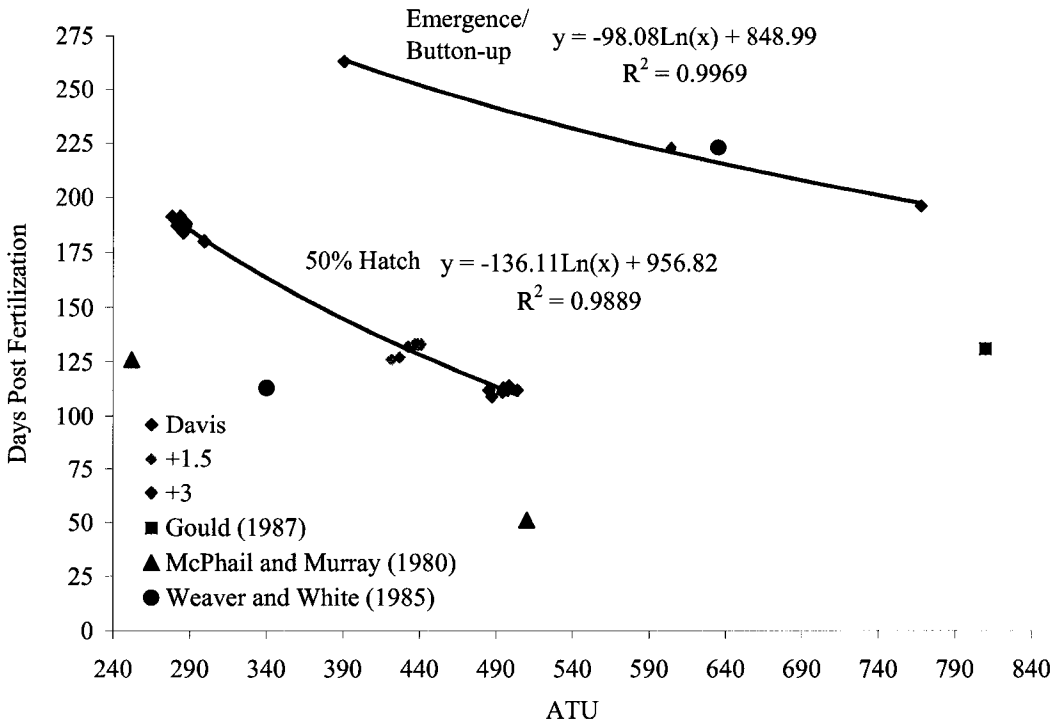


Figure 17. Duration of development (hatch and button-up) plotted against thermal history in accumulated thermal units (ATU). Data from the other authors were not included in the models for our data.

significant (F's < 3.32; P's > 0.05) (Appendix D Tables 26-28). Lengths and dry-body weights were greatest at the warmest temperatures (Figs. 18-20) and at the highest ATU's; although dry-body weights declined once yolk reserves were depleted in the warmest group. Maximum dry-body weights in the Davis and +1.5°C temperature groups were similar, despite differences in the number of ATU's (Figs. 19 and 20). Our estimates of the asymptotic length (length versus ATU from fertilization) for fish incubated in the cold ($\bar{x}$ = 24.41-mm; 95% CI 24.77- to 25.68-mm) were higher than for the +1.5°C ($\bar{x}$ = 23.11; 95% CI 23.96- to 23.25-mm) but were similar to the warmest group ($\bar{x}$ = 25.74; 95%CI 25.4- to 26.07-mm) (Fig. 22). The relationship between yolk utilization and ATU was also similar between the Davis and the +1.5°C temperature regimes (Fig. 21), however, at higher ATU's the rate of yolk utilization was more rapid for the +3°C regime. Higher temperatures also resulted in larger sizes and yolk utilization (Figs. 22-24). Maximum size was achieved in the warmest group by approximately 196 days after fertilization, while this did not occur until 263 days after fertilization for the Davis group. Yolk exhaustion occurred near day 223 in the warmest group, whereas yolk was still present by day 263 (button-up) for the Davis regime.

Mean white muscle fibre counts were highest in the heated groups (F's > 6.84; P's < 0.002) for each myofibre although there were no differences explained by parent or by tray or by developmental stage (Fig. 25) (Appendix D Tables30a-d). Post hoc examination between treatments revealed significant differences between the Davis River regime and warmest treatment (+3°C) (Tukey P = 0.0010); there were, however, no significant differences in myofibre density between the Davis and +1.5°C and between the +1.5°C and +3°C regimes (Tukey P's > 0.05).

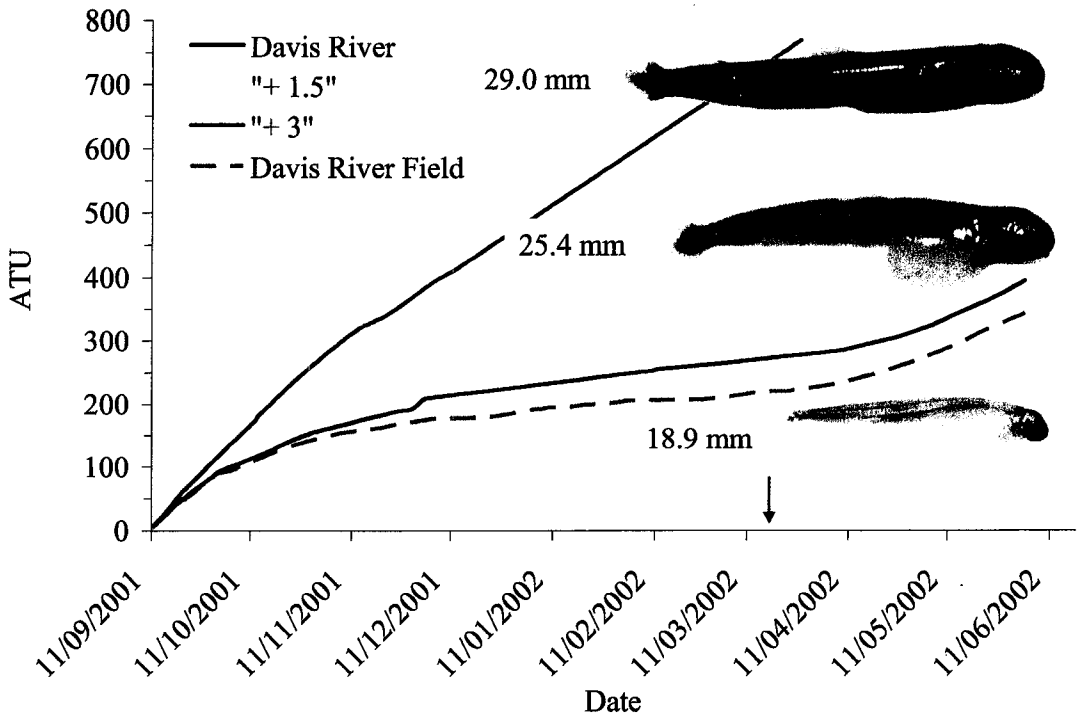


Figure 18. Thermal history of three alevins collected on March 18, 2002 under three different temperature regimes. The arrow show the sample date. Lengths of each individual alevin are presented for comparison and are representative of the average alevin in each lot. The dashed line "Davis River Field" is the temperature profile for a natural redd collected in winter 2000-2001. The arrow shows the precise collection date.

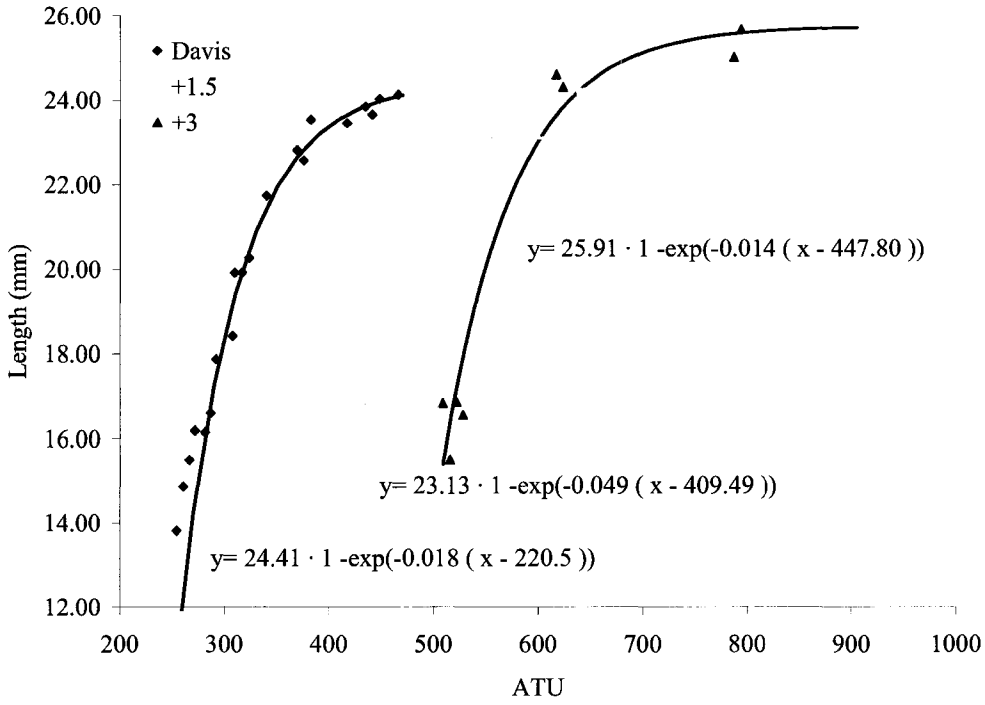


Figure 19. Length plotted against accumulated temperature units (ATU;°C·day) for each temperature replicate.

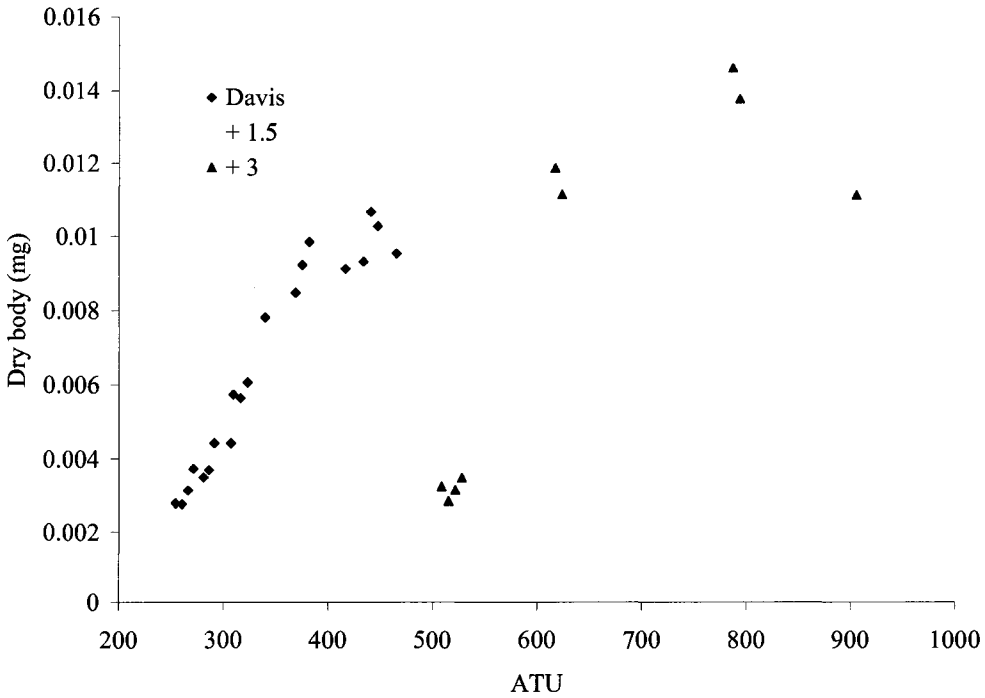


Figure 20. Dry-body weight plotted against accumulated temperature units

(ATU;°C·day) for each temperature replicate.

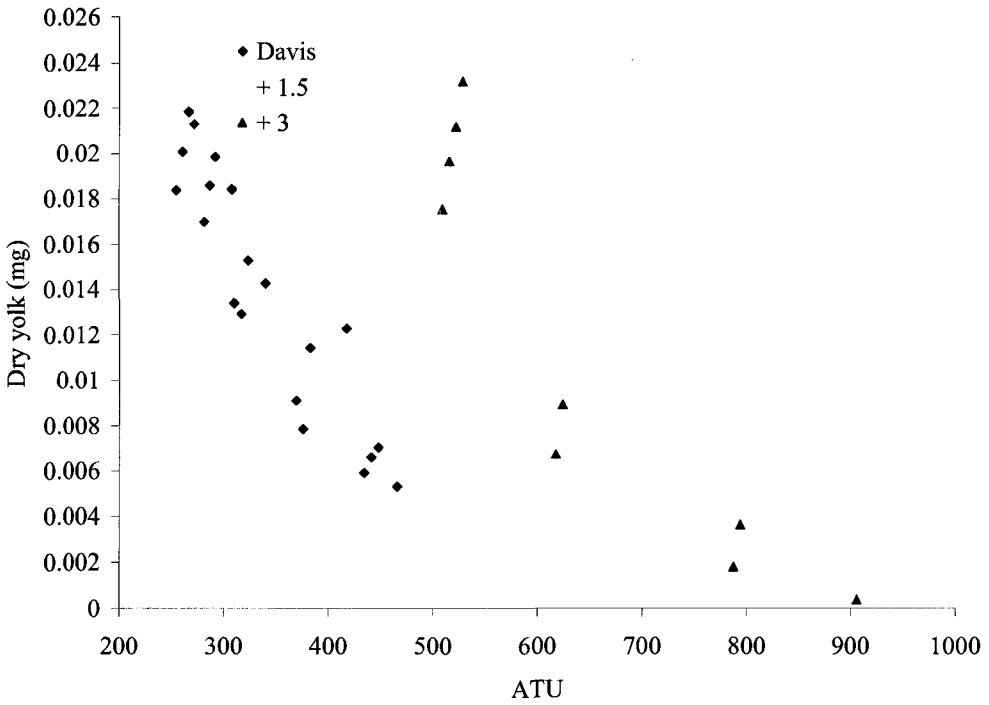


Figure 21. Dry yolk plotted against accumulated temperature units (ATU;°C·day) for each temperature replicate.

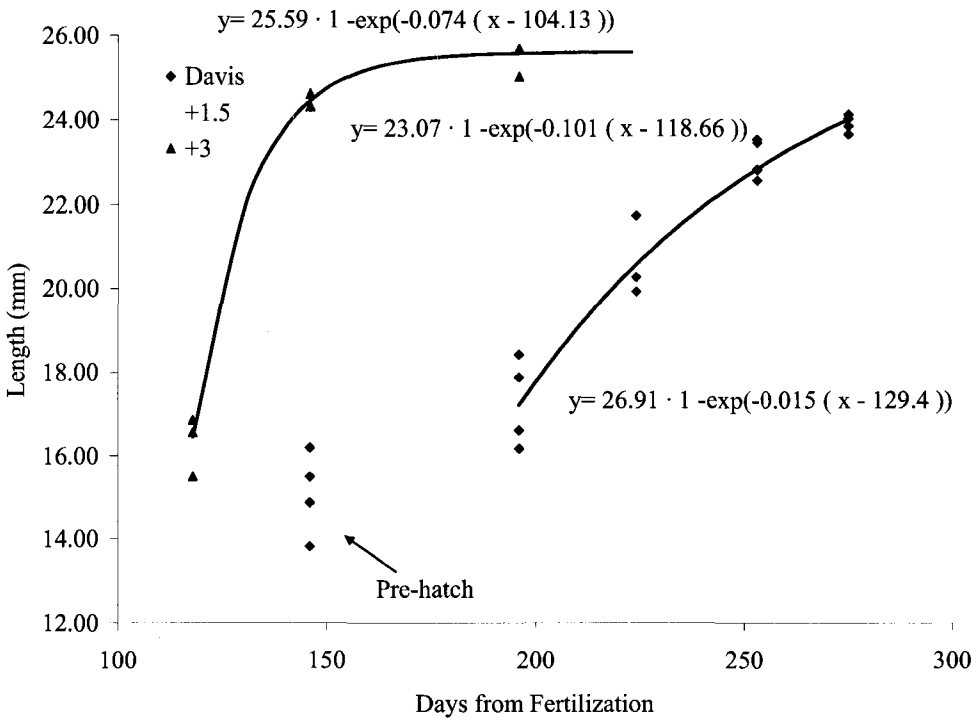


Figure 22. Length plotted against days from fertilization for each temperature replicate.

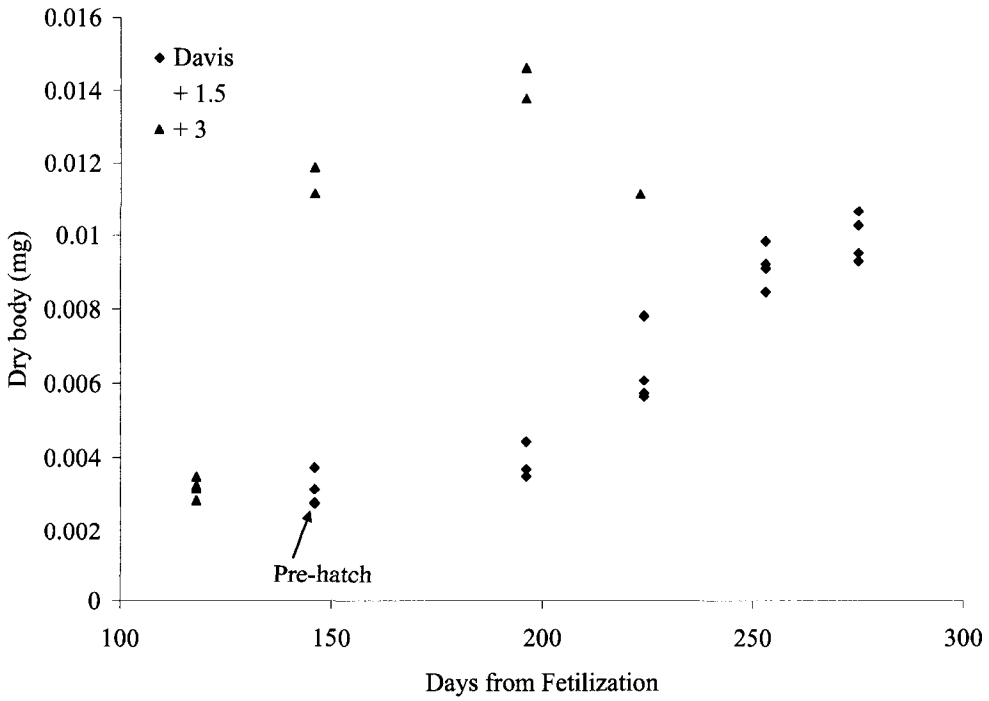


Figure 23. Dry-body weight plotted against days from fertilization for each temperature replicate

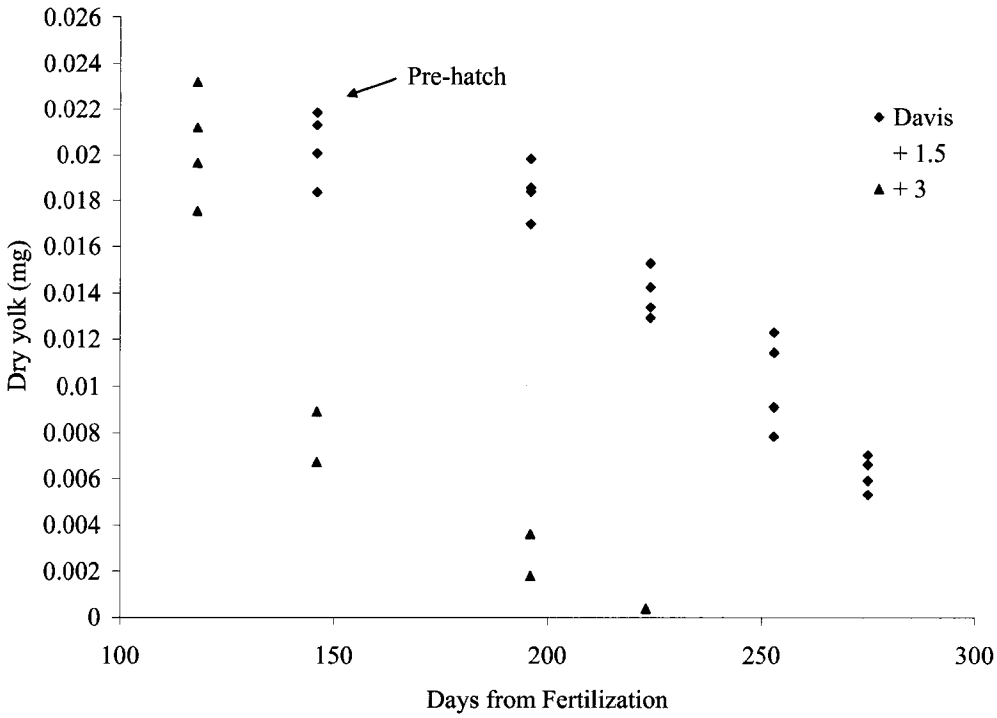


Figure 24. Dry yolk plotted against days from fertilization for each temperature replicate.

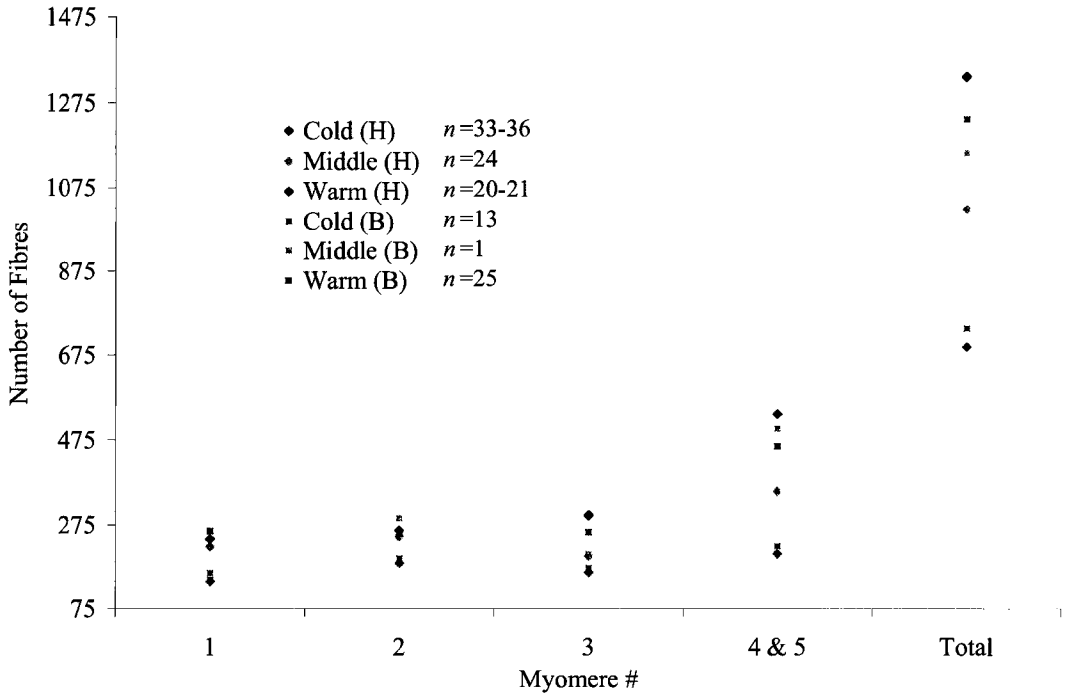


Figure 25. Mean muscle fibre (myofibre) count from each muscle myomere for each temperature replicate at hatch and at button-up. Some fish did not have a distinguishable fifth myomere, so 4 and 5 were combined. Sample sizes are provided for each temperature regime at hatch and button-up for each myomere. "H" designates hatch and "B" designates button-up.

Discussion

Temperature dominates the rate of early development in salmonids. Our results confirm and expand on existing work examining the effects of temperature on development in embryonic and larval salmonids in general and for bull trout in particular. The acceleration of development as well as the large differences in the size and yolk reserves resulting from small increases in temperature in our study were dramatic; similar effects have seldom been demonstrated previously (but see Rombough 1997). We also confirmed that bull trout can successfully tolerate incubation regimes with temperatures less than 1°C during incubation and may in fact require these temperatures for long periods during incubation to prevent premature exhaustion of yolk in northern streams.

A surprising result of our work is the total length of time required to reach developmental stages for these fish. Hatch and emergence were both substantially delayed compared to more southern populations of bull trout (Fig. 17; McPhail and Murray 1980; Weaver and White 1985; Gould 1987). This is the first reported account of bull trout incubation at controlled temperatures below 1°C. Weaver and White (1985) reported field incubation temperatures of 2°C to 5.4°C, although their thermographs were not used to record data during the coldest part of winter. Cope and Macdonald (1998) reported cold incubation temperatures approaching freezing in a sockeye salmon stream that is also known to be used by bull trout for spawning.

Additionally, time to hatch and emergence were not constant between temperature replicates, which suggests that bull trout show growth compensation (Brannon 1987) based on temperature. Longer incubation times likely represent adaptation to local thermal regimes and a longer winter period compared to populations in the south. Alteration of thermal regime during incubation may also lead to changes in growth, and

ultimately survival, of juvenile bull trout.

Rate of Development

Time-to-hatch ranged from 122-187 days and time to button-up ranged from 196- to 263 days (Table 9, Fig. 17). The duration of incubation for the natural temperature regime was approximately nine months. An example of the effect of the warmer temperatures on rate of development is seen in the time of button-up compared to hatch. Time of development differed considerably among the temperature treatments such that hatch for the control fish occurred within nine days of button-up for the warmest group (Table 8, Fig. 18). The large temporal differences in developmental stages we observed are similar to other studies. Time-to-hatch for Davis River fish was consistently two months longer than for bull trout from southern B.C. and the north-western United States, which incubated over a similar range of accumulated thermal units (ATU, measured in degree days) (Fig. 17; McPhail and Murray 1980; Weaver and White 1985; Gould 1987). Gould (1987) reported time-to-emergence was also about 2-months earlier than the time for button-up we recorded for Davis River fish. In contrast, Weaver and White (1985) reported a time-to-emergence (223 days) that was similar to that of our 1.5°C temperature treatment (Fig. 17). It was, however, unclear whether winter incubation temperature were recorded or estimated in their study. The other authors did not report button-up or emergence times. In our experiment we noted, but did not specifically record, that button-up and behaviours related to emergence such as positive or neutral photo-taxis (versus negative phototaxis prior to emergence) were roughly coincident. It is possible, therefore, that the differences in time to emergence we have noted between northern and southern populations could in part be explained by a difference between the timing of

button-up and the time of emergence from gravel nests.

Time-to-hatch is not necessarily a precise indicator of development and may be subject to external selective pressures such as changes in habitat quality that alter survival during incubation. Once hatch occurs, alevins may be mobile and can potentially avoid suboptimal or lethal conditions such as anchor ice formation. Cope and Macdonald (1998) found that after hatch, sockeye salmon alevins from an interior population had the capability of avoiding ice by moving through the gravel whereas a coastal population did not exhibit this behaviour. A relatively rapid time-to-hatch that occurred before freeze-up was also a characteristic of this interior stock. Bull trout in our study area do not appear to exhibit rapid development to hatch and hatch occurs well into the winter months (Fig. 17) when ice-formation in natural redds is possible. Interestingly, the late timing of hatch may be one of the reasons bull trout in particular seem to have a strong affinity for groundwater fed influenced spawning areas. Groundwater intrusions can provide stable thermal regimes and can prevent the formation of anchor ice (Power et al. 1999).

Time-to-emergence is likely subject to strong selective pressures that act to optimize juvenile survival and growth (Brannon 1987). Compared to hatch, however, there is likely less flexibility for emergence dates as yolk reserves are finite and emergence in salmonids often coincides with button-up, first feeding and increases in food availability during spring (Dill 1967). We observed times to hatch and potentially emergence in our study that were longer than for southern populations. The timing of emergence approximately coincides with spring freshet (mid-June) based on our field observations during 2001 and 2002. Both of these observations support the hypothesis

that this population of bull trout is adapted to local temperature regimes in order to maximize the growth and survival of juveniles. We observed distinct differences in the timing of specific life stages between our data and southern populations of bull trout (Fig. 17). Such adaptations have also been reported for populations of Pacific salmon (Brannon 1987; Beacham and Murray 1988; Leman 1993), brook trout (Curry et al. 1995) and Atlantic salmon (*Salmon salar*) (Webb and McLay 1996).

The number of degree days to achieve a specific developmental stage decreased with increases in temperature (Fig. 17). In the warmest temperature group, 768 degree days were required for complete button-up. In the mid temperature group, 604 degree days were required for complete button-up and 390 degree days were required in the natural regime. Thus, the relationship between ATU and development is not constant for larval development at very low temperatures for bull trout. A negative relationship between growth and time-to-development was described as growth compensation by Brannon (1987). Brannon speculated that compensation in growth is an adaptive mechanism that acts to stabilize emergence timing for fry under variable environmental conditions during spawning through emergence. Growth compensation has been observed intra- and inter-specifically in the development rates for four species of Pacific salmon (Brannon 1987; Beacham and Murray 1988; Murray and McPhail 1988). Our results show that bull trout may also be able to growth compensate; however, further study over a wider range of temperatures as well as at constant temperatures will be required for this comparison.

Embryonic Size and Yolk Utilization

Substantial differences in alevin size and yolk utilization rates were evident between each temperature regime throughout the experiment (Figs. 19-21), which if experienced in the natural environment may have lead to depletion of yolk reserve and pre-mature emergence. Warmer temperatures significantly increased lengths and weights of alevins among dates and even among trays within incubation stacks where there were small amounts of warming from top to bottom. We also found small maternal and some paternal effects. Female effects are common in salmonids as egg size has a direct bearing on growth until yolk reserves are exhausted (Heath et al. 1999). Alevins were collected on March 18, 2002, within a few days of the time of button-up for the warmest regime (+3°C regime) and 50% hatch for the natural regime (Davis River regime). A difference of >10-mm was observed for the warmest experimental group compared to the control (Fig. 18). The warmest temperature regime resulted in the longest and heaviest alevins, but this occurred earlier in time at the warmest temperature. Our observation contrasts with those previously reported. Heming (1982) noted that chinook salmon incubated at higher temperatures were smaller and had reduced yolks reserves at hatching and emergence than fish reared at cooler temperatures. Murray and McPhail (1989) made similar observations on five species of Pacific salmon, however, weights were lower at temperatures near 5°C for pink salmon compared to warmer temperatures. Hamor and Garside (1997) and Ojanguren et al. (1999) also found an inverse relationship between temperature and size in Atlantic salmon. Huuskonen et al. (2003) observed the same relationship for Arctic char for both length and weight. Considering that bull trout often incubate in cold water, it could be expected that low-temperature incubation should

produce large fry, however, it is unclear why our results differed from these reported studies. The coldest thermal regimes used in our study was close to the freezing temperature of water and this may be a common phenomenon of tissue growth and development at cold temperatures. It is possible, therefore, that bull trout embryonic growth is more efficient and that a growth optima exists at slightly warmer temperatures than we used in our experiment.

A possible confounding factor in the study was that both the middle and natural temperature replicate experienced relatively high levels of blue-sac disease. Blue-sac disease is characterized by increased fluid retention in the yolk sac as well as decreased growth and increased mortality (Peterson et al. 1977; Ihssen 1978). Peterson et al. (1977) observed that rapid decreases in temperature at the eyed and hatch stages increase the incidence of edema of the yolk sac in Atlantic salmon. In our study, both of these temperature treatments experienced equipment failures that resulted in temperature spikes (sharp increase and decrease; Fig. 16) during the course of the experiment which may have contributed to this effect.

Muscle Cellularity at Hatch and Button-Up

Previous work has demonstrated that changes to the thermal incubation environment of salmonids can alter muscle development in fish (reviewed in Johnston 2001). Nathanailides et al. (1995) found that Atlantic salmon alevins incubated at ambient temperatures had similar white muscle cross-sectional areas to fish incubated at higher temperatures, but fibre number was higher for the ambient group. In a similar study involving two different stocks of Atlantic salmon incubated under natural temperature regimes and under the regime of the other respective stock, Johnston et al.

(2000a) examined muscle cellularity at hatch and at button-up. They noted significant effects on both white muscle cross-sectional and fibre area that remained until the time of first feeding. In an extension of the previous study Johnston et al. (2000b) found that these effects were maintained into the juvenile stage for one population but not the other. Based in part on these results, Johnston (2001) speculated that different responses may reflect local adaptation in these fish. He further added that alterations to thermal regime or other factors affecting growth of muscle can cause effects that may be maintained until later in life; such effects could include changes in ultimate body size and swimming performance. To better understand how an altered temperature regime could affect development in bull trout, we examined differences in white muscle fibre density at the three different temperature regimes. We found highly significant differences in the number of muscle fibres existed between the temperature groups (Fig. 25); however, the results for white muscle area did not differ significantly. *Post hoc* examination of our results revealed that difference in muscle fibre number existed only between the warmest and natural temperature replicates.

Previous work on Atlantic salmon has shown the cross-sectional area of white muscle fibers and fiber number (myofibre hypertrophy) to be greater for embryos reared at lower temperatures (Johnston 2001). As a consequence, larval fish reared at lower temperature are not only larger at emergence, but also have a greater number of muscle fibers. The ecological implication of this finding could be that fish reared at lower temperatures have a greater chance of survival due to greater size and greater growth potential (Rombough 1987). Within the range of temperatures we examined, myofibre hypertrophy increased at elevated temperatures. This result contrasts with other

researchers who demonstrated decreases in muscle hypertrophy at higher temperatures. Differences may reflect adaptation to naturally low temperature incubation environments. We suggest that our observation of significant differences in white muscle fibre number in combination with our clear observation on growth indicate that incubation at temperatures near freezing has a profound effect on development in larval salmonids.

Yolk Utilization

Early exhaustion of yolk reserves in the warmest temperature group was another important finding of our study. Under the natural temperature regime, the depletion of yolk was initially slow in comparison to the two warmer groups and accelerated through time. We presume that the absorption rate would have decelerated after button-up, as the yolk depletion appears to be described well by a sigmoid function (Joworski and Kamler 2002). In the two warmer groups, yolk absorption was rapid in the period following hatch and the amount of yolk remaining at the time of emergence was inversely related to temperature (Figs. 21 and 24). Near the time of complete depletion of yolk in the warmest replicate (Figs. 21 and 24), the weight of these alevins had started to decline. The depletion of yolk represents an end-point for endogenous growth. The alevin must begin exogenous feeding to survive once yolk reserves are depleted. Exhaustion or depletion of yolk reserves leads to high mortality in salmonids in the natural environment due to early emergence and can also result in reduced body weight at emergence (Heming 1982; Ojanguren et al. 1999). The implications of small increases in temperature during incubation, therefore, are profound.

Summary

Our results confirm and expand on existing work examining the effect of temperature on larval development in salmonids. For bull trout, even apparently small differences in incubation temperature can have substantial impacts on growth and development from emergence to hatch. We have demonstrated that time-to-hatch, time-to-emergence, size, yolk utilization and potentially muscle fibre numbers are all affected significantly by relatively small changes to the incubation regime. As shown by previous researchers (see Rombough 1997) early developmental stages in fish are particularly sensitive to temperature changes, with the acceleration of development in salmonids being especially dramatic. Alterations in the stream environment related to industrial development or changes in global climate can cause stream warming or changes in groundwater flow patterns. The resulting changes may cause precocial exhaustion of yolk reserves and early emergence. Thus, changes to the incubation environment of bull trout, a cold water specialist may disrupt the timing of development, duration of incubation and size at emergence and ultimately long term reproductive success.

CHAPTER 5: GENERAL DISCUSSION

Bull trout are coldwater specialists that home to specific areas to spawn that are commonly influenced by groundwater (Baxter and McPhail 1996; Baxter and McPhail 1999; Baxter and Hauer 2000; Bahr and Shrimpton 2004). At the reach scale, my results demonstrate that bull trout spawn in groundwater-influenced valleys with extensive gravel deposits with high rates of hyporheic exchange. I also found evidence of localized groundwater influence at the habitat scale, where bull trout were spawning in a variety of habitats with both relatively warm upwelling groundwater or downwelling streamflow. This confirms the findings of Baxter and Hauer (2000) from a Montana population of bull trout, where the specific site chosen appeared to dictate the direction of the vertical hydraulic gradient. Sites located at the start of the transition between stream features exhibited downwelling whereas areas adjacent to banks or downstream of pool riffle sequences exhibited upwelling. The use of specific areas influenced by groundwater with specific thermal signatures may help to maximize reproductive success of fish by limiting the effects of stochastic environmental processes that lower survival during incubation. The presence of upwelling did not necessarily explain all the variation in site use as other authors have suggested (Baxter and McPhail 1996; Baxter and McPhail 2000); however, warm up-welling water appeared to have a positive bias on the sites used. Three of the warmest naturally-used sites in my study had the highest number of redds. In general, however, the warmest available sites appear to be avoided by spawners based on our observations. Other physical features linked to habitat quality may moderate site selection. I found that high intergravel-flow, high levels of oxygen and proximity to security cover all were features found at sites used for spawning.

In an effort to better understand the importance of habitat features at redd sites and the implications of habitat alteration on spawning and incubation success; I used a field incubation experiment to explore the effects of different habitat parameters on incubation success in an ecological context. I attempted to define the range of natural variation in habitat within my study area to determine the potential effects on bull trout survival, development size and yolk utilization.. I found that physical and chemical habitat quality appeared to be high at all sites, and, with the exception of temperature, all features I measured were poor predictors of survival, size and yolk utilization during incubation. In terms of thermal habitat, bull trout in my study area appeared to be well adapted to temperatures below 1°C after the onset of winter and for the majority of the incubation period. I also found relatively high survival at all sites, and growth was relatively constant at the sites naturally selected for spawning. Surprisingly, warm-incubation temperatures at non-used sites relative to used sites resulted in increased growth rates and advanced timing of hatch and button-up, despite the fact that the differences were relatively small. Thus the growth response as well as differences in the timing of developmental stages across habitats (reaches) that were used and not used for spawning were relatively large and significant.

Comparing growth between reaches, I found average lengths and body weights were highest at the two warmest sites that were also not used for spawning. Temperatures at these sites were more than 2°C warmer than three sites that were used extensively by spawning bull trout. In this context, yolk reserves were lowest at the warmest sites and were less than half of the reserves for alevins collected within the selected sites. Considering that spring freshet, normally a period of natural fry

emergence, was still at least a month and a half away, survival of the alevins at the warmest sites was unlikely as exogenous food resources are likely scarce prior to spring freshet. Furthermore, the digestibility of prey items is lower at low temperatures (Salvanes et al. 1995) and even if food resources were found, low temperature may limit growth from exogenous feeding. I found that bull trout selected spawning sites with low absolute temperature. The incubation temperatures at these sites throughout the winter were, however, intermediate within the range that was surveyed at all sites. The narrow range of temperatures throughout the winter for selected sites, therefore, suggests that a selective pressure for a specific thermal regime exists within these populations of bull trout. It is likely, that the incubation thermal regimes are directly related to survival post emergence and is suggestive that the mechanism for selection is through habitat preference for temperature differences among reaches. Based on my observation of an apparent preference for sites with low absolute temperature that were intermediate within the range I surveyed, as well as a pessimistic outlook for survival to emergence for alevins that were incubating in the warmest habitats, I hypothesize that for bull trout in my study area, habitat preference is strongly linked to temperature differences between reaches.

I also observed significant differences in length, weight and yolk utilization at lower factor levels than the reach, including differences between artificial redds and between families and individual sires and dams. Heterogeneity in temperatures within reaches and measurable differences in size at this scale suggests the possibility that site selection and adaptation to incubation temperature could be operating at a very fine scale in bull trout. Considering these results in the context of the synopsis of natural redd

habitat characteristics, it appears that the range of preferred temperatures is relatively narrow.

In the laboratory experiment, I examined the effects of small increases in temperature on size and development of bull trout that normally incubate at low temperatures to test their resiliency to change. My results confirm and expand on existing work examining the effect of temperature on larval development in salmonids. For bull trout, even apparently small differences in incubation temperature can have substantial impacts on size and development from emergence to hatch. I have demonstrated that time to hatch, emergence, size, yolk utilization and potentially muscle fibre counts are all affected significantly by relatively small changes to the incubation regime. As shown by previous researchers (see Rombough 1997), early-developmental stages in fish are particularly sensitive to temperature changes, with the acceleration of development in salmonids being especially dramatic. Alterations in the stream environment related to industrial development or changes in global climate can cause stream warming or changes in groundwater flow patterns. The resulting changes may cause precocial exhaustion of yolk reserves and early emergence. Thus, changes to the incubation environment of bull trout, a cold water specialist may disrupt the timing of development, duration of incubation, size at emergence and ultimately long term reproductive success.

In summary, based on the results of these studies, I recommend the following to resources managers seeking advice on protecting bull trout spawning habitat. Firstly, bull trout appear to home to and select specific reaches within stream with high fidelity. Features such as coarse woody debris, high intergravel permeability, and specific thermal

regimes appear to result in habitat selection at a fine scale. To maintain high quality spawning reaches, the structure, function and physical stream processes that govern the availability of course woody debris and channel features associated with bull trout redds should be protected and examined through further study.

Secondly, groundwater upwelling at the reach scale and hyporheic exchange within reaches both appear to be an important component of bull trout spawning habitat in northern B.C. Based on my work and the work of others, however, there appears to be considerable variation in the groundwater supply and function between watersheds. Thus I recommend, in the face of industrial developments that could alter fluvial and groundwater hydrology, steps should be taken on a watershed by watershed basis to better understand how groundwater influences and moderates spawning habitat at both the watershed and reach scale in significant bull trout spawning streams. These studies should be undertaken prior to any development and measures for mitigation should be incorporated into operational planning.

Finally, bull trout appear to be adapted to relatively narrow ranges of temperature for spawning with apparent differences between northern and southern populations. Small, changes to temperatures as the result of industrial developments or global warming have a strong potential to disrupt bull trout spawning and incubation success, therefore, it is incumbent that the proponents of such works undertake the necessary studies to ensure that their activities do not degrade the thermal habitat of bull trout spawning areas. Such work should include efforts to understand local adaptation within and between watersheds.

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APPENDIX A: ANOVA TABLES FOR ANALYSIS OF SURVIVAL.

Table 10. ANOVA table for year one analysis of survival, which includes the factor family in the test.

Source	DF	Adj SS	Adj MS	F	P
Date	1	0.94979	0.94979	12.61	0.001
Reach	2	0.18221	0.09110	1.21	0.303
Family	3	1.47025	0.49008	6.51	0.000
Redd(Reach)	5	0.37464	0.07493	0.99	0.425
Capsule(Reach Redd)	13	0.46330	0.03564	0.47	0.935
Error	99	7.45723	0.07533		

Table 11. ANOVA table for year two analysis of survival, which includes the factor family in the test.

Source	DF	Adj SS	Adj MS	F	P
Date	1	0.96516	0.96516	41.67	0.000
Reach	5	1.57559	0.31512	13.61	0.000
Family(Reach)	11	2.46987	0.22453	9.69	0.000
Redd(Reach)	12	0.63453	0.05288	2.28	0.011
Capsule(Reach Redd)	20	0.44578	0.02229	0.96	0.511
Error	148	3.42766	0.02316		

Table 12. ANOVA table for year two analysis of survival, which includes the factors male and female in the test.

Source	DF	Adj SS	Adj MS	F	P
Date	1	1.02249	1.02249	11.17	0.001
Reach	2	0.24930	0.12465	1.36	0.261
Male	1	0.25196	0.25196	2.75	0.100
Female	1	0.09818	0.09818	1.07	0.303
Redd(Reach)	5	0.40529	0.08106	0.89	0.494
Capsule(Reach Redd)	13	0.34446	0.02650	0.29	0.992
Error	101	0.09150			

Table 13. ANOVA table for year two analysis of survival, which includes the factor male in the test.

Source	DF	Adj SS	Adj MS	F	P
Date	1	0.98004	0.98004	38.50	0.000
Reach	5	1.50021	0.30004	11.79	0.000
Male(Reach)	5	1.97723	0.39545	15.53	0.000
Redd(Reach)	12	0.56639	0.04720	1.85	0.044
Capsule(Reach Redd)	20	0.43543	0.02177	0.86	0.643
Error	154	3.92030	0.02546		

APPENDIX A: CONTINUED

Table 14. ANOVA table for year two analysis of survival including the factor female in the test.

Source	DF	Adj SS	Adj MS	F	P
Date	1	0.94675	0.94675	26.38	0.000
Reach	5	1.50506	0.30101	8.39	0.000
Female(Reach)	5	0.36987	0.07397	2.06	0.073
Redd(Reach)	12	0.58192	0.04849	1.35	0.196
Capsule(Reach Redd)	20	0.40301	0.02015	0.56	0.933
Error	154	5.52766	0.03589		

APPENDIX B: ANOVA TABLES FOR ANALYSIS OF GROUNDWATER CHEMISTRY.

Table 15. ANOVA tables for examination of variance in specific discharge within three collection periods (September, March and April, respectively).

Source	DF	Adj SS	Adj MS	F	P
Use	1	0.0014488	0.0014488	6.32	0.020
Reach(Use)	4	0.0024253	0.0006063	2.65	0.061
Piezometer(Use Reach)	6	0.0067749	0.0011291	4.93	0.002
Error	22	0.0050423	0.0002292		

Source	DF	Adj SS	Adj MS	F	P
Use	1	0.04259	0.04259	0.94	0.343
Reach(Use)	4	0.41181	0.10295	2.27	0.094
Piezometer(Use Reach)	6	1.75580	0.29263	6.45	0.000
Error	22	0.99818	0.04537		

Source	DF	Adj SS	Adj MS	F	P
Use	1	0.015153	0.015153	2.84	0.109
Reach(Use)	4	0.039049	0.009762	1.83	0.167
Piezometer(Use Reach)	6	0.137054	0.022842	4.28	0.008
Error	18	0.096133	0.005341		

Table 16. ANOVA tables for examination of variance in specific conductivity within three collections periods (September, March and April, respectively).

Source	DF	Adj SS	Adj MS	F	P
Use	1	0.49779	0.49779	67.64	0.000
Reach(Use)	4	1.09618	0.27405	37.23	0.000
Piezometer(Use Reach)	6	0.06682	0.01114	1.51	0.218
Error	23	0.16928	0.00736		

Source	DF	Adj SS	Adj MS	F	P
Use	1	1.68652	1.68652	64.35	0.000
Reach(Use)	4	2.32312	0.58078	22.16	0.000
Piezometer(Use Reach)	6	0.06717	0.01120	0.43	0.853
Error	23	0.60278	0.02621		

Source	DF	Adj SS	Adj MS	F	P
Use	1	0.1669	0.1669	0.83	0.374
Reach(Use)	4	47.4355	11.8589	59.09	0.000
Piezometer(Use Reach)	6	0.8186	0.1364	0.68	0.668
Error	18	3.6125	0.2007		

Table 17. ANOVA tables for examination of variance in oxygen concentration within three collections periods (September, March and April, respectively).

Source	DF	Adj SS	Adj MS	F	P
Use	1	4.0778	4.0778	27.27	0.000
Reach(Use)	4	7.3322	1.8331	12.26	0.000
Piezometer(Use Reach)	6	2.3950	0.3992	2.67	0.040
Error	24	3.5890	0.1495		

Source	DF	Adj SS	Adj MS	F	P
Use	1	0.88602	0.88602	22.32	0.000
Reach(Use)	4	1.05606	0.26402	6.65	0.001
Piezometer(Use Reach)	6	0.27555	0.04592	1.16	0.363
Error	23	0.91292	0.03969		

Source	DF	Adj SS	Adj MS	F	P
Use	1	0.005629	0.005629	2.91	0.105
Reach(Use)	4	0.157701	0.039425	20.41	0.000
Piezometer(Use Reach)	6	0.009778	0.001630	0.84	0.553
Error	18	0.034778	0.001932		

Table 18. ANOVA tables for analysis of variance in specific discharge, specific conductivity, and oxygen concentration respectively, between two collections periods (September April).

Specific Discharge

Source	DF	Adj SS	Adj MS	F	P
Use	1	2.8228	2.8228	3.04	0.095
Reach(Use)	4	17.9182	4.4796	4.83	0.006
Depth(Use Reach)	6	4.7837	0.7973	0.86	0.540
Error	22	20.4166	0.9280		

Specific Conductivity

Source	DF	Adj SS	Adj MS	F	P
Use	1	2.31614	2.31614	26.52	0.000
Reach(Use)	4	1.27799	0.31950	3.66	0.021
Depth(Use Reach)	11	1.41522	0.12866	1.47	0.214
Error	21	1.83416	0.08734		

Oxygen

Source	DF	Adj SS	Adj MS	F	P
Use	1	0.01615	0.01615	0.59	0.450
Reach(Use)	4	1.56149	0.39037	14.29	0.000
Depth(Use Reach)	12	0.33695	0.02808	1.03	0.459
Error	22	0.60113	0.02732		

APPENDIX C: ANOVA TABLES FOR ANALYSIS OF EMBRYO SIZE AND YOLK UTILIZATION.

Table 19. ANOVA table for year one analyses of length with factors for male and female parent included in the test.

Source	DF	Adj SS	Adj MS	F	P
Date	1	17928.3	17928.3	12686.93	0.000
Female	1	5.8	5.8	4.08	0.044
Male	1	7.8	7.8	5.51	0.019
Reach	2	2666.8	1333.4	943.58	0.000
Female*Male	1	3.5	3.5	2.47	0.116
Redd(Reach)	5	78.7	15.7	11.13	0.000
Capsule(Reach Redd)	13	56.2	4.3	3.06	0.000
Error	1293	1827.2	1.4		

Table 20. ANOVA table for year one analyses of total dry weight with factors for male and female parent included in the test.

Source	DF	Adj SS	Adj MS	F	P
Date	1	0.0037224	0.0037224	1026.31	0.000
Female	1	0.0000156	0.0000156	4.31	0.038
Male	1	0.0000007	0.0000007	0.20	0.657
Female*Male	1	0.0039215	0.0039215	1081.20	0.000
Reach	2	0.0008054	0.0004027	111.03	0.000
Redd(Reach)	5	0.0001078	0.0000216	5.95	0.000
Capsule(Reach Redd)	13	0.0001322	0.0000102	2.80	0.001
Error	1246	0.0045192	0.0000036		

Table 21. ANOVA tables for year one analyses of dry-body weight with factors for male and female parent included in the test.

Source	DF	Adj SS	Adj MS	F	P
Date	1	0.434128	0.434128	5965.43	0.000
Female	1	0.000644	0.000644	8.85	0.003
Male	1	0.004301	0.004301	59.10	0.000
Female*Male	1	0.000253	0.000253	3.47	0.063
Reach	2	0.093867	0.046933	644.92	0.000
Redd(Reach)	5	0.002351	0.000470	6.46	0.000
Capsule(Reach Redd)	13	0.006025	0.000463	6.37	0.000
Error	1265	0.092059	0.000073		

Table 22. ANOVA tables for year one analyses of dry yolk weight with factors for male and female parent included in the test.

Source	DF	Adj SS	Adj MS	F	P
Date	1	0.0199154	0.0199154	3733.28	0.000
Female	1	0.0000014	0.0000014	0.26	0.608
Male	1	0.0000873	0.0000873	16.36	0.000
Female*Male	1	0.0039291	0.0039291	736.53	0.000
Reach	2	0.0049296	0.0024648	462.04	0.000
Redd(Reach)	5	0.0001863	0.0000373	6.99	0.000
Capsule(Reach Redd)	13	0.0002479	0.0000191	3.57	0.000
Error	1278	0.0068176	0.0000053		

Table 23. ANOVA tables for year two analyses of length with the factor family included in the test.

Source	DF	Adj SS	Adj MS	F	P
Date	1	2833.42	2833.42	3554.63	0.000
Reach	5	1682.13	336.43	422.06	0.000
Family(Reach)	11	78.61	7.15	8.97	0.000
Redd(Reach)	12	66.88	5.57	6.99	0.000
Capsule(Reach Redd)	20	63.10	3.16	3.96	0.000
Error	702	559.57	0.80		

Table 24. ANOVA tables for year two analyses of total dry weight with the factor family included in the test.

Source	DF	Adj SS	Adj MS	F	P
Date	1	0.0006681	0.0006681	211.47	0.000
Reach	5	0.0025632	0.0005126	162.26	0.000
Family	4	0.0006643	0.0001661	52.57	0.000
Redd(Reach)	12	0.0000914	0.0000076	2.41	0.005
Capsule(Reach Redd)	20	0.0000708	0.0000035	1.12	0.322
Error	701	0.0022147	0.0000032		

Table 25. ANOVA tables for year two analyses of dry-body weight with the factor family included in the test.

Source	DF	Adj SS	Adj MS	F	P
Date	1	0.0036610	0.0036610	3158.99	0.000
Reach	5	0.0018186	0.0003637	313.85	0.000
Family	4	0.0000052	0.0000013	1.13	0.342
Redd(Reach)	12	0.0000778	0.0000065	5.59	0.000
Capsule(Reach Redd)	20	0.0000519	0.0000026	2.24	0.002
Error	718	0.0008321	0.0000012		

Table 26. ANOVA tables for year two analyses of dry yolk weight the factor family included in the test.

Source	DF	Adj SS	Adj MS	F	P
Date	1	0.0072683	0.0072683	1956.05	0.000
Reach	5	0.0078339	0.0015668	421.65	0.000
Family	4	0.0007359	0.0001840	49.51	0.000
Redd(Reach)	12	0.0001459	0.0000122	3.27	0.000
Capsule(Reach Redd)	20	0.0001558	0.0000078	2.10	0.003
Error	702	0.0026085	0.0000037		

APPENDIX D: ANOVA TABLES FOR ANALYSIS OF LABORATORY GROWTH.

Table 27. ANOVA table for the laboratory analysis of length.

Source	DF	Adj SS	Adj MS	F	P
Date	8	5271.12	658.89	1153.07	0.000
Temperature	2	2308.54	1154.27	2020.00	0.000
Female	1	22.60	22.60	39.56	0.000
Male	1	0.29	0.29	0.50	0.479
Female*Male	1	0.02	0.02	0.04	0.847
Tray(Temperature)	9	42.81	4.76	8.32	0.000
Error	609	348.00	0.57		

Table 28. ANOVA table for the laboratory analysis of dry-body weight.

Source	DF	Adj SS	Adj MS	F	P
Date	8	0.0042494	0.0005312	441.19	0.000
Temperature	2	0.0021008	0.0010504	872.45	0.000
Female	1	0.0000029	0.0000029	2.39	0.123
Male	1	0.0000002	0.0000002	0.17	0.684
Female*Male	1	0.0000034	0.0000034	2.82	0.094
Tray(Temperature)	9	0.0000360	0.0000040	3.32	0.001
Error	618	0.0007440	0.0000012		

Table 29. ANOVA table for the laboratory analysis of dry-yolk weight.

Source	DF	Adj SS	Adj MS	F	P
Date	8	0.0148260	0.0018532	587.39	0.000
Temperature	2	0.0059096	0.0029548	936.52	0.000
Female	1	0.0005417	0.0005417	171.68	0.000
Male	1	0.0000001	0.0000001	0.03	0.855
Female*Male	1	0.0000105	0.0000105	3.32	0.069
Tray(Temperature)	9	0.0000850	0.0000094	2.99	0.002
Error	618	0.0019498	0.0000032		

Table 30. Four ANOVA tables for the laboratory analysis of muscle fibres for myomeres 1-3 and 4 & 5 combined, respectively.

Myomere 1

Source	DF	Adj SS	Adj MS	F	P
Treatment	2	85524	42762	6.95	0.002
Male	1	527	527	0.09	0.770
Female	1	1499	1499	0.24	0.623
Stage(Treatment)	3	10312	3437	0.56	0.644
Tray(Treatment)	9	49135	5459	0.89	0.539
Error	97	596752	6152		

Myomere 2

Source	DF	Adj SS	Adj MS	F	P
Treatment	2	62856	31428	6.84	0.002
Male	1	6121	6121	1.33	0.251
Female	1	13028	13028	2.84	0.095
Stage(Treatment)	3	1606	535	0.12	0.950
Tray(Treatment)	9	61611	6846	1.49	0.162
Error	97	445453	4592		

Table 30 continued.

Myomere 3

Source	DF	Adj SS	Adj MS	F	P
Treatment	2	82889	41444	6.88	0.002
Male	1	892	892	0.15	0.701
Female	1	18018	18018	2.99	0.087
Stage(Treatment)	3	8617	2872	0.48	0.699
Tray(Treatment)	9	80194	8910	1.48	0.166
Error	97	584033	6021		

Myomeres 4 and 5 Combined

Source	DF	Adj SS	Adj MS	F	P
Treatment	2	492627	246313	20.08	0.000
Male	1	109	109	0.01	0.925
Female	1	46645	46645	3.80	0.054
Stage(Treatment)	3	84264	28088	2.29	0.083
Tray(Treatment)	9	375380	41709	3.40	0.001
Error	97	1189692	12265		