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## Behavioural and physiological responses of brook trout *Salvelinus fontinalis* to midwinter flow reduction in a small ice-free mountain stream

A. N. KRIMMER\*, A. J. PAUL†, A. HONTELA\* AND J. B. RASMUSSEN\*‡

\*Department of Biological Sciences, 4400 University Dr. W., University of Lethbridge, Lethbridge, Alberta, T1K 3M4 Canada and †Fish and Wildlife Division, Alberta Sustainable Resource Development, Box 1420, 2nd Floor, Provincial Bldg., 213-1st Street W. Cochrane, Alberta, T4C 1B4 Canada

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This study presents an experimental analysis of the effects of midwinter flow reduction (50–75%, reduction in discharge in 4 h daily pulses) on the physical habitat and on behaviour and physiology of overwintering brook trout *Salvelinus fontinalis* in a small mountain stream. Flow reduction did not result in significant lowering of temperature or formation of surface or subsurface ice. The main findings were (1) daily movement by *S. fontinalis* increased (c. 2.5-fold) during flow reduction, but was limited to small-scale relocations (<10 m). (2) Undercut banks were the preferred habitat and availability of these habitats was reduced during flow reduction. (3) Although both experimental and reference fish did lose mass and condition during the experiment, no effects of flow reduction on stress indicators (blood cortisol or glucose) or bioenergetics (total body fat, water content or mass loss) were detected, probably because access to the preferred type of cover remained available. Like other salmonids, *S. fontinalis* moves little and seeks physical cover during winter. Unlike many of the more studied salmonids, however, this species overwinters successfully in small groundwater-rich streams that often remain ice-free, and this study identifies undercut banks as the critical winter habitat rather than substratum cover.

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Key words: flow manipulation; habitat selection; movement; salmonid; undercut banks.

### INTRODUCTION

Streams can be subjected to water withdrawals from a wide range of demands, leading to significant flow reduction and affecting fish communities (Kanno & Vokoun, 2010). Such demands include hydroelectric generation, municipal water supplies, oil and gas operations and other industrial uses, construction of winter ice roads and snow-making at ski hills (Cunjak, 1996). Many of these withdrawals occur in low flow periods including winter, and effective flow management requires understanding of the winter habitat requirements of fishes, a subject that has received increasing attention in the last decade (Alfredsen & Tesaker, 2002; Huusko *et al.*, 2007; Stickler *et al.*, 2008; Linnansaari & Cunjak, 2010).

‡Author to whom correspondence should be addressed. Tel.: +1 403 382 7182; email: joseph.rasmussen@uleth.ca

Behavioural responses to flow manipulation have been studied by monitoring fish movement and habitat preferences (Berland *et al.*, 2004; Murchie & Smokorowski, 2004; Scruton *et al.*, 2008). For example, Atlantic salmon *Salmo salar* L. 1758 increased exploratory behaviour in response to changes in flow regime (Armstrong *et al.*, 1998) and relocated in response to changes in the distribution of substratum cover (Scruton *et al.*, 2008). Juvenile brown trout *Salmo trutta* L. 1758 moved more during high flows and relocated in response to altered flow conditions (Bunt *et al.*, 1999; Vehanen *et al.*, 2000). Flow manipulation can thus be expected to change the distribution of suitable habitats, and fishes often relocate in response to these changes. While some flow manipulation studies have been carried out in winter, most studies have been focussed on the summer period, when fishes are generally most active.

Winter is the period where daily mean temperatures remain below zero, allowing snow to persist and accumulate, and standing waters to freeze over. During winter, the energetic budgets of stream salmonids are constrained by low feeding rates (Cunjak *et al.*, 1987; Metcalfe & Thorpe, 1992) and winter fishes remain sedentary (Huusko *et al.*, 2007; Enders *et al.*, 2008; Stickler *et al.*, 2008) to minimize energy expenditure (Heggenes *et al.*, 1993). Overwintering salmonids seek cover, either as shelter from current, social interactions or predators (Cunjak, 1996; Huusko *et al.*, 2007). Much of the focus of recent winter work on salmonids concerns the effect of ice conditions, particularly anchor and frazil ice, on the use of cover by *S. salar* (Stickler *et al.*, 2008; Linnansaari *et al.*, 2008). These fish overwinter mainly in very cold (<1° C) streams and take refuge either within the substratum or under the cover of surface ice (Maciolek & Needham, 1952; Finstad *et al.*, 2004). Less attention has been paid to species such as brook trout *Salvelinus fontinalis* (Mitchill 1814) or cutthroat trout *Oncorhynchus clarkii* (Richardson 1836), that overwinter in slightly warmer and usually ice-free groundwater-rich streams (Hunt, 1969; Brown & Mackay, 1995). These fishes seek winter refuge in deep pools, off-stream channels or undercut banks (Cunjak & Power, 1986; Chisholm *et al.*, 1987). Reduction in stream size and depth might reduce access to pools, bank cover or off-stream channels (Cunjak, 1996), or substratum cover through anchor ice development (Stickler *et al.*, 2008), and fishes with different winter cover requirements might respond differently to the same flow manipulation. Such responses should depend on the availability of critical habitats within the affected reach, and failure to locate suitable cover could potentially have energetic consequences in the face of constrained energy budgets.

Physiological responses of stream fishes to flow manipulation have been studied by monitoring fish growth, mortality and stress levels. Growth rates of rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) were lower in the summer when discharge was reduced (Harvey *et al.*, 2006), and an increase in the number of major discharge fluctuations downstream of a dam were correlated with mortality of winter-stocked *O. mykiss* (Annear *et al.*, 2002). A stress response, indicated by elevated cortisol levels, has often been found to accompany dewatering (Flodmark *et al.*, 2002; Arnekleiv *et al.*, 2004). Effects of flow fluctuations depend on the capability of fishes to respond to habitat alterations and to find appropriate refuges (Valentin *et al.*, 1996); therefore, physiological and behavioural changes in fishes caused by alterations in winter flow may be more pronounced in streams with less available habitat.

The purpose of this study was to experimentally evaluate the effect of severe (50–75% reduction of discharge), repeatedly applied (daily) withdrawals of a short duration (4 h), on movement and aspects of fitness of overwintering *S. fontinalis*

in a small mountain stream. These are types of water withdrawals that such small streams might be exposed to from hydropeaking (rapid changes in the release of water from hydroelectric power stations in response to fluctuations in the demand for power), temporary water diversions or snow making. Fish metrics evaluated included movement levels, habitat availability and use, levels of plasma cortisol and glucose, and body composition.

## MATERIALS AND METHODS

### STUDY AREA

The study was conducted in a first-order stream (width 0.8–3.0 m, depth 0.05–0.53 m, gradient 1–2%) in the Canadian Rocky Mountains (50° 59' 03.2" N; 115° 04' 48.2" W, elevation 1400 m), draining a bench of poorly sorted alluvial gravel, and entering the southern end of Barrier Lake, a hydroelectric reservoir on the Kananaskis River. The bench is covered by a thin layer of humic podzol, supporting dense pine and spruce, and the stream banks are deeply undercut and well shaded. The stream habitat is a mixture of riffles, runs and pools, with some woody debris; the substratum is a mixture of cobble and gravel, with a few boulders, strongly embedded with fine sand and silt, limiting access of the fish to substratum cover. The stream appears to receive a significant input of groundwater throughout the year, keeping flows stable, summer temperatures cool and preventing ice formation during winter. *Salvelinus fontinalis* were the only fish species within the stream (*c.* 3 g wet mass m<sup>-2</sup>), which is isolated from Barrier Lake by a series of beaver *Castor canadensis* dams downstream of the study site.

Climate in the Kananaskis valley is a transition between the continental and cordilleran; winters are long, the mean temperature remains below freezing from November to April, with continuous snow cover and all standing waters are ice-covered during this period (Janz & Storr, 1977). Lowest average temperatures (–26° C) occur during January, and agricultural development has not occurred in this valley because of long cold winters (Johnson & Fryer, 1987).

### EXPERIMENTAL PROTOCOL AND DESIGN

Water withdrawal experiments were conducted within two 100 m stream reaches fenced with metal screening (6 mm mesh) attached to T-bar posts. The upstream reach, termed the reference channel, was separated from the downstream reach, termed the experimental channel, by a 35 m reach where the stream ran through a culvert (Fig. 1). The same experimental and reference channel was used in both years. Flow was reduced by diverting water around the experimental channel through 100 m of 2 cm hose using two 10 cm 5.97 kW pumps. Stream discharge in both channels was monitored using four staff gauges and remained *c.* 45 ± 5 (mean ± S.E.) l s<sup>-1</sup> throughout both winters. Gauges were calibrated by measuring cross-sectional area and velocity with a hand-held Gurley mini-current meter (Model 625D, Gurley Precision Instruments; www.gurley.com) at two permanent transects. Water withdrawal achieved reductions of 50% (mean ± S.E. 22 ± 3 l s<sup>-1</sup>) and 75% (mean ± S.E. 34 ± 4 l s<sup>-1</sup>) of natural flow by regulating the pump throttles and monitoring with staff gauges downstream of the pumps.

In both years, flow reductions were applied in daily 4 h pulses (1000 to 1400 hours), and radio-tracking measurements in both channels were performed before, during and after water withdrawal. In 2007, flow manipulations were carried out in two, 2 week experiments (22 January to 5 February and 19 February to 4 March), with a 50% flow reduction. Both movement and bioenergetic results were somewhat equivocal in 2007. Therefore, the water withdrawal treatments were intensified (75% withdrawal) and the experimental design was altered for 2008, to provide increased statistical resolution of movement responses. By repeated application of the water withdrawal treatment (week 1, 3, 5 and 7), alternating with

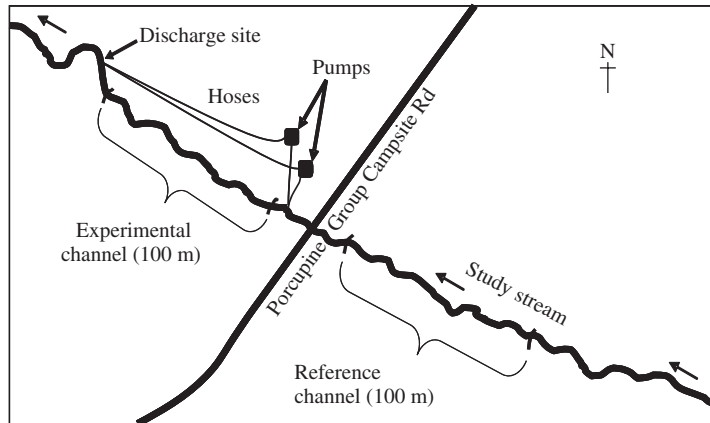


FIG. 1. Experimental site layout depicting the position of the experimental and reference channels, and the pumping arrangement. ←, stream-flow direction; |, fish fences.

no withdrawal (week 2, 4 and 6) in the experimental channel, with radio-tracking maintained throughout in both channels, the data for 2008 could be analysed using a repeated-measures design, since a withdrawal *v.* non-withdrawal comparison could be drawn for each individual. The number of *S. fontinalis* used in 2008 experiment was also larger than that in 2007 (Table I) increasing the d.f.

In 2007, the experiment was carried out using *S. fontinalis* obtained from the study stream by multi-pass electrofishing both within the study channels and in the neighbouring reaches. In 2008, abundance in the stream was insufficient to support the experiment, so all *S. fontinalis* used were obtained from a small stream near Canmore (51° 04' 43.7" N; 115° 23' 17.4" W, elevation 1390 m), with similar depth, width, flow, bank cover and elevation to the study stream and transported in 125 l containers to the study site.

## TAGGING

Coded radio Nano Tag transmitters (model NTC-3-M; Lotek Wireless Inc.; www.lotek.com) mass 0.5 g, volume 0.73 cm<sup>3</sup> (7.2 mm × 7.0 mm × 14.5 mm) were attached externally to the larger *S. fontinalis* (Table I), under anaesthesia from clove oil (40 ppm). The transmitters were manufactured with a small straw (10 mm in length) attached to the side and secured in place

TABLE I. A summary of *Salvelinus fontinalis* tagging information for the experimental and reference channels (Fig. 1) during 2007 and 2008. Fish were assigned randomly to the two channels

Year	Radio-transmitters number of fish			
	Tag ratio % range	Fish mass, range (g)	Experimental	Reference
Radio-transmitter tags				
2007	0.8–2.4	20.7–60.1	10	10
2008	0.6–1.6	31.1–78.7	15	15
Floy tags				
2007	<0.1%	9.5–19.5	10	12
2008	<0.1%	10.1–28.7	20	20

by heat shrink wrap. The straw on the transmitter was threaded with 0.225 mm diameter non-absorbable nylon suture thread (Supramid, S. Jackson Inc.; www.supramid.com). Two small gauged sewing needles were then threaded through each end of the suture thread. The transmitter was attached by simultaneously pushing the threaded needles through the dorsal musculature and then through the holes in a plastic back plate situated on the opposite side of the dorsal fin; the back plate (1.5 mm × 4 mm × 16.5 mm) was used to prevent tissue abrasion by the thread. Smaller *S. fontinalis* were tagged with Floy FTF-69 Fingerling Tags (Floy Tag Inc.; www.floytag.com) that were attached to the anterior of the dorsal fin through the dorsal musculature (Table I). Following tagging, fish were placed in large holding tanks of fresh stream water and were monitored for 15 min to ensure that they had recovered. They were placed into the fenced reaches of the stream and allowed 1 week to recover and acclimatize before water withdrawals commenced.

## RADIO-TRACKING MEASUREMENTS

Movement and habitat selection was monitored using manual radio telemetry and tracking on foot. A receiver with a hand-held H antenna (Lotek Wireless Inc.) was used to track the coded transmitters. Each individual was located three times daily: at 0830–0900 hours prior to water withdrawal, 1130–1200 hours (during water withdrawal) and again at 1430–1500 hours (after water withdrawal), and experimental and reference fish were tracked concurrently. Linear longitudinal movement was determined relative to the previous position occupied, and thus the set of three measurements yielded two estimates of movement for each fish each day. Location (range ± 0.5 m) of every fish was determined during each tracking event. Most fish were not observed visually during the experiment.

## BEHAVIOURAL AND PHYSIOLOGICAL RESPONSES

Blood samples were taken in the 2007 field season in order to determine plasma cortisol and glucose levels. At the end of the experiment, *S. fontinalis* were caught by electrofishing and transported in a bucket to the sampling station (<5 min). All individuals large enough (>100 mm fork length,  $L_F$ ) to provide a blood sample (41 fish) were first anaesthetized using clove oil (0.04 ml l<sup>-1</sup>) and blood was taken from the caudal vessels using 1 ml heparinized plastic syringes. Blood was transferred to a 0.5 ml tube, centrifuged and plasma stored in liquid nitrogen until transferred to a -80° C freezer prior to analysis. Cortisol levels were measured in duplicate with a radioimmunoassay kit (catalogue # 07-221102 Mediacorp; www.mediacorp.com). Glucose was measured in duplicate in a spectrophotometric assay (510 nm) using the glucose oxidase-protein precipitation (GOD-PAP) method (Boehringer Mannheim Corp; www.boehringer-ingenheim.com), when samples contained a sufficient amount of plasma.

In 2008, total body fat (TBF) and total body water (TBW) were estimated using bioelectrical impedance analysis (BIA) by measuring resistance and reactance of the fish with a tetrapolar, Quantum X bioelectrical body composition analyser (RJL Systems; www.rjlsystems.com). The analyser was adapted for use on fishes by using 28 gauge 12 mm stainless steel needle electrodes (Grass Telefactor; www.grasstechnologies.com). The anaesthetized *S. fontinalis* were patted dry and placed on a dry paper towel. The electrodes were placed ipsilaterally midway between the lateral line and the dorsal midpoint (Cox & Hartman, 2005). To calibrate the BIA readings, resistance and reactance were measured from 40 live fish collected from nearby streams. After taking the BIA measurements, fish were euthanized, frozen, and lipid and water content were measured using standard biochemical methods. Calibration equations between the BIA and the biochemical measurements were developed ( $r^2 = 0.77$  for both TBF and TBW). While only radio-tagged *S. fontinalis* were used for estimates of movement and habitat selection, physiological measurements as well as changes in mass  $L_F$  and BIA (condition indices) were determined for all tagged individuals.

## HABITAT CHARACTERISTICS

Descriptions of habitat are based on physical variable of the stream and its surroundings that affect its suitability for *S. fontinalis*. Air temperature (range ± 0.1° C) was recorded at

the stream site three times daily at 0830, 1130 and 1430 hours. These temperatures were correlated with three nearby weather stations: University of Calgary Kananaskis Field Station, Kananaskis (Nakiska Ridgetop) and Bow Valley (Provincial Park). Weather data from Bow Valley (Provincial Park) were most closely correlated with data from the stream site and were therefore used to indicate approximate hourly air temperature at the study site. Stream temperature (range  $\pm 0.1^\circ\text{C}$ ) was recorded every 10 min using four digital temperature loggers (HOBO Water Temp Pro, Onset Computer Corporation; www.onsetcomp.com). The loggers were placed upstream and downstream of the two reaches, in the middle of the stream channel.

To determine how available habitat changed as discharge was manipulated, stream width (range  $\pm 1\text{ cm}$ ), depth (range  $\pm 0.5\text{ cm}$ ) and velocity (range  $\pm 0.1\text{ cm s}^{-1}$ ) were measured along a series of 24 transects across four different mesohabitat types: pools, riffles, runs with deeply undercut banks and runs without undercut banks. Twelve transects were located within the experimental channel and 12 within the reference channel. Depth and velocity was measured at 0.2 m increments along each transect. Mean water velocity readings were taken at 0.6 of the stream depth, measured from the surface. The surveys were completed at four levels of discharge in the experimental channel (100, 75, 50 and 25% of the normal stream discharge), and in the reference channel, only at the normal discharge. In addition to measurements taken across transects, habitat was recorded longitudinally for every metre along both the experimental and the reference channel. Measurements or descriptions of stream width, water depth, substratum composition, bank shape, undercut bank depth and instream cover were recorded. Substratum was recorded according to a modified Wentworth scale as silt and sand ( $<2\text{ mm}$ ), gravel (2–64 mm), cobble (65–256 mm) or boulder ( $>256\text{ mm}$ ).

## DATA ANALYSIS

Statistical analyses were completed using JMP IN version 8 (www.jmp.com) software. Variables and their  $\log_{10}$  transformations were tested for normality using the Shapiro & Wilk (1965), quantile test. Movement data for both 2007 and 2008 were first analysed using one-way ANOVA, using the equation:  $x_{ij} = \mu + \alpha_i + \varepsilon_{ij}$ , where  $x_{i,j}$  is the mean movement ( $\text{m day}^{-1}$ ) of fish  $j$  given treatment  $i$ ,  $\mu$  is the mean movement of fish across all treatments,  $\alpha_i$  is the effect of treatment  $i$  on mean movement and,  $\varepsilon_{i,j}$  is the residual error. There were two treatments, no water withdrawal ( $i = 0$ ; reference channel) and water withdrawal ( $i = 1$ ; experimental channel), with  $j = 20$  in 2007 and  $j = 30$  in 2008. Movement for each of the  $j$  fish was a single value calculated as the daily movement estimate averaged across the period of each treatment. The one-way ANOVA did not utilize information from fish during weeks of no water withdrawals.

To account for variability in movement among individuals, two-way (repeated-measures) ANOVAs were carried out with the 2008 data using the model:  $x_{i,j,k} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$ , where  $x_{i,j,k}$  is the mean movement of fish  $j$  given treatment  $i$  in week  $k$ . In this analysis, each channel (experimental or reference) is treated as a separate ANOVA experiment with: replication through time (weeks,  $k = 7$ ), treatment ( $\alpha$ ) for weeks with ( $i = 1$ ) or without withdrawals ( $i = 0$ ) in the experimental channel; and the effect of variability among individuals ( $\beta$ ;  $j = 15$ ). The analysis was run as a mixed model with the withdrawal effect ( $\alpha$ ) analysed as a fixed effect and the fish effect ( $\beta$ ) analysed as a random effect (Zar, 1999). Thus, significant  $\alpha$ ,  $\beta$  and possibly  $\alpha\beta$  interaction effects were expected in the experimental channel, whereas in the reference channel only a significant  $\beta$  effect was expected. A significant  $\alpha\beta$  interaction would indicate that individuals differed significantly in their level of response to flow reduction. The repeated-measures analysis was expected to reveal  $\alpha$  effects similar in magnitude but statistically much stronger than those of the one-way ANOVA, where effects of individual differences could not be factored out. The 2007 data could not be analysed using the two-way (repeated-measures) ANOVA since in the experimental channel although the water removal treatment was replicated, the non-removal treatment was not.

Movement estimates are expressed as metres moved (sum of two measurements) per daily measurement bout. Movement estimates from individual days generally failed to comply with normality requirements for ANOVA owing to a preponderance of 0 values. That is, a substantial fraction of fish radio-tracked on any given day exhibited no detectable change in position. When movement estimates were averaged over 1 week (2008) or 2 week (2007) intervals,

most fish did show movement; however, the residuals of the ANOVAs still violated the Shapiro–Wilk test. Analyses on transformed movement [ $\log_{10}(x + 1)$ ] are therefore presented, as well as the untransformed estimates of movement per daily tracking session, averaged over 1 week periods. The error residuals from these analyses did not depart significantly from normality and satisfied the homoscedasticity requirement as well. Non-parametric analyses were also used. While there are no direct non-parametric alternatives to two-factor ANOVA, Friedman's test (Zar, 1999) involves ranks within blocks and can therefore be used in a paired design to factor out individual differences. Estimates of median movement have been analysed in this way in several studies (Stickler *et al.*, 2008). Movement data were also analysed in terms of the proportion that moved, with individual days as replicates, in order to see whether the integration had any effect on the estimated effects of flow manipulation on movement.

Because of the intensive habitat sampling grid described above, mean ( $\mu$ ) and s.d. ( $\sigma$ ) of available habitat variables were considered to be well characterized ( $n > 30$ ). Characteristics of sites selected by fish were averaged ( $\bar{x}$ ) and compared to the mean habitat available ( $\bar{x} - \mu$ ) using Z-scores calculated as  $(\bar{x} - \mu)(\sigma\sqrt{n}^{-1})^{-1}$ . Although values were obtained for each radio-location measurement (*e.g.* three times daily over the course of each experiment), and as such,  $\bar{x}$  represents the average of all measurements, in order to avoid pseudoreplication,  $n$  represents the number of fish and not the total number of measurements. Differences between habitat selected and habitat available were also assessed using the non-parametric Kolomogorov–Smirnov two-sample test, as described by Conover (1980).

Estimates of habitat used by individual *S. fontinalis* were regressed against individual fish movement, both using daily estimates, and averages pooled for each fish. Individual estimates of movement and habitat use were also regressed against physiological variables and mass changes.

## RESULTS

### MOVEMENT RESPONSE

In 2007, the mean  $\pm$  s.e. level of movement observed among the 10 radio-tagged *S. fontinalis* in the experimental channel during 4 h bouts of flow reduction was  $3.4 \pm 0.7$  m day<sup>-1</sup> compared to  $1.8 \pm 0.7$  m day<sup>-1</sup> in the reference channel. These differences were not statistically significant [one-way ANOVA on  $\log_{10}(x + 1)$  transformed values where  $x =$  movement,  $F_{1,18} = 3.90$ ,  $P < 0.05$ ]. In 2008, the mean  $\pm$  s.e. level of movement in the experimental channel during flow reduction was  $5.0 \pm 0.9$  m day<sup>-1</sup> in 2008 compared to  $1.7 \pm 0.9$  m day<sup>-1</sup> of the reference channel for the same weeks (Fig. 2). These differences were statistically significant when analysed as a one-way ANOVA on  $\log_{10}(x + 1)$  transformed values ( $F_{1,28} = 11$ ,  $P < 0.01$ ).

Individual *S. fontinalis* differed greatly in their tendencies to move, and in the 2008 experiment, it was possible to compare movement of individuals during weeks of water withdrawal (weeks 1, 3, 5, 7) to that of the same individuals in weeks with no water withdrawal (weeks 2, 4, 6), using two-way ANOVA testing for both effects of water withdrawal ( $\alpha_{1,0}$ ) as well as individual differences ( $\beta$ ) and their interactions ( $\alpha\beta$ ). For the experimental channel:  $\log_{10}(x + 1) = 0.30 (\pm 0.04) + 0.30 (\pm 0.04) \alpha_1$  ( $F_{1,67} = 33$ ,  $P < 0.001$ ); the  $\beta$  effect, analysed as a random effect, was significant ( $F_{14,14} = 3.6$ ,  $P < 0.05$ ), but the  $\alpha\beta$  interaction was not ( $F_{14,67} = 0.8$ ,  $P > 0.5$ ). The residuals of the model ( $\varepsilon = 0.26$ ) were normally distributed (Shapiro–Wilks'  $W = 0.99$ ,  $P > 0.5$ ). The corresponding analysis on the reference channel, where no flow manipulations were imposed, yielded no significant  $\alpha$  effect ( $F_{1,75} = 0.1$ ,  $P > 0.5$ ); however, the  $\beta$  effect was again significant ( $F_{14,14} = 3.3$ ,  $P < 0.05$ ), but

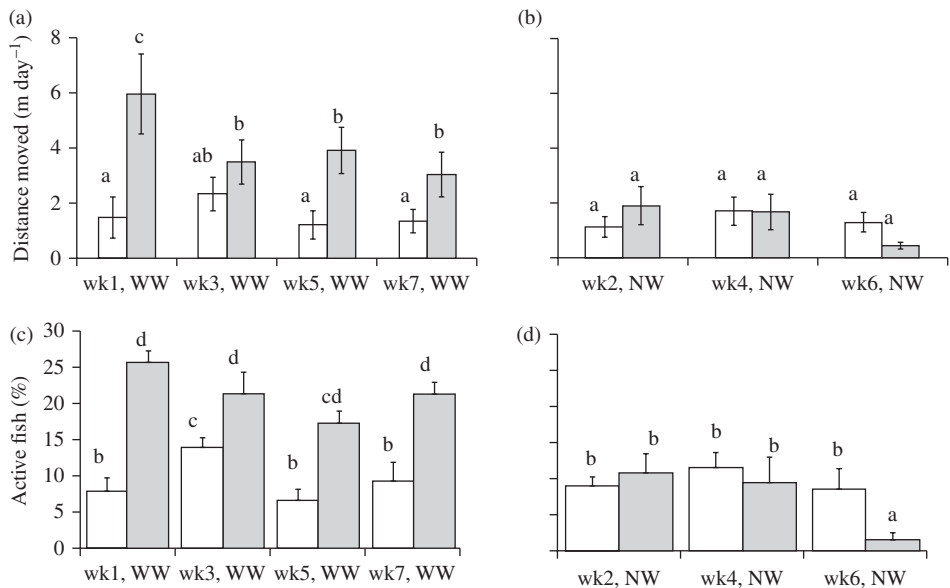


FIG. 2. Analysis of *Salvelinus fontinalis* movement using tracking (2008 data) in experimental channel (■) and reference channel (□), (a) average distance moved per daily 4 h water-withdrawal session, during 4 weeks (wk) where flow reduction was taking place in the experimental channel (WW) compared to (b) weeks of no water withdrawal (NW), (c) % active fish (movement >2 m) during flow reduction weeks and (d) weeks of no flow reduction. Values are means  $\pm$  s.e.; bars with the same lowercase letter are not significantly different across panels (a), (b) and across panels (c), (d) (Tukey test at  $P < 0.05$ ). For both reference and experimental channels  $n = 15$  fish.

the  $\alpha\beta$  interaction was not ( $F_{14,75} = 0.9$ ,  $P > 0.5$ ). The residuals of the model ( $\varepsilon = 0.26$ ) were again normally distributed (Shapiro–Wilks'  $W = 0.97$ ,  $P > 0.10$ ). The number of error d.f. for the experimental group was eight less than that of the reference group, as four *S. fontinalis* were killed by a mink *Neovison vison* in week 5, and thus the last 2 weeks of observations were lost for these individuals.

Average daily movement estimates spanned about one order of magnitude, from a few tenths to several metres in one daily measuring bout. Mean levels of movement were much higher than medians, reflecting the strong positive skew in the distributions. The skewed distributions, both under normal flow and under water withdrawal fitted the  $\log_{10}$ -normal model, and the transformed estimates of mean movement were very close to the median values [Fig. 3(a), (b)]. The movement of individuals under normal and reduced flow was well correlated [Fig. 3(c)], and thus the large individual differences tended to limit resolution of differences resulting from flow alteration.

The movement response was also significant using Friedman's non-parametric repeated-measures test, since flow manipulation yielded movement increases for each of the fish in the experimental channel; median movement estimates were 3.7 m for altered flow and 1.2 m for normal flow ( $\chi^2_{r-1,14} = 15$ ,  $P < 0.01$ ).

While movement levels rose and fell in the experimental channel, as water withdrawal was imposed in alternating weeks, the baseline movement level during week 2, 4 and 6 was very similar to that constantly recorded within the reference channel



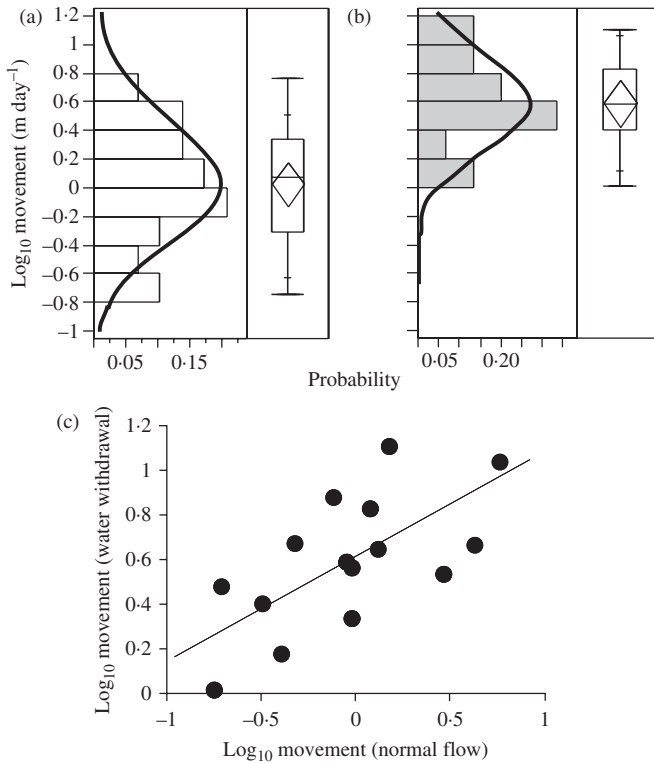


FIG. 3.  $\text{Log}_{10}$ -normal frequency distributions of observed movement for individual *Salvelinus fontinalis* in bins of 0.2 for (a) normal flow (NF) and (b) water withdrawal (WW). Box plots show the interquartile range (25–75%) and the median (50%), with bars denoting the minimum and maximum values and the 10% and 90% quantiles.  $\diamond$ , estimated mean and 95% C.L. of the mean. Curves represent fitted normal distributions; distribution parameters are: (a) NF mean = 0.03,  $s^2 = 0.39$ , s.e. mean = 0.08,  $n = 30$ , skewness =  $-0.27$ , kurtosis =  $-0.68$ . Shapiro–Wilk fit to the normal distribution,  $P > 0.50$ . Untransformed mean = 1.54, median = 1.18, back transformed mean = 1.10. (b) WW mean = 0.59,  $s^2 = 0.30$ , s.e. mean = 0.08,  $n = 15$ , skewness =  $-0.12$ , kurtosis = 0.04. Shapiro–Wilk fit to the normal distribution,  $P > 0.50$ . Untransformed mean = 4.84, median = 3.86, back transformed mean = 3.89. (c) The relationship between fish movement under water withdrawal and movement of the same fish under normal flow. The curve was fitted  $y = 0.61 (\pm 0.06) + 0.43 (\pm 0.14) x$  ( $r^2 = 0.41$ ,  $P < 0.01$ ).

(Fig. 2), indicating that behavioural responses to flow manipulation do not persist for long after flows return to normal. The level of movement exhibited by individuals, although highly variable was not significantly correlated with any of the measured habitat variables associated with the sites that they occupied, either during flow manipulation or under normal flow regimes; moreover, neither the individual estimates of movement nor the habitat use were correlated with either mass changes, changes in condition indices or any of the other physiological indices.

During each round of radio-tracking, there was a sizeable fraction of fish that did not move or moved very little. Thus, movement was analysed both in terms of distance moved and in a discrete manner, by testing whether the proportion that moved was affected by flow manipulation. This analysis was performed using one-way

ANOVA, with the individual tracking rounds as replicates (d.f. = 278), and the proportional data were arc sine transformed. A fish was considered to have moved if movement over the day was >2 m [Fig. 2(c), (d)]. The analysis of movement as a discrete variable revealed similar patterns to the previous analysis where movement was treated as a continuous variate (*i.e.* distance moved). More fish moved during the water withdrawal treatments ( $F_{1,278} = 33.5$ ,  $P < 0.001$ ) and the proportion that moved was not significantly different in the experimental and reference channel during weeks when no water manipulation was being imposed [Fig. 2(b), (d)]; movement showed no apparent upstream or downstream trend.

## HABITAT

Mean  $\pm$  s.e. water temperature was  $3.0 \pm 0.1^\circ$  C, (range +1 to +4.9 $^\circ$  C); no significant temperature differences were recorded either within or among the channels, even when water withdrawal was in effect. The stream never developed surface ice or anchor ice, even during the most severe weather, and this appeared to be a result of a significant and continuous input of groundwater to the stream. As stream flow was reduced, there was a reduction in stream width, depth and the quantity of available undercut bank cover [Fig. 4(a)–(c)]. Mean water velocity also declined with flow; at base flows mean  $\pm$  s.e. velocity was  $0.20 \pm 0.03$  m s $^{-1}$  (range = 0.0–0.8 m s $^{-1}$ ), at 50% reduction was  $0.10 \pm 0.02$  m s $^{-1}$  (range = 0.0–0.8 m s $^{-1}$ ) and at 75% reduction was  $0.10 \pm 0.02$  m s $^{-1}$  (range = 0.0–0.6 m s $^{-1}$ ) [Fig. 4(d)–(f)].

Stream habitat type was characterized primarily as runs with some riffles and pools, with substratum largely composed of fines and gravel with some cobbles

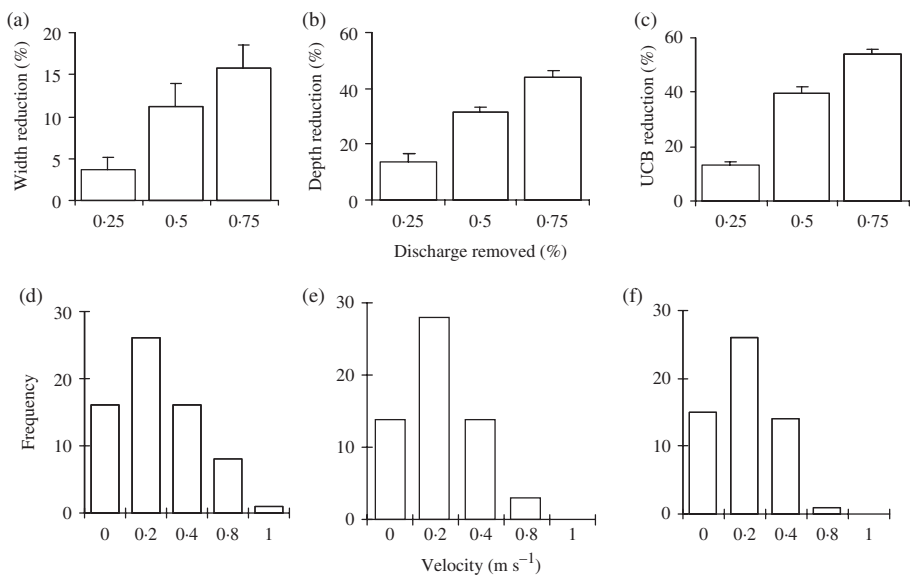


FIG. 4. Effect of reduced flow on (a) stream width, (b) stream depth and (c) undercut bank (UCB) availability (values are mean  $\pm$  s.e.) for occupation by *Salvelinus fontinalis*. Frequency distribution of velocity at (d) normal flow (e) 50% discharge reduction, and (f) 75% discharge reduction. Velocity measurements were taken every 0.2 m across the width of the stream on 12 transects.

TABLE II. Characteristics of stream habitat used by *Salvelinus fontinalis* compared to those available as measured along study transects. Results are provided for the experimental channel under natural flow conditions.  $Z = \bar{x} - \mu(\sigma\sqrt{n}^{-1})^{-1}$ . KS, Kolomogorov–Smirnov two-sample test, and the probability of its  $D$ -statistic. All length measurements are in cm, and substratum composition is in % of total. Wood measurements are % of substratum covered by branches and other coarse woody material

Habitat variable	2007, $n = 10$			2008, $n = 15$		
	Habitat selected-present $\bar{x} - \mu, (\sigma\sqrt{n}^{-1})$	KS-test, $P$	$Z, P >  Z $	Habitat selected-present $\bar{x} - \mu, (\sigma\sqrt{n}^{-1})$	KS-test, $P$	$Z, P >  Z $
Depth	29.0–25.0, (2.5)	>0.05	1.68, 0.10	29.5–25.0, (1.9)	>0.05	2.09, 0.04
Width	152–150, (11)	>0.05	0.18, 0.86	155–150, (9)	v	0.59, 0.55
UCB	42–23, (5)	<0.01	4.11, <0.001	38–23, (4)	<0.01	4.19, <0.001
Fines	54–49, (11)	>0.05	0.45, 0.66	51–49, (8)	>0.05	0.17, 0.86
Gravel	38–42, (8)	>0.05	–0.55, 0.29	40–42, (6)	v	–0.41, 0.68
Cobble	6–7, (3)	>0.05	–0.43, 0.66	8–7, (2)	v	0.46, 0.64
Boulders	0.2–0.5, (0.6)	>0.05	–0.48, 0.64	0.6–0.5, (0.5)	>0.05	0.21, 0.84
Wood	15–13, (8)	>0.05	0.26, 0.80	12–13, (6)	v	–0.10, 0.92

UCB, undercut bank depth.

and boulders. Undercut banks and woody debris provided a large amount of cover throughout both reaches (Table II). No significant difference between the amount of undercut bank cover, boulder substratum, or stream width was found between the experimental or the reference channel; however, the experimental site was slightly deeper, with slightly more fine substratum, more woody debris cover, less gravel substratum and less cobble substratum (Table II).

The habitat characteristics of the locations used by *S. fontinalis* in the experimental and reference channels were compared to the habitat available over each reach. Width, depth, substratum composition and woody debris cover at utilized locations were not different from the average available; however, the average extent to which banks were undercut at sites used by fish greatly exceeded the average availability and was statistically significant (Fig. 5). These patterns were not different between the reference and the experimental channels, however, only values for the latter are presented in Table II.

## PHYSIOLOGICAL RESPONSE

There was no significant difference in the levels of blood cortisol between *S. fontinalis* from experimental (mean  $\pm$  s.e.  $13.7 \pm 4.5$  ng ml<sup>-1</sup>) and reference channels ( $14.1 \pm 5.4$  ng ml<sup>-1</sup>;  $F_{1,39} = 0.9$ ,  $P > 0.05$ ) or in levels of blood glucose (experimental fish,  $0.70 \pm 0.02$  mg ml<sup>-1</sup> v. reference fish  $0.70 \pm 0.02$  mg ml<sup>-1</sup>;  $F_{1,32} = 0.87$ ,  $P > 0.05$ ). Most *S. fontinalis* lost mass over both winters. In 2007, body mass declined by 6.3% ( $F_{1,18} = 93.5$ ,  $P < 0.001$ , reference;  $F_{1,18} = 62.9$ ,  $P < 0.001$  experimental), and in 2008 mass declined by 10.2% ( $F_{1,28} = 93.5$ ,  $P < 0.001$ , reference;  $F_{1,24} = 62.9$ ,  $P < 0.001$ , experimental). Mass loss in reference and experimental channels were not significantly different ( $F_{1,19} < 1$ ,  $P > 0.05$ , 2007;  $F_{1,52} = 2.07$ ,  $P > 0.05$ , 2008). Bioelectrical impedance analysis indicated that lipid

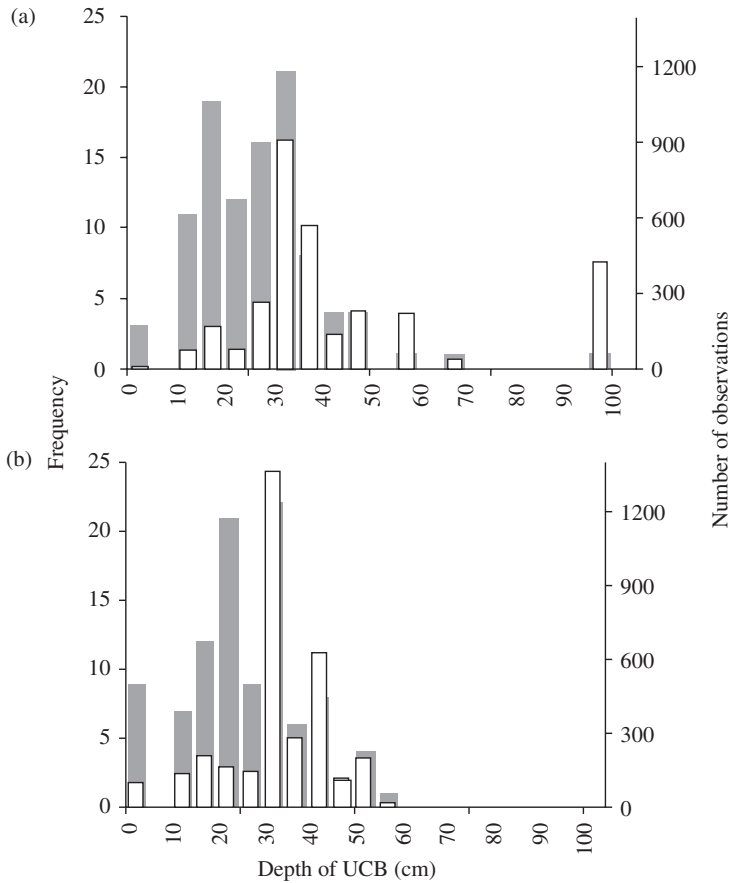


FIG. 5. Frequency of undercut bank depth (UCB) cover available (■) and the frequency of UCB cover used (□) by *Salvelinus fontinalis* within the (a) experimental reach and (b) the reference reach. Used locations were determined three times daily over 2007 and 2008, and estimates were averaged for each *S. fontinalis*.

content declined by 21% ( $F_{1,52} = 6.24$ ,  $P < 0.05$ ) and water content increased by 1% ( $F_{1,52} = 7.12$ ,  $P < 0.01$ ). The changes that took place in the experimental and reference channels were not different (TBF:  $F_{1,52} = 1.02$ ; TBW:  $F_{1,52} < 1$ ).

## DISCUSSION

The experimental flow reduction imposed on *S. fontinalis* in this study resulted in increased levels of movement by *c.* 2.5 fold; however, it should be noted that the levels of movement during daily recording bouts were limited to a few metres, similar to those recorded in previous studies of midwinter salmonids (Huusko *et al.*, 2007; Scruton *et al.*, 2008). The increased movement under changing flow regimes is probably a combination of directed responses to the changing distribution of suitable habitat, combined with undirected responses resulting from a cascade of social interactions. As flow regimes change, altering habitat availability, dominance hierarchies

can break down, destabilizing habitat use and resulting in increased social stress and reduced growth rates (Sloman *et al.*, 2001, 2002).

Individual fish differed significantly in their tendency to move, with a strong positive skew in the distribution. Similar individual differences have been observed in other studies (Scruton *et al.*, 2008; Stickler *et al.*, 2008) and the differences in movement among individuals have been suggested to reflect dominance status, with more dominant fishes holding stable territories, and moving less than subordinate individuals (Mäki-Petäys *et al.*, 1997; Scruton *et al.*, 2003).

By exposing the same individuals repeatedly to different flow regimes, as was done in 2008, the individual variability was factored out using a repeated-measures design, increasing statistical resolution. The movement response to flow manipulation was greater and more statistically significant in 2008 than in 2007, a combined effect of the improved statistical design, the more severe level of flow reduction imposed (75% in 2008 *v.* 50% in 2007), and possibly, the higher densities of fish used in that year. Although individuals differed greatly in their tendency to move, the absence of a significant interaction between the response to water withdrawal and the fish effect indicates that individuals did not differ greatly in their response to flow reduction. In addition, no correlations could be detected between the level of movement of individuals and the habitat variables they selected, or their energetic status at the end of the experimental period.

The magnitude of flow fluctuations imposed in the experimental channel was large (50–75% reductions relative to base flow), but of fairly short duration (4 h). In terms of relative magnitude and timing, these experimental manipulations are comparable to those associated with hydropeaking operations such as the one on Kananaskis River, of which the experimental stream is a tributary. The Kananaskis River supports three hydroelectric facilities with daily fluctuations of discharge exceeding two orders of magnitude ( $0.2\text{--}25\text{ m}^3\text{ s}^{-1}$ ) (Bunt *et al.*, 1999). Although these authors did not report an increase in large-scale movements by *S. trutta* ( $>100\text{ m}^2$ ), they did observe localized movements in response to the changing habitat conditions associated with these flow fluctuations (Bunt *et al.*, 1999), which is consistent with the present study. Increased movement in response to flow alteration has also been observed in other studies; *S. fontinalis* increased movement during up and down-ramping of hydroelectric facilities (Scruton *et al.*, 2003; Murchie & Smokorowski, 2004), and *S. salar* parr moved more under changing flows than at stable flows (Berland *et al.*, 2004).

During winter, most salmonids gravitate strongly to cover, either as refuge from current or from predators (Huusko *et al.*, 2007), and are more sedentary than at other times of the year. The strong selectivity for undercut banks exhibited by *S. fontinalis* is consistent with this pattern. Although the availability of undercut bank habitat was greatly reduced under water withdrawal, fish that moved were always able to locate this type of habitat. While most studies of winter salmonid biology involve *S. salar*, which seek out mainly substratum cover (Linnansaari *et al.*, 2008) or cover from surface ice, most studies that have examined winter habitat use by *S. fontinalis* show strong attraction either to pools or to bank-associated cover (Hunt, 1969; Cunjak & Power, 1986; Chisholm *et al.*, 1987). Consistent with this, *S. fontinalis* in the present study selected bank cover; however, the option of using substratum cover was not available due to the embedded nature of the substratum, and surface ice was never present. Like most charrs, *S. fontinalis* are strongly dependent on groundwater

upwelling for spawning, and streams suitable for this species often lack significant surface ice cover (Cunjak & Power, 1986). The movement responses that salmonids make in response to altered flow conditions in winter will reflect the need for suitable cover. Species with different winter cover requirements might be expected to respond differently to flow manipulation, depending on the availability of critical habitats within the affected reach, and inability to make appropriate responses can potentially have energetic consequences.

Movements, whether in search of new habitat or resulting from social disturbance, can impose significant energetic costs (Flodmark *et al.*, 2002). Experimentally reduced flow during the summer dry season significantly reduced growth of small *O. mykiss* in a California stream (Harvey *et al.*, 2006), and other studies have demonstrated that sedentary juvenile *O. mykiss* maintain their lipid levels better than active ones (Simpkins *et al.*, 2003). In the present study, despite the increase in movement, there was no significant relationship between mass loss and water withdrawal. While body mass and condition declined during winter, neither of it increased in response to flow manipulation. This could indicate that the movements were not of a sufficient distance or frequency to be a major energy drain, possibly because critical habitat was sufficiently available even under reduced flow. In situations where adaptive responses are not possible, either due to habitat shortage or high population density, an energetic toll affecting growth and possibly survival is expected (Scruton *et al.*, 2003). It is also possible that had the altered flow regime been imposed for a longer period, energy stores may have been reduced.

Although salmonids have not always been found to deplete lipid reserves over winter (Webster & Hartman, 2007), several studies have reported declines in lipid content and increases in water content in overwintering fishes similar to those reported here (Cunjak & Power, 1986; Biro *et al.*, 2004). Salmonid winter feeding is restricted because low water temperatures limit appetite, digestion and gastric evacuation rates (Cunjak *et al.*, 1987; Sweka *et al.*, 2004); consequently they primarily rely upon stored fat to meet energetic demands, and thus can lose mass during this period (Cunjak *et al.*, 1987). Although no loss of mass could be associated with water withdrawal in the present study, reduced growth by *O. mykiss* in response to summer flow reduction has been observed (Harvey *et al.*, 2006), possibly reflecting the greater importance of the summer season for feeding and growth. In summer, fishes need to select habitat not only to minimize costs and maximize cover which are probably the major winter issues, they must also trade these off against food supply. Thus, habitat selection problems encountered during winter, although potentially consequential, might be in some ways simpler than those encountered during summer, since winter salmonids are essentially on hold with regard to feeding and growth.

Not only did the increased movements resulting from the imposed flow reduction not deplete energy stores, they elicited no significant physiological stress responses either. Thus, the imposed regime was either not stressful to *S. fontinalis* or the imposed stress level was low enough to allow rapid habituation. In order to avoid disturbing fish behaviour throughout the study, blood sampling for analysis was carried out only once at the end of the experiment and no differences in cortisol and glucose levels between experimental and reference fish could be detected at this point. A stressor may initially elicit elevated levels of cortisol and glucose which may then subside despite continued administration of the stressor (Pickering & Pottinger, 1989; Scruton *et al.*, 2005). Such habituation responses for cortisol and glucose have

been observed in several flow manipulation studies (Barton *et al.*, 1987; Flodmark *et al.*, 2002; Arnekleiv *et al.*, 2004) and may have occurred in the present experiment, but would not have been detected. Whether habituation occurs, or whether a stress response becomes chronic, depends on many aspects of the stress, including its intensity, rate of onset and duration (Scruton *et al.*, 2005).

It might be expected that the scale of movements that would be observed within a small stream, with enclosed channels and limited habitat range, might be smaller than those expected in large rivers, where many of the previous studies have been done (Bunt *et al.*, 1999; Huusko *et al.*, 2007). In rivers, larger scale movements have mainly been recorded in summer (Scruton *et al.*, 2008), and in the early winter (Cunjak, 1996) or late in winter, associated with the break-up of winter ice (Brown *et al.*, 2001). In midwinter, however, studies on unconstrained winter movements of salmonids over a large range of river sizes, either in the presence of flow manipulation (Scruton *et al.*, 2005, 2008) or not (Hiscock *et al.*, 2002; Enders *et al.*, 2008; Stickler *et al.*, 2008) have shown daily movement distances of no more than a few metres. These involve small relocations among substratum cover sites, similar to those described here, and thus within the midwinter context there is no reason to consider these results to be an artefact of the small stream or experimental channels.

Although there was no detectable effect of flow reductions on energetic condition, increased movement associated with daily flow changes may have increased the vulnerability of *S. fontinalis* to predation. Mink predation has often been observed to be a major contributor to winter fish mortality of radio-tagged salmonids (Hiscock *et al.*, 2002; Lindstrom & Hubert, 2004), and the seasonal shift in diel behaviour, characterized by day time concealment (Heggenes & Dokk, 2001; Annear *et al.*, 2002), may be an adaptive behaviour to minimize predation risk (Heggenes *et al.*, 1993; Valdimarsson & Metcalfe, 1998).

Although most fish were rarely seen while tracking, *S. fontinalis* in the experimental channel were more often seen than those in the reference channel, probably because they moved more. In fact reference *S. fontinalis* were never observed in the centre of the stream channel, whereas six of the 15 experimental *S. fontinalis* with transmitters were observed regularly in the centre of the channel during tracking, sometimes near woody debris, and four of these were eventually killed by mink. While a number of studies have shown that foraging movements expose organisms to predation risk (Werner & Anholt, 1993; Biro *et al.*, 2003), movements directed towards habitat search should also increase the risk of detection by predators. At low temperatures responses of ectothermic prey are slowed, making them highly vulnerable to homeothermic predators (Huusko *et al.*, 2007).

Studies on instream flow requirements of fishes are vital to environmental management due to increasing water demands and changing hydrological regimes. Identification of critical habitats is crucial for habitat management and conservation of freshwater fish populations. Salmonids in winter strongly orient to cover (Huusko *et al.*, 2007), and whether the preferred cover is from undercut banks as in the present study, the interstitial spaces in coarse unimbedded substratum (Linnansaari *et al.*, 2008; Scruton *et al.*, 2008), surface ice (Maciolek & Needham, 1952; Finstad *et al.*, 2004) or the shelter of deep water in pools, back eddies or off-stream channels (Cunjak & Power, 1986; Chisholm *et al.*, 1987), flow alteration can be expected to change the distribution and availability of each of these kinds of cover. The small-scale nature of the movements shown in this and other studies on midwinter

salmonids, however, indicates that fishes are usually able to locate acceptable cover even if the flow manipulation alters the distribution and availability of preferred habitat. The fact that no chronic stress or energetic impairment was detected suggests that when flow manipulations induce only small-scale relocations, fishes are not energetically affected. Under some situations, however, salmonids have been observed to undergo fairly large-scale habitat shifts during winter, and such responses would be expected in situations where the preferred cover has been eliminated by flow manipulation, or in situations where access to habitat may be seriously restricted by the build-up of frazil or anchor ice (Stickler *et al.*, 2008). Understanding of the type of winter cover that different salmonids seek, and the ability to model the changes in distribution and availability of this critical habitat at different temporal and spatial scales, and under different flow regimes, is essential for establishing instream flow criteria for this critical time of year. The experimental set-up and protocols used in this study proved workable under winter conditions and could be used to assess the effect of a broad range of water withdrawal regimes from small streams.

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