

The energetic equivalence of changing salinity and temperature to juvenile salmon

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Summary

1. The influence of salinity, temperature and physiological development on habitat choice by juvenile salmon is poorly understood. We measured differences in the net energetic cost of habitats that differed in salinity or temperature using behavioural titration of juvenile salmon and correlated these costs with body size and osmoregulatory enzyme activity to quantify how costs change with physiological development.
2. Juvenile salmon showed a strong preference for saline water (27‰ or 15‰ vs 0‰) and for cold water (9 °C vs 14 °C). It was estimated to be 0.15 W and 0.11 W more costly for salmon to forage in fresh water than in 15‰ and 27‰ salt water, respectively, and 0.09 W more costly to forage in warm than in cold water.
3. We expected fish to prefer 15‰ salt water to fresh water regardless of enzyme activity because they are iso-osmotic with this salt concentration. In contrast, preference for higher salt concentrations should increase with enzyme activity. Consistent with our expectations, enzyme activity was not correlated with preference for 15‰ salt water, but was positively correlated with preference for 27‰ salt water.
4. The cost of changing salinity vs changing temperature were very similar, indicating that knowledge of both of these costs are necessary to understand juvenile salmon habitat choice.

Key-words: Foraging, habitat choice, ideal free distribution, Na⁺, K⁺-ATPase activity

Functional Ecology (2006) **20**, 621–629
doi: 10.1111/j.1365-2435.2006.01128.x

Introduction

Foraging theory predicts that animals will attempt to maximize their rate of energy intake by foraging in areas of high prey density (Stephens & Krebs 1986). However, areas with the highest prey density may not be preferred if it is too energetically costly to forage there. High energetic costs may be the result of abiotic factors that alter metabolic requirements, such as temperature (Wildhaber & Crowder 1991; Hilton, Ruxton & Cresswell 1999; Hughes & Grand 2000) or salinity (McGaw 2001). Hummingbirds, for instance, will not forage in habitats that are colder than 13 °C, even when ample food is available, because metabolic costs are too high (Lopez-Calleja & Bozinovic 2003).

To predict habitat choice, the relationship between abiotic conditions and net energy gain must be quantified. Physiologists often infer differences in net energy gain by contrasting metabolic rates (e.g. Kirschner 1993; Morgan & Iwama 1998) between different abiotic con-

ditions. However, in addition to increasing direct costs, changes in abiotic conditions may impose indirect costs. For example, changing conditions may cause a decrease in net energy gain by decreasing intake rates (Pravosudov & Grubb 1998; Brown 1999). If only direct costs are considered, habitat-specific estimates of net energy gain will be inaccurate and lead to incorrect predictions about how varying abiotic conditions should affect habitat choice.

An alternative means of estimating direct and indirect energetic costs is to use behavioural titration (Kotler & Blaustein 1995). Behavioural titration is based on the assumption that animals will leave a habitat when the net benefit of foraging there falls below the net benefit of foraging in an alternative habitat (Brown 1988). A commonly used behavioural titration technique is based on Fretwell & Lucas's (1970) ideal free distribution theory (IFD) (e.g. Abrahams & Dill 1989; Nonacs & Dill 1990; Grand & Dill 1997; Abramsky, Rosenzweig & Subach 2000). IFD theory assumes that animals have perfect information about the distribution of competitors and resources ('ideal'), and can move to the habitat where their fitness will be the highest

('free'). If costs are equal in all habitats, IFD predicts an equilibrium distribution at which individuals cannot increase their fitness by switching habitats and the density of animals in each habitat will match resource availability (input matching). If the relative cost of one habitat is increased, animals will redistribute themselves until their net benefits are equalized again, but they will no longer exhibit input matching (e.g. see Abrahams & Dill 1989). The total (direct and indirect) difference in cost between the habitats can be quantified in units of energy by calculating how much food must be added to the more costly habitat to equalize the animal's net benefits and return them to their original distribution.

Behavioural titration has been used to quantify costs associated with a number of different biotic factors, such as predation risk (Abrahams & Dill 1989; Nonacs & Dill 1990; Grand & Dill 1997; Brown & Kotler 2004) and competition (Abramsky *et al.* 2000), but few studies have used titration to quantify abiotic costs. Hughes & Grand (2000) partially addressed this gap by modelling the effect of varying water temperature and competition on salmonid habitat choice. Consistent with the effects of biotic conditions, their model predicted that fish should rarely exhibit input matching when abiotic conditions differ between habitats.

Pacific salmon (genus *Oncorhynchus*) provide a convenient system to evaluate behavioural titration as a means of quantifying the effects of different abiotic factors on habitat choice. Pacific salmon migrate from freshwater natal streams through estuaries composed of complex temperature and salinity gradients associated with different energetic costs. Foraging in areas that are more or less saline than the fish's internal osmotic state will result in reduced growth rates because of the increased metabolic costs associated with maintaining homeostasis (Morgan & Iwama 1991). A brackish habitat, on the other hand, will result in lower metabolic costs than salt water because it is osmotically neutral to the juvenile salmon's internal state (Morgan & Iwama 1998).

The costs paid by salmon in habitats of different salinities and temperatures also vary depending on the salmon's physiological state. For instance, if there is insufficient food in a warm habitat to maintain metabolic demands, the fish will have to move to a cooler habitat (Brett 1971; Elliott 1982; Hughes & Grand 2000). Variations in the fish's developmental stage will also affect energetic costs. The development of fry and parr into smolts is characterized by an increase in the activity of gill Na^+ , K^+ -ATPase, an enzyme that allows fish to eliminate excess salts through active ion transport (McCormick 2001; Marshall 2002). Fry and parr with low Na^+ , K^+ -ATPase activity pay higher energetic costs trying to maintain homeostasis in salt water than do smolts and ocean-going adults with high enzyme activity (McCormick *et al.* 1987; McCormick, Moyes & Ballantyne 1989).

We used behavioural titration to quantify the energetic costs of different water salinities and temperatures

to juvenile salmon and to test if these costs vary with the salmon's physiological state. Specifically, we test whether: (1) differences in energetic cost between habitats associated with salinity or temperature will result in the violation of the input-matching prediction of IFD (Hughes & Grand 2000); (2) as Na^+ , K^+ -ATPase activity increases, the energetic cost of foraging in fresh water will increase relative to salt water; and (3) fish will prefer cold to warm water habitats when foraging rate is limited.

Methods

We used a two-part experiment to quantify the energetic costs paid by juvenile Chinook Salmon (*Oncorhynchus tshawytscha*, Walbaum) in habitats that varied in either temperature or salinity. In part 1, groups of 10 salmon were free to choose between one of two habitats, each providing equal amounts of food and identical abiotic conditions. Under these conditions fish should demonstrate input matching (Fig. 1a). Fish were then allowed to choose between two habitats that differed in either salinity or temperature. Under these conditions a greater proportion of fish should choose the habitat that maximizes their net energetic gain, not necessarily their food intake rate (Fig. 1b). The amount of food each salmon was willing to forgo to feed in the preferred habitat (that chosen by the majority of fish) was used as a measure of the energetic cost of being in the other habitat.

In Part 2 of the experiment we calculated how much food should be moved from the preferred, to the less preferred, habitat to equalize net benefits (Fig. 1c). The return of the fish to an equal distribution would confirm the cost estimate. If the fish do not return to an equal distribution, then one of the assumptions of our test is probably invalid.

EXPERIMENTAL SET-UP

Experiments were conducted on young-of-the-year, Chinook Salmon (average fork length: salinity trials, 70.2 ± 8.2 mm; temperature trials, 88.7 ± 3.1 mm; average mass: salinity trials, 3.6 ± 1.3 g; temperature trials, 7.0 ± 0.8 g; means \pm SD) collected during April 2003 from Chilliwack Hatchery, Chilliwack, BC. Chinook were used in this study because they reside in the estuary longer than other species (Taylor 1990; Groot & Margolis 1991) and typically migrate through estuaries during the study period.

Fish were kept in outdoor, 2000-l flow-through tanks maintained under natural photoperiod and continuously supplied with air-equilibrated well water (salinity 0‰ (parts per thousand); temperature 12.0 ± 0.9 °C and dissolved oxygen $90.6 \pm 3.3\%$ air saturation). Fish were fed twice daily on a maintenance ration (2% biomass day^{-1}) of commercial salmon pellets (EWOS Canada, Surrey, British Columbia).

Each trial consisted of ten fish of similar body mass (average within group variation: 0.3 ± 0.0 g SD) and

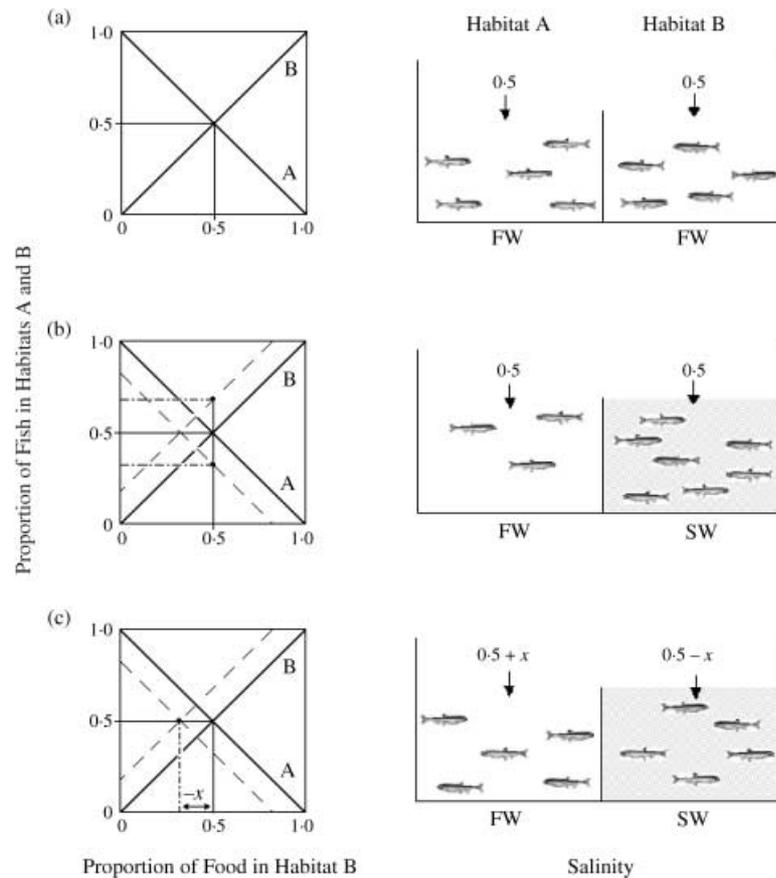


Fig. 1. Quantifying energetic cost in habitat A relative to habitat B. Lines A and B represent the proportions of juvenile salmon in habitats A and B as a function of the proportion of food in habitat B. (a) When habitats A and B are identical, fish should distribute themselves evenly between the two habitats (input matching). (b) When food distribution is kept constant but the cost associated with foraging in habitat A is greater than that in habitat B (dashed lines; in this case because salt water was added to habitat B), fish will redistribute themselves to equalize net benefits (dot-dash lines). (c) Food is then redistributed (proportion x shifted from B to A) such that the fish can only equalized net benefits by distributing themselves equally between the two habitats. The required proportion is determined from the fish distribution observed at step (b); see calculations in text. FW = fresh water, SW = salt water.

fork length (average within-group variation: 2.2 ± 1.3 mm SD). Different fish were used in each trial. Variation in body mass and length within trials was minimized to reduce competitive differences between fish. Trials were conducted in 75-l ($0.60 \times 0.30 \times 0.38$ m³) experimental aquaria divided into two equal-sized habitats by a 0.20 m \times 0.30 m clear Plexiglas™ divider (Fig. 2). The two habitats could be kept at either different temperatures or different salinities using a flow-through system. A 'bridge' of water across the divider was formed by creating a layer of warm or fresh water over the denser cold or salt water, respectively, allowing the fish to move between the two habitats.

Fish food pellets were delivered throughout the feeding component of the trials. The fish had about 20 s to capture the food before it passed through a grate at the bottom of the aquarium. To minimize defecation of the food, it was delivered to four different locations in each habitat. Aquaria were maintained on a 16:8 h light : dark cycle. Water quality characteristics during all phases of the study are given in Table 1.

PART 1: ESTIMATING THE ENERGETIC COST OF SALINITY AND TEMPERATURE

Salinity

Trials in part 1 of the salinity experiment consisted of three phases (acclimation, control and test) conducted over a 4-day period. During the 2-day acclimation phase both habitats contained fresh water and equal food was provided to both habitats for 3 h each day. On the third day (the control phase) conditions remained the same, but the number of fish in each habitat was recorded eight times every 20 min between 10.00 and 13.00. At the end of the third day test-phase conditions were created by gradually adding salt water of either 15‰ or 27‰ to one of the habitats. Fish were left overnight to acclimate and the following day their distribution was recorded in the same way as in the control phase. Fifty-six salinity trials were conducted over 11 weeks ($N = 34$ 27‰ salinity trials and $N = 22$ 15‰ salinity trials).

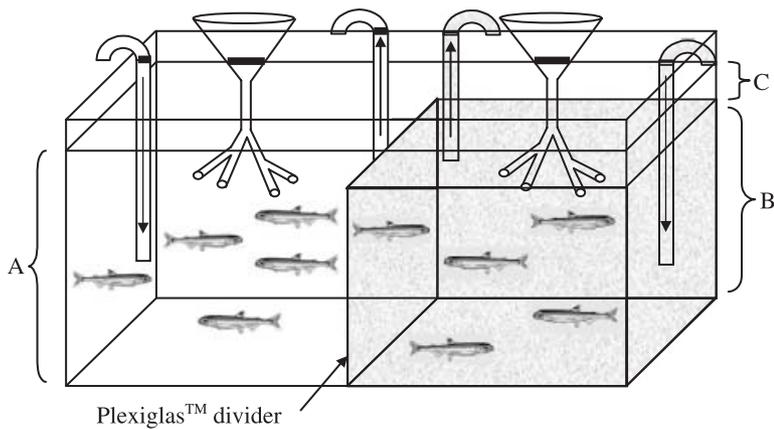


Fig. 2. Apparatus used for experiments 1 and 2. Habitats A and B are separated by a clear Plexiglas™ divider and differ in either salinity or temperature. The less dense water in habitat A (fresh water in salinity experiments and cold water in temperature experiments) overflowed the barrier forming a fresh water or cold water bridge (C) that the fish could use to move between habitats at any time. Temperature, salinity and oxygen concentrations were maintained using a duplex flow through the system (arrows indicate water flow) that replaced the water on either side of the apparatus. Feeders located on either side of the centre divider provided food pellets to both habitats.

Temperature

Trials in part 1 of the temperature experiments consisted of the same three phases as the salinity trials. To minimize temperature shock, the fish were introduced to the experimental aquaria when the temperature in both habitats was similar to the stock tank temperature (11.4 ± 0.3 °C; mean \pm SD). The acclimation and control phases followed the same procedures as in the salinity trials except that the acclimation phase was reduced to 1 day in an attempt to decrease territoriality. Over a 3-h period prior to the test phase the two habitats were chilled or heated gradually until they differed by approximately 4.8 °C. Once the habitats reached their experimental temperatures, temperature trials followed the same procedures as the salinity trials. Thirty-five temperature trials were conducted over a 7-week period. A maintenance ration (2% biomass day⁻¹) based on fish acclimated to 9 °C was delivered

to each habitat. As a result, food would have been somewhat limited in the warm water habitat where energetic needs are higher.

Estimating costs

The amount of food provided to each habitat (F) during the test-phase was the same, and therefore the availability of food to each fish in the alternate habitat (that chosen by the minority of fish; indexed by subscript A) was necessarily greater than in the preferred habitat (that chosen by the majority of fish; indexed by subscript P). We estimated the intake of fish in each as the total amount of food available, divided by the number of fish (N_A or N_P). The extra intake of fish in the alternate habitat (E) is:

$$E = F/N_A - F/N_P \quad \text{eqn 1}$$

On the assumption that the total net benefit to each individual is equalized, the higher intake in the alternate habitat is required to offset the salinity-related costs of foraging there. The energetic equivalent of these costs is estimated by the value of E (cf. Abrahams & Dill 1989). All estimates of E were translated into watts (W) by multiplying by the caloric content of the food (18 kJ g⁻¹).

PART 2: USING A BEHAVIOURAL TITRATION TO OFFSET COSTS

In part 2 of the experiment we test our cost estimate by using E to predict the amounts of food in the preferred and alternate habitats that would be required to equalize the number of fish in each habitat ($N_A = N_P = N$). The total amount of food in the system is $2F$. Call the redistributed amount of food in the alternate habitat F'_A ; the amount of food in the preferred habitat is $2F - F'_A$.

After redistribution, each individual in the alternate habitat has intake F'_A/N , but must expend E to offset the temperature- or salinity-related costs of foraging there. Each individual in the preferred habitat has intake

Table 1. Summary of water quality (means \pm SD) as a function of trial phase and habitat during salinity and temperature experiments. Bold numbers represent the habitat conditions that were changed between the acclimation/control phases and the test and titration phases

Trial Phase	Habitat	Salinity (‰)	DO ₂ (% air saturation)	Temperature (°C)	
Acclimation/Control 15‰ and 27‰	A	0.1 \pm 0.0	101.2 \pm 1.7	10.4 \pm 0.7	
	B	0.1 \pm 0.0	101.3 \pm 1.8	10.5 \pm 0.7	
Temperature	A	0.1 \pm 0.0	101.6 \pm 1.0	11.0 \pm 0.2	
	B	0.1 \pm 0.0	101.7 \pm 1.3	10.8 \pm 0.2	
Test/Titrations 1 & 2	15‰	A	0.4 \pm 0.7	100.7 \pm 2.1	10.5 \pm 0.7
		B	14.5 \pm 1.6	100.4 \pm 2.1	10.7 \pm 0.7
	27‰	A	0.6 \pm 0.9	101.3 \pm 2.0	10.5 \pm 0.7
		B	26.9 \pm 1.4	100.6 \pm 1.7	10.3 \pm 0.7
Temperature	A	0.1 \pm 0.0	100.4 \pm 1.6	14.0 \pm 0.5	
	B	0.1 \pm 0.0	100.1 \pm 1.1	9.3 \pm 0.6	

$(2F - F'_A)/N$. At equilibrium, the net benefit to each individual is assumed to be the same, and therefore:

$$(F'_A/N) - E = (2F - F'_A)/N \quad \text{eqn 2}$$

To find the food required in the alternate habitat to equalize the number of fish in each habitat we solve for F'_A , which yields:

$$F'_A = (N/4)(E) + F \quad \text{eqn 3}$$

Note that in contrast to Abrahams & Dill (1989) we have kept the total amount of food constant, and adjusted the proportion of food in the two habitats.

Each habitat received food amounts equal to F'_A and F'_p during the fifth (hereafter referred to as titration 1) and sixth (hereafter referred to as titration 2) days of the salinity trials, and the fourth and fifth days of the temperature trials. Fish distribution during these conditions was recorded using the same procedures as in phases 2 and 3 of part 1. The groups of fish used in part 2 were the same as those in part 1.

STATE VARIABLES

At the end of all temperature and salinity trials the fork length (FL; to the nearest 1.0 mm) and wet mass (M; to the nearest 0.1 g) of each fish was recorded. The condition factor (CF) of each fish was then calculated using the formula $CF = (M/FL^3) \times 100$ (McCormick & Naiman 1984).

At the end of all salinity trials we also measured gill Na^+ , K^+ -ATPase activity on crude gill homogenates following McCormick (1993). Gills were dissected from the fish (killed by a blow to the head), immediately frozen in liquid nitrogen and subsequently stored at -70°C . ATPase activity was normalized to total homogenate [protein] (measured using the bicinchoninic acid method with bovine serum albumin standards; Sigma-Aldrich Canada Ltd., Oakville, Ontario). All samples were run in triplicate and the coefficients of variation were $\leq 10\%$. Ouabain-sensitive ATPase activity is expressed as micromoles of ADP per milligram of protein per hour.

Three days in salt water should not result in a significant change in Na^+ , K^+ -ATPase activity (Quinn, Veillette & Young 2003). Consequently, we considered individual differences in Na^+ , K^+ -ATPase activity to be a predictive measure of fish habitat preference rather than a consequence of habitat preference.

DATA ANALYSIS

Data were analysed using two statistical techniques. Wilcoxon signed-rank tests were used to determine if changes in experimental conditions altered the distribution of fish between habitats. Forward step-wise regression was used to assess the relationship between changes in the physiological state of the salmon and their energetic costs.

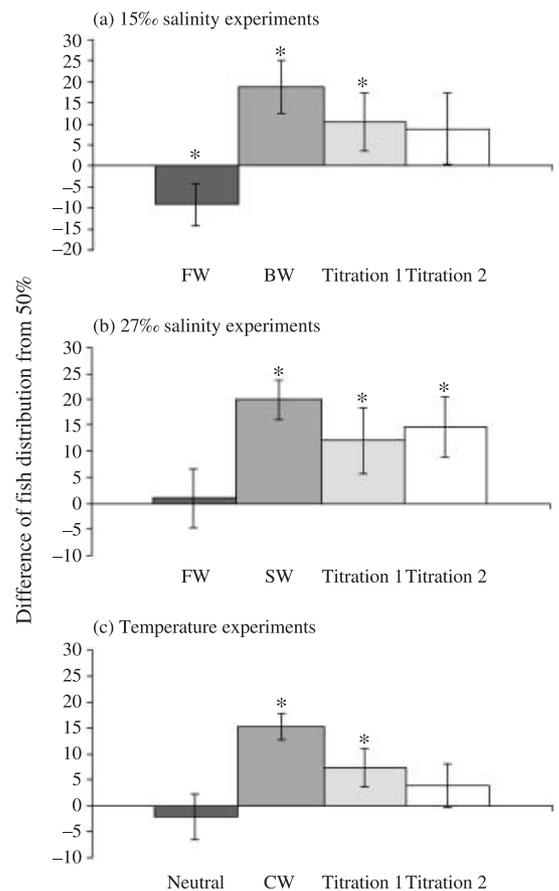


Fig. 3. The proportion of juvenile salmon above or below 50% that resided in the preferred habitat during control, test, titration 1 and titration 2 phases. (a) Distribution of fish during 15‰ salinity experiments. (b) Distribution of fish during 27‰ salinity experiments. (c) Distribution of fish during temperature experiments. FW = equal food, both habitats are fresh water; BW = equal food, preferred habitat is brackish water, the alternative habitat is fresh water; SW = equal food, the preferred habitat is salt water, the alternative habitat is fresh water; neutral = equal food, both habitats are at 10.8°C ; CW = equal food, the preferred habitat is 9.3°C and the alternative is 14.0°C . Titrations 1 and 2 = food is redistributed between habitats to offset the cost of the alternative habitat. Bars give the 95% confidence interval. * indicates significantly different from 50%, the predicted value.

Results

PART 1: THE ENERGETIC COST OF SALINITY AND TEMPERATURE

Control phase

During 27‰ salinity and temperature experiments, when conditions in the two habitats were identical, fish distributed themselves equally between the two feeders (27‰ salinity: $z = 0.4$, $N = 34$, $P = 0.732$, Fig. 3b; temperature: $z = 0.7$, $N = 35$, $P = 0.502$, Fig. 3c). However, during identical conditions in 15‰ salinity experiments fish demonstrated a 6:4 preference for the habitats that would remain at 0‰ during the test-phase ($z = 2.8$, $N = 22$, $P = 0.005$).

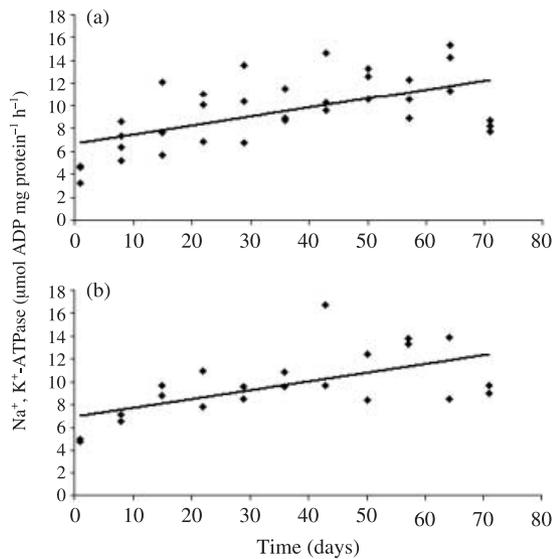


Fig. 4. Change in average gill Na⁺, K⁺-ATPase activity per trial as a function of time during (a) 27‰ salinity experiments and (b) 15‰ salinity experiments. Solid lines represent regression lines with $R^2 = 0.341$ and $R^2 = 0.351$, respectively.

Effects of different salinities

When food was distributed equally between the two habitats, but habitats differed in salinity, fish preferred the more saline habitats (0‰ vs 15‰ salinity: $z = 3.815$, $N = 22$, $P < 0.001$, Fig. 3a; 0‰ vs 27‰ salinity: $z = 3.2$, $N = 34$, $P = 0.002$, Fig. 3b). The energetic cost of feeding in the alternate habitat was $0.11 \text{ W} \pm 0.02 \text{ SE}$ in 27‰ trials, and $0.15 \text{ W} \pm 0.03 \text{ SE}$ in 15‰. A comparison of fish movement in the 15‰ vs 27‰ salinity experiments demonstrated that a greater proportion of fish switched from the fresh water feeder to the feeder in 15‰ salinity (brackish water) than from the fresh water feeder to the feeder in 27‰ ($F_{1,54} = 5.6$, $P = 0.022$).

Results from the forward stepwise regressions indicated that the proportion of fish choosing to feed in 27‰ salinity was best explained by a negative effect of body mass and a positive effect of Na⁺, K⁺-ATPase activity ($F_{2,31} = 8.5$, $P = 0.001$). The regression coefficient for body mass was -0.009 , suggesting that differences in body mass were small. None of the variables were correlated with the proportion of fish choosing to feed in 15‰ salinity.

Gill Na⁺, K⁺-ATPase activity increased throughout the course of both the 27‰ ($F_{1,32} = 16.6$, $P < 0.001$) and 15‰ ($F_{1,20} = 10.8$, $P = 0.004$) salinity experiments (Fig. 4). Increased Na⁺, K⁺-ATPase activity was positively correlated with preference for salt water during 27‰ salinity trials (Fig. 5a: $F_{1,32} = 10.2$, $P = 0.003$), however, there was no relationship between Na⁺, K⁺-ATPase activity and preference for 15‰ in 15‰ salinity experiments (Fig. 5b: $F_{1,20} = -0.1$, $P = 0.784$). This indicates that preference for brackish water is constant over time, but that preference for salt over fresh water increased as a function of the fish's developmental stage.

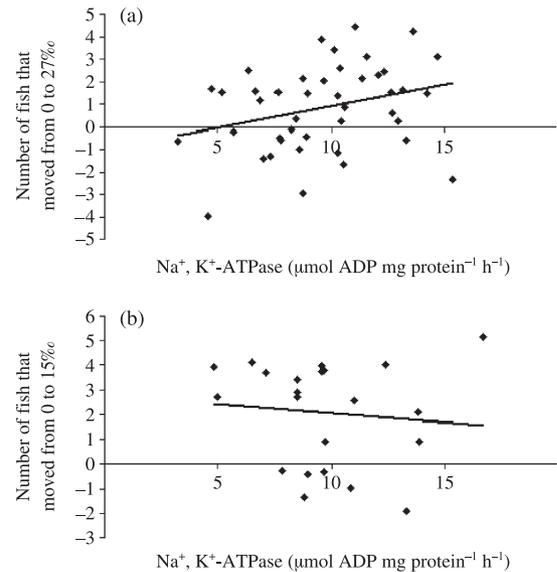


Fig. 5. Relationship between average gill Na⁺, K⁺-ATPase activity per trial and the number of juvenile salmon that moved from fresh water to either 27‰ salinity (a) or 15‰ salinity (b) during the trial phase. The relationship (regression line) is positive, and significant, for 27‰ trials ($P = 0.003$), but insignificant for 15‰ trials ($P = 0.784$).

Effects of different temperatures

When food was distributed evenly between cold and warm water habitats fish preferred the cold water habitat (Fig. 3c; $z = 3.4$, $N = 35$, $P = 0.001$). The energetic cost of feeding in the alternate habitat was $0.09 \text{ W} \pm 0.01 \text{ SE}$. Forward stepwise regression analysis indicated that the proportion of fish foraging in cold water habitat was best predicted by condition factor, but this relationship was not significant ($F_{1,32} = 2.8$, $P = 0.103$).

PART 2: OFFSETTING THE ENERGETIC COST OF SALINITY AND TEMPERATURE

Equation 3 was used to calculate how food should be redistributed to equalize the net energetic benefits between habitats. Owing to the increase in body size over the course of the experiments food ration increased with time. To account for this increase, food availability in the two habitats was expressed using proportions.

We calculated that to offset the high energetic costs associated with foraging in the alternate habitat during the 15‰ and 27‰ salinity experiments, $74.2 \pm 7.5\%$ and $69.7 \pm 5.4\%$ (mean \pm SE) of the food should be delivered to these habitats, respectively. In the temperature experiment $68.5 \pm 2.0\%$ of the food should be in the alternate habitat to offset the increased energetic cost. These proportions did not differ among the three experiments (ANOVA: $F_{2,88} = 0.3$, $P = 0.728$).

The addition of food to the alternate habitat during titration 1 resulted in an increase in the number of fish foraging there, but the fish did not reach an equal distribution (15‰ experiments: $z = -2.1$, Fig. 3a; $N = 22$,

$P = 0.023$, Fig. 3b; 27‰ experiments: $t = -3.4$, $N = 34$, $P = 0.001$; temperature experiments: $t = -3.2$, $N = 35$, $P = 0.001$, Fig. 3c). During titration 2 the addition of food to the alternative habitat did result in fish moving to an equal distribution in 15‰ salinity and temperature experiments (15‰ experiments: $z = 1.6$, $P = 0.101$, Fig. 3a; temperature experiments: $z = -1.7$, $N = 35$, $P = 0.090$, Fig. 3c), but not in the 27‰ experiments (Fig. 3b $z = -4.0$, $N = 34$, $P < 0.001$).

Discussion

When given a choice between two identical habitats juvenile salmon distributed themselves in a pattern consistent with the input-matching prediction of IFD. However, when salinities or temperatures differed between habitats, fish distribution no longer matched food availability, but instead revealed a preference for more saline conditions in salinity experiments and colder conditions in temperature experiments. These results indicate that energetic costs differed between different salinities and temperatures and that these differences resulted in the violation of the simple input-matching predictions of IFD. Further, in two of three cases we were able to predict the amount of additional food necessary to induce fish to pay the higher costs associated with the less preferred habitat and return them to an equal distribution, thus demonstrating that the costs associated with different salinities and temperatures can be estimated in units of energy equivalents using behavioural titration.

Previous studies that have attempted to understand habitat preference of juvenile salmon in estuaries have considered the effect of distance from shore and water velocity, and how the stress of adapting to rising salinity may result in increased vulnerability to predation (Järvi 1989; Handeland *et al.* 1996). However, even though juvenile salmon in estuaries are often confronted with changes in both temperature and salinity, little attention has been paid to temperature or to differences in costs associated with salinity and temperature. By defining all costs in common units of energy we can compare and contrast cost estimates for different salinities and temperatures and thus hope to obtain a better understanding of the influence of these factors on salmonid habitat choice. Our comparison of energetic cost equivalents demonstrates that the cost paid by fish exposed to different salinities and temperatures are similar. This suggests that when a smolt is exposed to habitats that differ in salinity and temperature, but have similar, but limited, food availability, fish should prefer cooler and more saline habitats. However, if food abundance is increased by 50–60% in the warmer or freshwater habitats, fish should prefer these habitats to the colder and/or more saline ones.

The energetic costs experienced by an animal will not necessarily be constant over time or between individuals because energetic costs can be influenced by changes in the animal's developmental or physiological

state (Abrahams & Dill 1989; Morgan & Iwama 1998). Much of the discrepancy in metabolic cost estimates associated with osmoregulation is thought to be the result of not considering differences in the physiological condition of the fish being studied (Morgan & Iwama 1998; Swanson 1998), such as stage of smoltification (Otto & McInerney 1970; Morgan & Iwama 1991, 1998) and body size (Macdonald & Levings 1988; Levings *et al.* 1989). Results from our salinity experiment revealed a positive relationship between changes in the fish's developmental stage and preference for 27‰ salinity over fresh water. In contrast, and consistent with our predictions, there was no relationship between developmental stage and energetic costs during the brackish water trials. The salinity concentration of a salmon's internal fluid is similar to that of brackish water and therefore fish residing in a brackish habitat can dedicate less energy to maintaining homeostasis than if it were in a more or less saline habitat (Glova & McInerney 1977; Febry & Lutz 1987; Morgan & Iwama 1998). As a result, fish should always prefer brackish water to both fresh and salt water.

In our temperature experiments we used water temperatures and food rations similar to those modelled by Hughes & Grand (2000). Our results supported their prediction that salmon should prefer the colder water habitat. This preference for lower temperatures is not consistent with the 12–14 °C preference range found in laboratory studies when food is unlimited (Brett 1971). However, it does fall within the 7–12 °C preference range observed in field studies when food is unpredictable (Belvelhimer & Adams 1993; Orsi & Wertheimer 1995; Hinke *et al.* 2005). Such wide variation in temperature preferences is expected because of the relationship between food availability and metabolic rate: fish should not forage in warm water if the food available there is insufficient to meet their metabolic demands (Elliott 1976, 1982). Therefore, if a salmon has a choice between habitats that differ in temperature it should choose the habitat in which it can maximize its net energetic gain, not necessarily its metabolic rate.

A number of studies have successfully predicted the distribution of fish based on IFD theory predictions (e.g. Tyler & Gilliam 1995; Grand & Dill 1997; Giannico & Healey 1999), and some have demonstrated that energetic costs can be offset if sufficient food is added to the more costly habitat (e.g. Abrahams & Dill 1989; Grand & Dill 1997). However, there have also been a number of studies in which the distribution of fish has deviated from IFD predictions (e.g. Godin & Keenleyside 1984; Talbot & Kramer 1985; Sargent, Gross & van den Berghe 1986). When we tried to offset freshwater costs during the 27‰ salinity experiments by increasing the amount of food in the fresh water habitat, fish did not return to an equal distribution. This is probably because our experimental system failed to meet one, or both, assumptions of IFD theory. If the fish failed to perceive the true costs or benefits of either habitat (i.e. fish were not ideal) (Spencer,

Kennedy & Gray 1995; Cartar & Abrahams 1997; Hakoyama & Iguchi 1997), or competition between fish increased costs or decreased benefits (i.e. fish were not free), then our estimate of E would be incorrect.

Interestingly, only the 27‰ salinity experiments did not titrate back to an even distribution, suggesting that the fish's perception of costs or benefits, or competitive differences, were a function of differences in the concentration of salt in the two habitats. We observed a disruption of neutral buoyancy when a fish changed habitats (in either direction) during salinity experiments, and this was significantly more pronounced in 27‰ than 15‰ trials. Fish compensate for negative buoyancy by increasing upward propulsion with their pectoral fins, a behaviour referred to as compensatory swimming (Harvey & Bothern 1972). Compensatory swimming will result in increased energetic costs until the fish adjusts its swim bladder volume to compensate for the change in water density (Alexander 1972). In addition to these direct energetic costs, the loss of neutral buoyancy may have put fish at a competitive disadvantage compared with fish already acclimated to the habitat. The combination of these costs could have caused the fish to spend less time in the alternate habitat, leading to an incorrect perception of net energetic gain there. Though changes in buoyancy also may have affected the costs of fish in the 15‰ salinity experiments, this effect would have been smaller due to the smaller difference in salinity.

A sudden spike in costs following the transition between salinities would have made it more difficult for us to correctly estimate the amount of food necessary to titrate the fish back to an even distribution. The food we added may have been sufficient to compensate for increased costs following acclimation to the change in salinity, but insufficient to account for the initial, short-term increase in costs following transition to salt water.

The current study has demonstrated that behavioural titration can be used as a tool to quantify abiotic costs, but can be misleading if the assumptions of IFD are not met. Fortunately, titrations have the additional benefit of testing the accuracy of the cost estimate. In situations where the estimate is deemed inaccurate, such as was the case in the 27‰ experiments, it may be possible to identify previously unknown costs. This study also demonstrates the importance of considering both salinity and temperature when assessing habitat quality for salmon, and how the costs and benefits associated with these factors will change depending on the fish's developmental stage and food availability. This knowledge should improve our ability to predict salmon distribution, abundance and behaviour and to understand how these factors will change as the result of natural and anthropogenic changes to their environment.

Acknowledgements

We would like to thank J. Korstrom and I. Birtwell for providing us with access to facilities at the West Van-

couver, Fisheries and Oceans Canada laboratory, P. Walton for her assistance with data collection, J. Richard and H. Jarrard for their assistance with the gill Na^+ , K^+ -ATPase analysis, and B. Gurd, S. Gray, C. Kennedy and R. Ydenberg for comments on an earlier version of the MS. This work was funded by PADI A.W.A.R.E. Foundation, Association of Professional Biologists of British Columbia, Simon Fraser University, and National Science and Engineering Research Council (NSERC) Canada scholarships to S.J.W., and NSERC operating (A6869) and Watershed Watch grants to L.M.D. The experiments conducted in this study comply with the regulations of the Canadian Council for Animal Care (permit 649B-02).

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