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SHORT COMMUNICATION

Can smolting be assessed by food intake in steelhead trout, *Oncorhynchus mykiss* (Walbaum)?

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Salmonid smolts under cultivation must often be able to tolerate abrupt transfer from freshwater (FW) to seawater (SW). Traditionally, smolt readiness for SW transfer has been estimated using terminal methods. In view of the increasing regard for animal welfare it would be desirable to develop a reliable, non-lethal method for assessing smolt readiness in hatcheries and for research.

One potential non-lethal smolt index could be the measurement of changes in food intake. In Atlantic salmon, *Salmo salar* L., only some individuals of a population are able to feed soon after transfer to sea (Usher, Talbot & Eddy 1991; Arnesen, Johnsen, Mortensen & Jobling 1998), likely those individuals that have a better capacity to osmoregulate in SW. However, earlier studies did not reveal a correlation between feed intake and physiological parameters in SW (Damsgård & Arnesen 1998; Arnesen *et al.* 1998). Nobody has heretofore attempted to correlate feed intake and smolting physiology in FW, or feed intake in FW and physiology in SW. The purpose of the present experiment was to monitor whether feed intake could be used as an indicator of smolting with respect to physiology in steelhead trout, *Oncorhynchus mykiss* (Walbaum), during the spring.

The experiment was carried out at the Hatfield Marine Science Center (Newport, OR, USA), Oregon State University, between 14 April and 22 June 1999. The fish were 1+ steelhead from Eagle Creek (WA) strain, offspring of wild parents. Fish were held in two holding tanks with single pass, flowthrough dechlorinated (by activated carbon) tap water. Each of the five separate experiments (referred to as runs) consisted of a 10-day acclimation period of 30 fish in each of the six experimental tanks (1 m in diameter, water depth 0.33 m) with a water flow of 6 L min^{-1}. The sampling days for each run are given in Table 1. The fish weight ranged from 50.2 to 149.8 g during the first run in mid-April and from 88.8 to 311.3 g during the last run in mid-June. On the 10th day after acclimation for each run, three tanks were switched to running UV-sterilized SW. Maximum salinities (Fig. 1) were achieved in 2 h. Salinity and temperature were measured using a YSI model 30 (YSI, Yellow Springs, OH, USA). Because of the differences in oceanic upwelling, the temperature and salinity varied between runs; the temperature of FW also rose between spring and summer (Fig. 1). The fish were fed by hand twice daily according to a hatchery feeding table.

Sampling was accomplished by netting 15 fish from each tank as quickly as possible into buffered ($NaHCO_3$) tricainemethanesulphonate (MS-222, 200 mg L^{-1}). SW-exposed fish were sampled 6 h after SW exposure started. FW control tanks were sampled immediately before the SW tanks. After anaesthetization, fish were dried, weighed (to 0.1 g) and measured (total length to 1 mm). A blood sample was then withdrawn from the caudal vessel via a vacutainer. Plasma was separated and stored at − 80 °C for later analysis of sodium, potassium (NOVA 1 Na^+, K^+ analyser, Nova biomedical, Waltham, MA, USA) and cortisol. The plasma cortisol concentration was analysed in duplicate by radioimmuno-assay

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Table 1 Linear correlations between feed intake and condition factor (*K*), plasma sodium and muscle water at five different runs in FW and SW sampled at 6 and 96 h after SW entry

		K					Plasma sodium					Muscle water				
		Run					Run					Run				
	Time (h)	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
FW	6					+										
FW	96			+	+				+					−		
SW	6										+					
SW	96															

Fish were fed the marker feed at 0 h (in FW) and at 96 h. $N = 15$ in each tank.
FW, freshwater; SW, seawater.
+ = 2 tanks of three replicated tanks showed a significant positive correlation.
− = 2 tanks of three replicated tanks showed a significant negative correlation.
Run 1: 15 and 19 April, run 2: 30 April and 4 May, run 3: 14 and 18 May, run 4: 28 May and 1 June, run 5: 18 and 22 June for 6 and 96 h respectively.

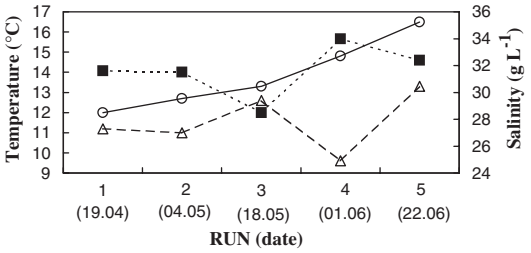

Figure 1 Temperatures (○ , freshwater; △, seawater; left *y*-axis) and salinity (■ , right; *y*-axis) at the termination (at 96 h) of each of the five runs.

according to Redding, Schreck, Birks & Ewing (1984) from seven fish from each tank, except for the first run, where smaller numbers of samples were available from most tanks because of small sample volumes. A small sample of dorsal muscle was excised and dried at 60 °C to a constant weight in a tarred microtube for determination of moisture. This sampling procedure was repeated for the remaining fish in each tank 96 h later. The condition factor (*K*) was calculated as $100W * L^{-3}$, where *W* is the weight in g and *L* is the total length in cm.

The feed was a commercial dry feed (BIO DRY 1000 by Bio-Oregon, Warrenton, OR, USA). The experimental diet containing X-ray dense lead glass beads (ballotinis) was made as described in Pirhonen, Schreck & Gannam (2000). On the 10th day of the acclimation period, the fish were fed in excess in the morning with the X-ray dense feed, and then three of the tanks had their inflow water switched to SW. After sampling, carcasses were X-rayed and the number of ballotinis in each fish was calculated for estimation of the amount of food eaten. Over the following 96 h, the experimental fish were deprived of food, and then were fed the X-ray dense feed in excess again.

Statistical differences between the fish in SW and FW, and between 6 and 96 h were tested using a nested ANOVA model, where individuals within the tank were nested under each treatment. The possible correlation between feed intake and other variables was estimatedusing a linear regression model at an individual level within each tank. The relationship was regarded as meaningful if fish in at least two out of three replicated tanks showed a significant correlation in the same direction and the correlation in the third replicate was insignificant.

During the first runs, two fish died during the SW exposure, one individual in one of the FW tanks during the second run was moribund at 96 h and during the third run three fish died during the fasting period in FW tanks. Scale loss was observed in many individuals, especially during the second and third run. No fish died in larger holding tanks or in any of the test tanks at any other time during the experiment. The condition factor (*K*) was relatively stable, varying between 1.0 and 1.1; however, *K* was significantly lower during run 3 than run 2.

The feed intake in FW during the morning feeding at time 0 (immediately before the change to SW) was relatively low (group means 0.06–0.48% body weight) during the first three runs, but higher during the last two runs (0.26–0.91% body weight) (Fig. 2a). Fish in SW always ate significantly less than fish in FW at 96 h (Fig. 2b); the average percentages of FI of SW fish compared with FW fish were 45%, 34%, 51%, 13% and 9% for the five runs respectively. In terms of osmoregulatory capacity, the population appeared to

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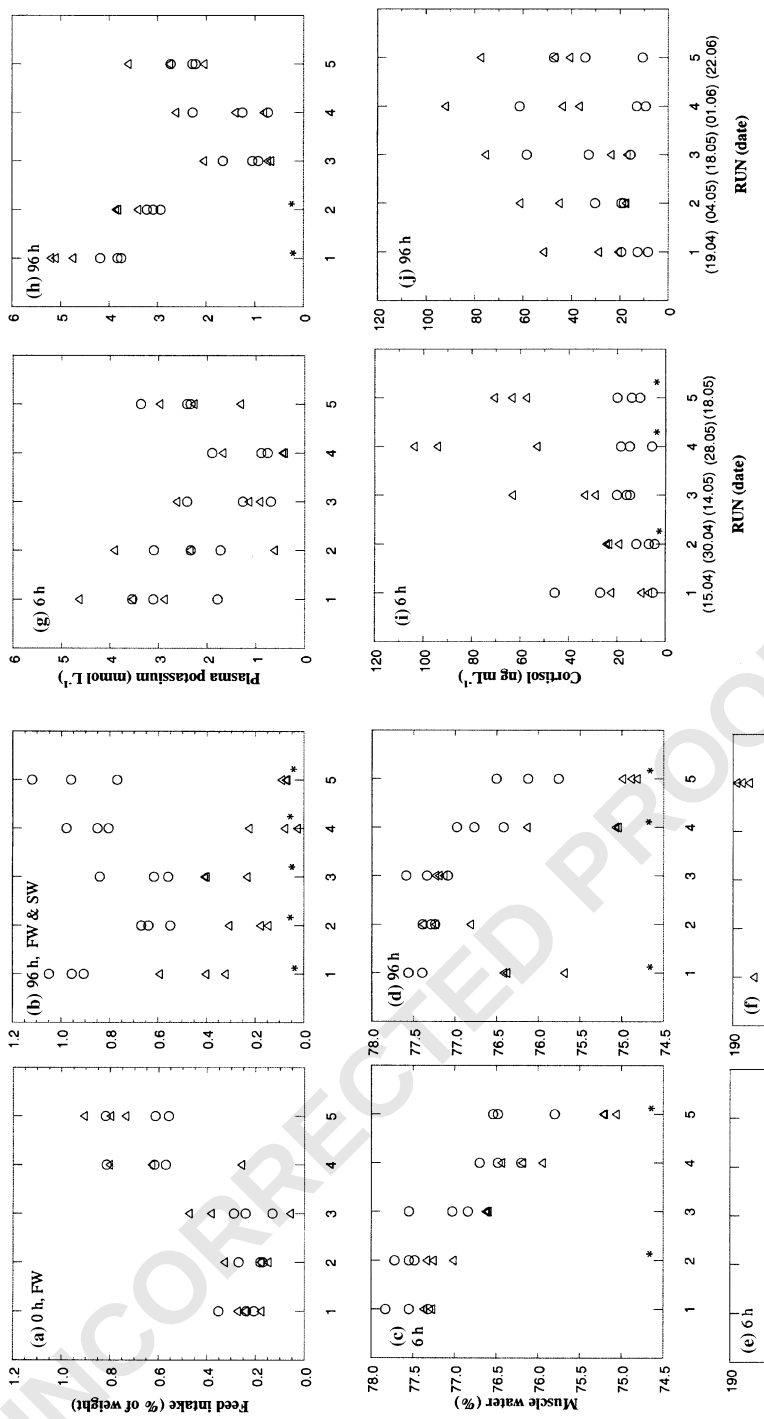

Figure 2 Feed intake (% of body weight per feeding; a and b), muscle water content (c and d), plasma sodium concentration (e and f), plasma potassium concentration (g and h) and plasma cortisol concentration (i and j) during each of the five runs in FW (○) and SW (△), at 6 and 96 h after the switch to SW. The first feeding was at 0 h (a). Data are shown as a mean for each replicated tank: ○, freshwater; △, seawater. Statistical differences between means ($P < 0.05$) between FW and SW are shown with an asterisk.

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be best smolted in mid-May (third run; Fig. 2c–h), which is also supported by the drop in *K*. In general, plasma cortisol concentrations were relatively steady through-out the experiment and also quite low (usually below 20 ng mL^{-1}) in FW fish, even though occasionally higher mean values were recorded, especially in the 96-h sample (Fig. 2i–j).

We found little evidence that feed intake could be correlated with the osmoregulatory ability or plasma cortisol concentrations. The only time when FI was correlated with other measured parameters in SW was the 6-h sample during the fifth run, when a positive correlation between FI in FW and plasma sodium was found in two of the SW groups (Table 1). For fish in FW, we found significant positive correlations between FI and plasma sodium (third run at 96 h) and the condition factor (fifth run at 6 h, fourth run at 96 h) and a negative correlation between feed intake and muscle moisture (third run) (Table 1). We did not find any evidence of a relationship between cortisol concentrations and food consumption in SW or FW.

There was some variation in salinity and temperature between different runs, and also a difference in temperature between SW and FW within each run. Water temperature is a critical factor affecting the food intake and growth of fish (e.g. Jobling 1993), and temperature rise during the experiment was most likely one of the causes of increased feed intake in FW. However, based on the feed intake model of Baltic salmon (*S. salar*) (Koskela, Pirhonen & Jobling 1997), which takes into account both fish size and temperature, it is probable that the 3.5 °C temperature rise in FW between the third and fifth run had minimal ($< 10\%$) effect on the rise of feed intake. The variations in salinity between different runs may also have affected feed intake; however, oncorhynchids are relatively tolerant to changes in salinity (Hoar 1988). For example, McKay & Gjerde (1985) found no significant difference in the growth of juvenile rainbow trout (*O. mykiss*) held at salinities of 24, 28 and 32 g L^{-1}, or in appetite between salinities 10, 20 and 32 g L^{-1}. Based on earlier observations of the effect of salinity on physiology (Handeland, Berge, Björnsson & Stefansson 1998), we assume that the fluctuation in environmental parameters had only a slight, if any, influence on our results.

At each run, there was a significant difference in feed intake between fish in SW and FW. Even if good smolts, as judged by physiological measurements, the decrease in feed intake appears to be an inevitable response of the steelhead after transfer to SW, which has also been reported for salmon (Usher *et al.* 1991; Arnesen *et al.* 1998).

An indication of a correlation between feed intake and osmoregulatory ability in SW was observed during the fifth run; when fish were fed in FW and sampled 6 h later in SW, a positive correlation between feed intake and plasma sodium concentrations in two out of three replicated tanks was found. The plasma sodium concentration decreases and muscle water content increases in smolts in FW (Virtanen 1987); thus physiological parameters (sodium and muscle water) correlating with feed intake in FW during the third run of our experiment can be linked with smolting; i.e., those individuals possibly experiencing greatest ionic imbalance in FW were most reluctant to feed there. However, we could not demonstrate an increase in muscle water or sodium loss in FW at the population level. Regardless of some correlations between feed intake and some of the physiological parameters, we conclude that feed intake has limited value as an indicator of smolting in steelhead trout. This likely means that the suppression of feed intake after SW entry is dictated by some physiological process other than osmoregulation. It is possible that the positive correlations between feed intake and condition factor after the 96 h fasting in FW are linked to smolting, indicating the reluctance of smolts to eat in FW, because smolting fish become streamlined and typically, a decrease in the condition factor is observed (Boeuf 1993). In addition, the absence of correlation between feed intake and cortisol concentrations suggests that feeding rates were unaffected by cortisol in our experiment, a result that is contradictory to that of Gregory & Wood (1999).

Physiological changes observed during the experiment were usually typical for smolts, except that we did not find a clear increase in plasma cortisol in FW fish during the experiment, which is often evident in smolts (Specker 1982). The restoration of the muscle water balance in our fish in SW was quite rapid compared with the results of Handeland *et al.* (1998), who reported that Atlantic salmon smolts regain water balance at 8 °C only after 14 days. Our values for potassium were relatively low compared with the concentrations reported elsewhere (Houston 1959; Folmar & Dickhoff 1981), even though low average values for post-smolts have also been reported (Holmes & Steiner 1966).

Interestingly, we also observed a negative correlation at 96 h between plasma sodium and muscle water in fish in SW during the runs 1, 2, 4 and 5 (in all three tanks, except in two tanks during the second

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run), but only at 96 h during the third run in FW (all three tanks). In most cases, the correlation between muscle water and plasma sodium was very significant ($P < 0.001$) and the coefficient of determination was at least moderate (range 0.47–0.93 in SW and 0.32–0.86 in FW). This correlation in SW can be interpreted as an indication of incomplete smolting, because it can be supposed that the more the fish are dehydrating during SW exposure, the more they have to drink and consequently, the concentrations of sodium should be higher due to incomplete osmoregulatory ability. The negative correlation between muscle water content and plasma sodium concentrations during the third run in FW is likely attributable to the tendency for increased ionic losses and increased water gain in FW during the smolting period, as suggested by Virtanen (1987).

In conclusion, correlations of feed intake with the condition factor, muscle water or plasma sodium indicate that the more imbalanced a fish's physiology in FW is, or the lower the condition factor is, the less food the fish will eat. However, the lack of a consistent relationship between FI and physiological parameters implies that FI is not a reliable indicator of smolting in steelhead trout.

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