Future migratory behaviour predicted from premigratory levels of gill Na$^+$/K$^+$-ATPase activity in individual wild brown trout (*Salmo trutta*)

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Summary

The relationship between premigratory gill Na$^+$/K$^+$-ATPase activity, determined at two dates during spring, and future migratory behaviour was investigated using non-lethal gill biopsies and PIT-tagging in wild brown trout (*Salmo trutta*) from two tributaries. No significant relationship between future migratory strategy (individuals eventually becoming migrants or residents) and gill Na$^+$/K$^+$-ATPase activity was found in late February–early March. By contrast, in mid-April, a highly significant logistic regression equation identifying the migratory strategy in 93% of the 75 individuals was obtained. The ability of this regression model from the tributaries to predict future migratory behaviour in an independent group of trout caught in early April in the mainstream was evaluated. A threshold probability of migration was used to predict the behaviour of the mainstream individuals as either future migrants or residents. The maximum percentage of correct predictions of future migratory behaviour in mainstream fish was observed at threshold probabilities between approximately 0.15 and 0.45 (corresponding to threshold gill Na$^+$/K$^+$-ATPase activities between 2.7 and 3.7 μmol ADP mg^{-1} protein h^{-1}), with an average of 91% of the predictions being correct. The present study shows that a non-lethal premigratory biochemical measurement can successfully select individual brown trout with high probability of migration.

Key words: brown trout, *Salmo trutta*, migration, prediction of migratory behaviour, gill, Na$^+$/K$^+$-ATPase.

Introduction

The brown trout, *Salmo trutta* L., is a widespread European salmonid with a great diversity in life history strategy compared with other more obligatory anadromous salmonid species (L'Abée-Lund et al., 1989). In wild populations of brown trout within coastal river systems, freshwater (FW) residents as well as sea-run individuals may develop within the same genetic population (L'Abée-Lund et al., 1989; Hindar et al., 1991). This phenotypic divergence becomes obvious during springtime where the population differentiates into migratory and FW-resident fractions. Before entering the sea, the migrating individuals undergo extensive morphological, physiological and behavioural changes, termed smoltification, in preparation for life in the marine environment (McCormick and Saunders, 1987). The smoltification process is regulated by changes in environmental factors, such as light and temperature (Björnsson et al., 1989; McCormick et al., 1995), and is mediated and integrated by the endocrine system (Hoar, 1988). As part of the process of maintaining homeostasis in seawater (SW), teleosts actively excrete ions across the gill epithelium, and the activity of the membrane-spanning enzyme Na$^+$/K$^+$-ATPase in the gills creates an electrochemical gradient needed for this process (Marshall, 1995).

The existing knowledge of smolt physiology and migration in several species is primarily based on comparisons of migrants and residents at the population level, with residents having lower average gill Na$^+$/K$^+$-ATPase activity than migrants at the time of migration (Hart et al., 1981; Zaugg, 1981; Ewing et al., 1984; Rodgers et al., 1987; McCormick and Björnsson, 1994; Ewing and Rodgers, 1998).

The aims of the present study were (1) to investigate the potential of regression analysis for describing the relationship between premigratory gill Na$^+$/K$^+$-ATPase activity, measured at two dates during spring, and future migratory behaviour of individual wild brown trout and (2) to evaluate the ability of the regression equation obtained for trout caught in two tributaries to predict future migratory behaviour in an independent group of trout caught in a distinct location in the mainstream. This was done by repeatedly measuring gill Na$^+$/K$^+$-ATPase activity in both descending (i.e. caught in a trap) and non-descending (caught by upstream electro-fishing) individuals of passive integrated transponder (PIT)-tagged wild brown trout before the smolt run in a large Danish river system.

Materials and methods
Study area

The study was conducted in River Lille Aa, River Granslev Aa and Haldum Brook. River Granslev Aa and Haldum Brook are tributaries to River Lille Aa. The distance between the outlets of Haldum Brook and River Granslev Aa into the River Lille Aa is approximately 25 km. In the present study, individuals from Haldum Brook and River Granslev Aa are treated as one group originating from tributaries, while individuals from River Lille Aa are referred to as mainstream trout. Descending fish were caught in two traps placed in connection with a weir approximately 2 km downstream from the outlet of River Granslev Aa. The traps were operating from late February till early June and were emptied on a daily basis. Tagging and initial sampling in the two tributaries was conducted on 29 February and 1 March 2000.

Capture, tagging and sampling

Tributaries

Electro-fishing (1000 W pulsed DC-current) was conducted over a stretch of 3 km of Haldum Brook and River Granslev Aa on 29 February 2000 and 1 March 2000, respectively. The captured wild brown trout were size selected, and 200 fish between 11.3 cm and 18.4 cm were chosen in both of the tributaries. The fish were anaesthetised in a 5 mg l^{-1} methomidate (Marnil TM, Wildlife labs, Inc., Fort Collins, USA) solution and the total length (L_T) recorded to the nearest millimetre. Each fish was implanted peritoneally with a PIT tag. Just prior to the implantation of the PIT tags, while the fish was under anaesthesia, a small gill biopsy (four or five tips of gill filaments) was removed from the first gill arch of each fish and frozen in SEI buffer (300 mmol l^{-1} sucrose, 50 mmol l^{-1} imidazole, 20 mmol l^{-1} Na-EDTA, pH 7.3) using a non-lethal gill biopsy method (McCormick, 1993). This method is reported to have no effect on gill Na$^+$/K$^+$-ATPase activity (Rodgers et al., 1987) or the subsequent growth and survival of the fish (McCormick, 1993). The tagging and sampling procedure was conducted at the river banks of the tributaries, and prior to release the fish were allowed to recover at the banks in tanks supplied with freshwater from the rivers. On 11 April 2000 (Haldum Brook) and 12 April 2000 (River Granslev Aa), a second round of electro-fishing was conducted over the same stretches of the tributaries as during the first sampling. All caught wild trout were anaesthetised and examined for PIT tags. A small gill biopsy was taken from the other first gill arch of each PIT-tagged fish and the L_T was measured. Fish were allowed to recover as described above and were subsequently released.

Mainstream

Electro-fishing was conducted on 6 April 2000 over a stretch of 3 km of the River Lille Aa approximately 22–25 km upstream from the traps. Two hundred wild trout between 11 cm and 20.5 cm were selected, implanted with PIT tags, sampled and further treated in the same way as the trout from the tributaries. Fish from the mainstream were only sampled once.

Migratory behaviour

Migratory behaviour was established when PIT-tagged individuals were either caught in the traps (i.e. migrants) or by electro-fishing after the smolt run had ceased (i.e. residents) in early June 2000.

Analysis

Na$^+$/K$^+$-ATPase activity was analysed in gill homogenates at 27°C by the method of McCormick (1993) using a microtitre plate reader (Spectramax, Molecular Devices, Sunnyvale, CA, USA). Protein content in the tissue homogenates was measured by the Lowry method (Lowry et al., 1951), modified for the microtitre plate reader, i.e. reagent volumes being adjusted for 96-well plates. Experiments were carried out subject to a licence from the Animal Experimentation Inspectorate, Danish Ministry of Justice.

Statistics

To ascertain if future seaward migration or freshwater residency in wild brown trout can be related to a premigratory measurement, the relationship between premigratory determinations of either gill Na$^+$/K$^+$-ATPase activity or L_T and the future migratory behaviour (i.e. migration or residency) in individual trout from the tributaries and from the mainstream was investigated. A logistic regression model was used to investigate the relationship between a continuous independent variable (i.e. premigratory gill Na$^+$/K$^+$-ATPase activity or L_T) and a categorical dependent variable with only two possible outcomes (i.e. migration or residency). Logistic regression analysis was performed on pooled data from the two tributaries and subsequently on data from the mainstream.

The logistic regression procedure involves the iterative fitting of a linear function of the independent variable (X), i.e. premigratory gill Na$^+$/K$^+$-ATPase activity or L_T, to the natural logarithm of the odds ratio for the dependent variable, i.e. probability (P) of migration divided by probability of residency ($1–P$), using the following equation:

$$\log_e\left(\frac{P}{1-P}\right) = aX + b \, .$$

Linear regression analysis was used to evaluate the relationship between premigratory L_T and gill Na$^+$/K$^+$-ATPase activity, and for migrants only between premigratory gill Na$^+$/K$^+$-ATPase activity and the number of days from premigratory sampling to migration. In all cases, a significance level of P=0.05 was used.

Results
Is premigratory gill Na$^+$/K$^+$-ATPase activity related to migratory behaviour?

In the two tributaries, no relationship between future migratory strategy (i.e. whether individuals became migrants or residents) and gill Na$^+$/K$^+$-ATPase activity was found in late February–early March (P>0.05, N=188=163 migrants + 25
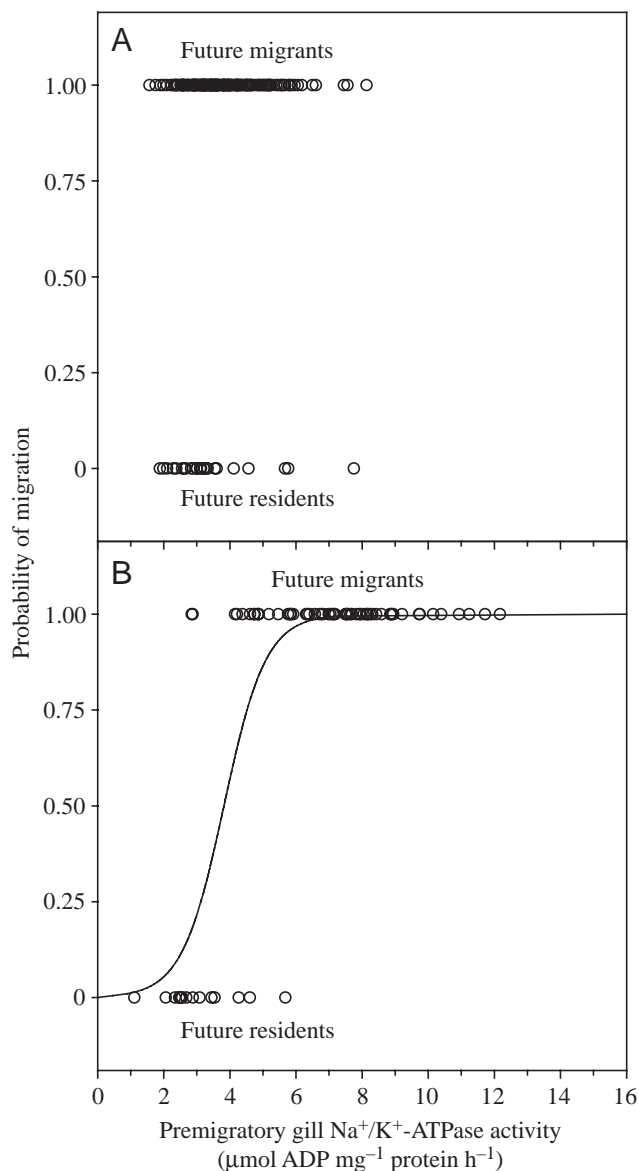
Fig. 1. Premigratory gill Na$^+$/K$^+$-ATPase activity and future migratory behaviour in wild brown trout from the two tributaries (River Granslev Aa and Haldum Brook) in (A) late February–early March (163 future migrants and 25 future residents) and (B) mid-April (60 future migrants and 15 future residents). The solid line in B indicates the probability of future migration as a function of premigratory gill Na$^+$/K$^+$-ATPase activity, as determined by logistic regression analysis.

Fig. 2. Premigratory gill Na$^+$/K$^+$-ATPase activity and future migratory behaviour in wild brown trout from the mainstream (River Lille Aa) in early April (88 future migrants and 16 future residents). The solid line indicates the probability of future migration as a function of premigratory gill Na$^+$/K$^+$-ATPase activity, as determined by logistic regression analysis on mainstream data. For comparison, the broken line indicates the logistic regression curve for mid-April in the tributaries.

residents; Fig. 1A). By contrast, in mid-April, a highly significant logistic regression equation was obtained ($P<0.000001$, $r^2=0.64$, $a=1.573$, $b=-6.009$, $N=75=60$ migrants + 15 residents; Fig. 1B). Based on the single mid-April measurement of gill Na$^+$/K$^+$-ATPase activity of individual fish, the fitted regression equation correctly identified 97% of the 60 future migrants, meaning individuals that actually migrated 1–42 days (median 13 days) after the gill biopsies were taken in mid-April. Of the 15 future residents, meaning individuals
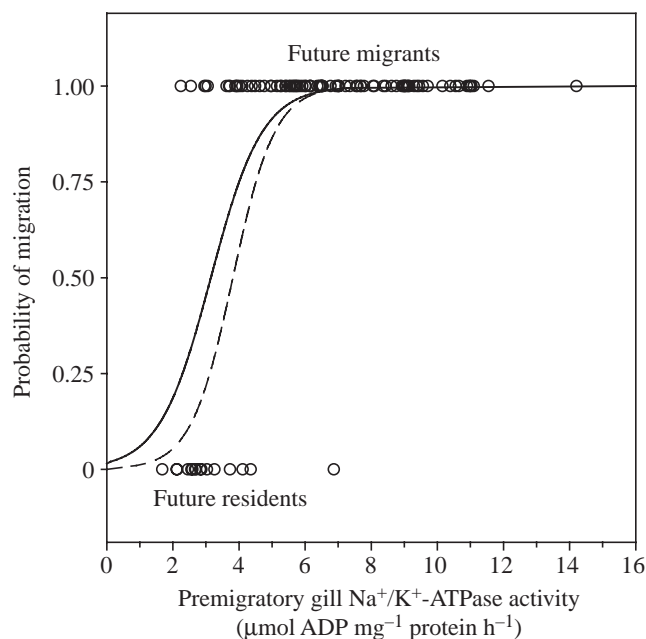
that remained resident 56–57 days after the mid-April sampling, 80% were correctly identified. Hence, the regression equation correctly identified the migratory strategy in 93% of the 75 individuals studied.

The logistic regression equation relating future migratory behaviour of trout from the tributaries to the change in gill Na$^+$/K$^+$-ATPase activity between the first and second sampling was highly significant ($P<0.000001$, $r^2=0.64$, $a=2.549$, $b=-1.242$, $N=75=60$ migrants + 15 residents). However, no improvement in the ability of the regression model to identify individuals as migrants or residents was observed when data from both sampling periods were combined, as compared with using only the second sampling: 95% of the 60 future migrants, 80% of the 15 future residents, and 92% of the total of 75 individuals were correctly identified.

In the mainstream, a highly significant logistic regression equation describing future migratory behaviour as a function of gill Na$^+$/K$^+$-ATPase activity was found ($P<0.000001$, $r^2=0.48$, $a=1.283$, $b=-4.040$, $N=104=88$ migrants + 16 residents; Fig. 2). The regression model correctly identified 94% of the 88 future migrants, i.e. individuals that actually migrated 14–54 days (median 19 days) after sampling. Of the 16 future residents, i.e. individuals that remained resident 63 days after sampling, 69% were correctly identified. In summary, the regression equation correctly identified the migratory strategy in 90% of the total of 104 individual fish.
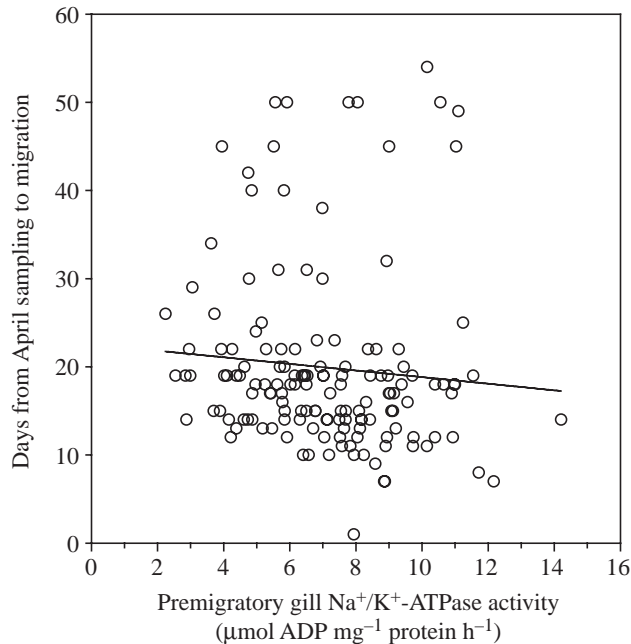
Fig. 3. Premigratory gill Na$^+$/K$^+$-ATPase activity and number of days from April sampling to migration (147 migrants in total from tributaries, River Granslev Aa and Haldum Brook, and mainstream, River Lille Aa). The solid line indicates the non-significant (P=0.31) linear regression curve.

Is L_T related to migratory behaviour or to gill Na$^+$/K$^+$- ATPase activity?

No relationship between future migratory strategy and L_T was found for either of the two samplings in the tributaries or for the mainstream sampling (P>0.05). Furthermore, no relationship between the April measurements in both tributaries and mainstream of premigratory L_T and gill Na$^+$/K$^+$-ATPase activity was observed (linear regression, P=0.26, N=178).

Is gill Na$^+$/K$^+$-ATPase activity related to the number of days from sampling to migration?

For migrants only from both tributaries and mainstream, the premigratory gill Na$^+$/K$^+$-ATPase activity was not related to the number of days from premigratory sampling in April to migration (linear regression, P=0.31, N=147; Fig. 3).

Can the regression model for trout from the tributaries predict future migratory behaviour of trout from the mainstream?

The ability of the highly significant logistic regression model obtained from the tributary-collected trout sampled in mid-April to predict future migratory behaviour in the independent group of trout caught in early April in the mainstream (River Lille Aa) was explored. The regression curve for the tributaries is included for comparison in Fig. 2.

A threshold probability of migration was used to predict the behaviour of the mainstream individuals as either future
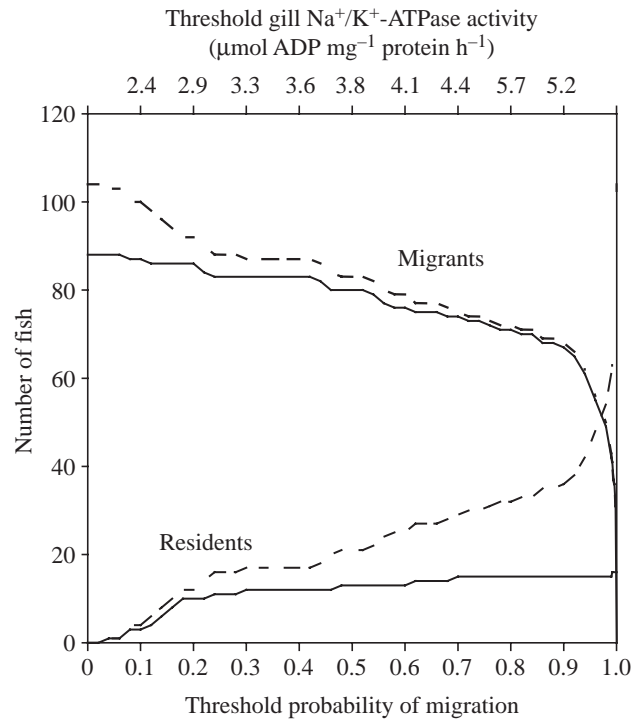

Fig. 4. Predicting migratory behaviour in wild brown trout in the mainstream (River Lille Aa). Numbers of predicted (broken lines) and actual (solid lines) future migrants and residents in the mainstream (River Lille Aa) as a function of threshold probability of migration (or threshold gill Na$^+$/K$^+$-ATPase activity), as predicted from the logistic regression equation relating premigratory gill Na$^+$/K$^+$-ATPase activity in mid-April and future migratory behaviour of wild brown trout from the two tributaries (Haldum Brook and River Granslev Aa).

migrants, comprising fish with probabilities of migration higher than or equal to the threshold, or future residents, consisting of fish with probabilities of migration lower than the threshold.

Predicting migration

The effect of changing the threshold probability of migration on the ability of the logistic regression model to predict future migration is illustrated in Fig. 4. Setting the threshold probability at 0, i.e. predicting all individuals as future migrants, resulted in a predicted number of migrants equal to 104. The 104 predicted migrants, of course, included the 88 actual migrants but also the 16 actual residents. Hence, 85% of the predictions, i.e. 88 out of 104, regarding future migrants among the mainstream individuals were correct using this threshold probability. As the threshold probability was increased, changes in predictive ability were observed. The first improvement occurred at a threshold probability of migration of 0.04 (corresponding to a threshold gill Na$^+$/K$^+$-ATPase activity of 1.8 µmol ADP mg^{-1} protein h^{-1}), where the number of predicted migrants decreased to 103, including all 88 actual migrants. As the threshold was increased further, the

number of falsely predicted migrants continued to decrease. The number of actual migrants included in the predicted migrants remained at 88 until a threshold probability of 0.06 (corresponding to a threshold gill Na+/K+-ATPase activity of 2.1 μmol ADP mg^{-1} protein h^{-1}), with 87% of the predictions regarding future migrants being correct, and then subsequently decreased. At a threshold probability of 0.70 (corresponding to a threshold gill Na+/K+-ATPase activity of 4.4 μmol ADP mg^{-1} protein h^{-1}), the 75 predicted migrants included only one individual that actually remained resident (with 99% of the predictions regarding migrants being correct). At a threshold probability higher than 0.99 (corresponding to a threshold gill Na+/K+-ATPase activity of 6.9 μmol ADP mg^{-1} protein h^{-1}), all predicted migrants actually migrated.

Predicting residency

Fig. 4 illustrates the effect of changing the threshold probability of migration on the ability of the logistic regression model to predict future residency in mainstream individuals. Setting the threshold probability at 1, i.e. predicting all individuals as future residents, resulted in a predicted number of residents equal to 104. These 104 predicted residents included the 16 actual residents but also the 88 actual migrants, with only 15% of the predictions regarding residents being correct. Lowering the threshold probability of migration results in a decrease in the number of falsely predicted future residents. At a threshold probability of 0.70 (corresponding to a threshold gill Na+/K+-ATPase activity of 4.4 μmol ADP mg^{-1} protein h^{-1}), all but one of the 16 actual residents were included in the number of predicted residents, along with 14 actual migrants (with 52% of the predictions regarding residents being correct). At a threshold probability of 0.20 (i.e. a threshold gill Na+/K+-ATPase activity of 2.9 μmol ADP mg^{-1} protein h^{-1}), the 12 predicted residents included two individuals that actually migrated (with 83% of the predictions regarding residency being correct). Only at a threshold probability of 0.06 (corresponding to a threshold gill Na+/K+-ATPase activity of 2.1 μmol ADP mg^{-1} protein h^{-1}) were these two actual migrants separated from the one remaining actual resident.

Overall predictive ability of the logistic regression model for migratory behaviour of trout from the tributaries

The maximum percentage of correct predictions of future migratory behaviour in fish from the mainstream was observed at threshold probabilities between approximately 0.15 and 0.45 (corresponding to threshold gill Na+/K+-ATPase activities of 2.7–3.7 μmol ADP mg^{-1} protein h^{-1}), with an average of 91% of all predictions being correct.

Discussion

The present study directly links, by means of a predictive model, a premigratory biochemical factor of an individual animal to the subsequent migratory behaviour of that same individual. The level of gill Na+/K+-ATPase activity in mid-April was a highly successful biochemical identifier of the future migratory behaviour of wild brown trout in the tributaries at the individual level. Based on a single measurement of gill Na+/K+-ATPase activity in mid-April, a logistic regression model was very accurate and correctly identified migration in 97% and residency in 80% of the cases. Identification of future migratory choice was not possible using Na+/K+-ATPase activities in gill biopsies taken in late February–early March and there was no marked improvement in the predictive ability when data from samplings in both late February–early March and mid-April were included. For the mainstream fish, a logistic regression model based on a single premigratory gill Na+/K+-ATPase activity measurement in early April correctly identified migration in 94% and residency in 69% of the cases. No relationship between L_T and either future migratory strategy or gill Na+/K+-ATPase activity was observed. Furthermore, the premigratory gill Na+/K+-ATPase activity was not related to the number of days from premigratory sampling to migration.

The robustness of the logistic regression analysis approach in predicting future migration or residency was illustrated when the future migratory behaviour of the mainstream individuals was successfully predicted using the logistic regression equation from the tributaries. Depending on the threshold probability of migration chosen, up to 91% of the predictions of future migratory strategy (migration or residency) were correct.

The rationale for using the level of gill Na+/K+-ATPase activity to predict the future migratory behaviour of individual brown trout was primarily based on previous reports of correlations between gill Na+/K+-ATPase activity and migratory behaviour at the population level in several different salmonid species [e.g. rainbow trout (*Oncorhynchus mykiss*), Zaugg and Wagner, 1973; chinook salmon (*Oncorhynchus tshawytscha*), Buckman and Ewing, 1982; coho salmon (*Oncorhynchus kisutch*), Rodgers et al., 1987; Atlantic salmon, Nielsen et al., 2001; brown trout, Aarestrup et al., 2000]. Furthermore, the ability of a smolt to acclimate to seawater may be enhanced by premigratory physiological priming and by coupling of migration with elevated gill Na+/K+-ATPase activity, as the level of gill Na+/K+-ATPase activity is positively coupled to SW-tolerance in salmonids (e.g. Nielsen et al., 1999).

The high degree of accuracy of the predictive model is interesting in several respects. Firstly, the method used for determining gill Na+/K+-ATPase activity is an *in vitro* assay using homogenized solutions of a few gill filaments under standardized conditions, representing the functional capacity under optimized *in vitro* conditions and not necessarily the true *in vivo* activity. Secondly, the premigratory enzyme measurement correctly predicts a future behaviour occurring days or weeks later. Finally, the smolt transformation consists of a series of more or less independent and concurring changes in physiology, morphology, metabolism and behaviour, being individually controlled and coordinated by several hormones

(e.g. Hoar, 1988). Migratory behaviour could be dependent upon the synchronization of several of the changes associated with smoltification and it is unlikely that an increase in gill Na^+/K^+-ATPase activity is the physiological factor initiating migration. A single premigratory measurement of gill Na^+/K^+-ATPase activity did, however, prove to be a very accurate determinant of future migratory behaviour of wild brown trout.

Migration may be seen as the climax of smolt development in the river and it has been hypothesized that migration only occurs when the smolt has passed a physiological threshold condition that assures responsiveness to external release factors (Solomon, 1978; Aarestrup et al., 2000). The transition from nearly no migrants below gill Na^+/K^+-ATPase activities of approximately 2.5 µmol ADP mg^{-1} protein h^{-1} to almost all migrants above approximately 4 µmol ADP mg^{-1} protein h^{-1} (Figs 1B, 2) suggests a threshold value for differentiation of wild brown trout into migrant and resident individuals occurring within this range of enzyme activities.

A note of caution regarding the general applicability of predictive logistic regression models obtained using the methods described in the present study is appropriate. Whether a regression equation for fish from one river system can be utilized to predict the behaviour of fish from a separate river system within a given year remains to be established. It is, however, questionable as the physiological state (e.g. level of gill Na^+/K^+-ATPase activity) at which the fish become responsive to triggering factors and the nature of these factors must be adapted to local conditions. Fish from different river systems may therefore respond to different proximate factors in order to reach sea during an optimal period. Heggberget et al. (1993) showed that smolts of Atlantic salmon in three different river systems had developed a specific proximate trigger system for migration adapted to local conditions, but whether the physiological state of the smolts at the time of the initiation of migration differed as well was not investigated. Smolts of five strains of Atlantic salmon originating from different river systems have been shown to initiate downstream migration differently when released into the same foreign river (Nielsen et al., 2001). However, the level of gill Na^+/K^+-ATPase activity at the time of initiation of migration was approximately the same in the different strains, suggesting a comparable threshold level of gill Na^+/K^+-ATPase activity in populations of salmonids of the same species living in different river systems.

Similarly, the ability to predict behaviour in a given river system from a regression model obtained in the same river in another year should be ascertained. Photoperiod and, to a lesser degree, temperature regulate the neuroendocrine alterations that result in the physiological changes during smolting (Hoar, 1988). Water temperature is believed to be the rate-determining factor of the physiological development, and once a certain physiological smolt condition is reached (Solomon, 1978), water temperature (Jonsson and Ruud-Hansen, 1985) and discharge (Hansen and Jonsson, 1985) are factors known to have a rapid stimulatory effect on the

initiation of downstream migration in salmonids. The response to these cues may have evolved by natural selection to increase the survival of the smolts, because the timing of the environmental cues changes from year to year (McCormick et al., 1998; Wedemeyer et al., 1980). The timing of smolt migration is reported to be controlled by a combination of actual temperature and temperature increases in the water during spring (Jonsson and Ruud-Hansen, 1985). As the temperature profile in a particular river varies from year to year, the time when the physiological development is reached is likely to differ and the time of the main run of Atlantic salmon smolts is reported to vary between 0 and 14 days between different years (Jonsson and Ruud-Hansen, 1985; Whalen et al., 1999).

Finally, the data obtained in the present study regarding the temporal applicability of the regression model should be considered. In the tributaries in late Feruary–early March, i.e. two months before the main run, physiological differentiation in terms of gill Na^+/K^+-ATPase activity was not yet initiated and it was not possible to predict future migratory behaviour from gill Na^+/K^+-ATPase activity. In comparison, the premigratory measurement of gill Na^+/K^+-ATPase activity from mid-April, i.e. 2–3 weeks (median time before capture in trap) before the main run, very successfully predicted the future migratory behaviour of the individual fish from the tributaries. In Fig. 2, the displacement along the x-axis of the regression curve for the tributaries compared with the curve for the mainstream probably reflects that the mainstream sampling was performed 5–6 days before the mid-April sampling in the tributaries.

In conclusion, a non-lethal premigratory biochemical measurement of gill Na^+/K^+-ATPase activity was successful in selecting individual brown trout with a high probability of future migration. The present findings should encourage similar studies of the migratory behaviour in other species.

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