ELSEVIER

Aquaculture 189 (2000) 177–188

Aquaculture

www.elsevier.nl/locate/aqua-online

Effects of photoperiod manipulation on development of seawater tolerance in Arctic charr

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Received 14 September 1998; accepted 8 March 2000

Abstract

Effects of photoperiod manipulation on the development of seawater tolerance were studied in Arctic charr. Three groups of fish, previously reared under natural photoperiod and ambient water temperature conditions, were subjected to a constant short daylength, 4L:20D, from 21 December to 30 January, followed by exposure to either 4L:20D, continuous light (24L:0D) or simulated natural photoperiod (nLD). Temperature of the fresh water was held constant at 4°C until mid-May, after which it increased gradually to reach 8.5°C at the termination of the experiment on 2 July. All groups displayed improved seawater tolerance during the course of the study, assessed as changes in plasma chloride and osmolality concentrations following 72-h exposure to seawater (33–34‰). The tolerance to seawater was positively related to fork length within some sampling dates in all groups. Exposure to 24L:0D advanced the development of seawater tolerance by approximately 6 weeks, compared to the nLD group. Both groups displayed increases in gill Na^+/K^+-ATPase activity that coincided with the period of improved seawater tolerance. Seawater tolerance of the 4L:20D group was delayed by 6 weeks in comparison with that of the nLD group, but without any concomitant increase in gill Na^+/K^+-ATPase activity. The results corroborate previous findings, and suggest that the seasonal changes in seawater tolerance of Arctic charr are controlled by an endogenous, circannual timing mechanism that is entrainable by artificially extended daylengths in spring. Our data further suggest that development of seawater

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PII: S0044-8486(00)00370-7

tolerance in Arctic charr may occur independently of changes in gill Na^+/K^+-ATPase activity.

Keywords: Arctic charr; *Salvelinus alpinus*; Photoperiod; Osmoregulation; Smoltification; Gill Na^+/K^+-ATPase

1. Introduction

The Arctic charr (*Salvelinus alpinus)* is found as anadromous and resident forms, which differ in morphology and life history pattern (Johnson, 1980; Dempson and Kristofferson, 1987). Anadromous charr migrate downstream in late spring, and spend the summer months feeding in coastal waters before returning to fresh water in the autumn (Mathisen and Berg, 1968; Berg and Berg, 1993). Both juvenile and mature fish may participate in the sea-run, and each individual usually makes several annual migrations during its lifetime (Johnson, 1980).

Recent studies indicate that anadromous Arctic charr display seasonal variations in hypo-osmoregulatory capacity (Finstad et al., 1989; Arnesen et al., 1992; Halvorsen et al., 1993; Staurnes, 1993). Seawater tolerance improves in spring whilst the fish are still in fresh water, and this improved seawater tolerance correlates with increases in gill Na^+/K^+-ATPase activity (Finstad et al., 1989) and chloride cell number (Damsgård, 1991). These observations suggest that Arctic charr undergo preparatory physiological changes prior to the sea-run, and that these changes resemble those seen during the parr–smolt transformation displayed by other salmonids, i.e. members of the genera *Oncorhynchus* and *Salmo* (Hoar, 1988).

The physiological events associated with parr–smolt transformation in salmonids are thought to reflect endogenous circannual rhythms which are controlled by the seasonal photoperiod cycle (Wagner, 1974; Eriksson and Lundvist, 1982). It is assumed that increasing or long photoperiods in spring stimulate these processes (McCormick et al., 1987; Hoar, 1988). Photoperiod, or daylength, is thought to act as a synchronizer (Duston and Saunders, 1990), and there is good evidence to suggest that the effects of photoperiod are mediated via a light-pituitary axis (Komourdjian et al., 1976) involving several endocrine factors, such as growth hormone, thyroid hormones and cortisol (Björnsson et al., 1989; Young et al., 1989; McCormick et al., 1995). Photoperiod manipulation alters the timing of the parr–smolt transformation of several species of salmonids within the genera *Oncorhynchus* and *Salmo* (Hoar, 1988), but only circumstantial (Arnesen et al., 1992) or equivocal information (McCormick and Naiman, 1984) exists for *Salvelinus* species. The present study was, therefore, undertaken to investigate the effects of photoperiod manipulation on the development of seawater tolerance in anadromous Arctic charr.

2. Methods

The study was carried out at the Aquaculture Research Station, Tromsø (69°N), in the period December 1995–July 1996, using 2-year-old hatchery-reared Arctic charr (*S.*

alpinus) of the ''Hammerfest-strain''. Eggs were hatched at the research station during the winter of 1993–1994, and the fish were reared under natural light conditions in fresh water at ambient water temperature (min.: 0.6°C in January–February; max.: 12°C in July–August) until the start of the experiment. On 21 December 1995, a total of 1020 fish of mixed sex (average weight 140 g, fork length > 20.0 cm) was divided into three groups and transferred to each of three circular rearing tanks (500 l capacity, water depth 50 cm) supplied with a circumferential flow of fresh water (Christiansen and Jobling, 1990). Water temperature was increased from 0.5°C to 4°C during the first week of a 14-day acclimatization, and was then held constant until ambient fresh water temperature rose to above 4°C in mid-May. From this time onwards the temperature of the fresh water increased gradually to reach 8.5°C at the termination of the experiment on 2 July. Temperature was recorded (ADAM 400 Series Data Acquisition Modules) every hour during the course of the study. Rates of water flow were adjusted to maintain oxygen above 90% of the air saturation level.

Each tank was shielded by a black, light-proof plastic canopy, and lighting was provided by three 10 W, 24 V bulbs giving a light intensity of approximately 80 lx at the water surface. Photoperiod was controlled by automatic timers without a twilight period. All three groups were held on a constant short day photoperiod of 4L:20D until 30 January. Between 30 January and 2 July, the three experimental groups were exposed to one of the following photoperiods: simulated natural photoperiod (nLD), corresponding to that for Tromsø (69°N), continuous light (24L:0D) or 4L:20D.

To ensure similar feeding conditions for the fish held under the three photoperiod regimes food was offered for a restricted period of 4 h each day set to match the light period of the 4L:20D group. The fish were fed commercial dry feed (Felleskjøpet) using automatic disc feeders. Daily feed requirements were calculated from predicted growth rates (Jobling, 1983) assuming a feed:gain ratio of 1.

The following procedures were used to evaluate the development of hypo-osmoregulatory ability. Ten fish from each group were taken at 3-week intervals for blood and gill sampling. At the same time, 20 fish from each group were subjected to a 72-h seawater challenge test (SWCT) in a separate 200 l tank supplied with seawater (33–34‰) at ambient temperature (min.: 3.0°C in late February; max.: 6.4°C in early July). Mortality was recorded and blood samples were taken from fish that survived the test.

The fish were killed by a blow to the head, and body weight (W; nearest 0.5 g) and fork length (L; nearest 0.1 cm) measured. A blood sample was then collected from the caudal vein using lithium-heparinized (30 USP units) 2 ml vacutainers. The blood samples were held on ice for a maximum of 30 min before being treated further. Blood plasma was separated by centrifugation for 8 min at 3850 rpm ($2700 \times g$) and was then frozen at −70°C for later determination of Cl^- (Corning chloride analyser, mod. 925) and osmolality (Fiske ONE–TEN osmometer). Samples for measurements of gill Na^+/K^+-ATPase activity were taken from the first gill arch on the left side of each fish. A 0.5-cm piece of tissue was dissected from the middle of the gill arch, placed in 1 ml SEI solution (0.3M sucrose, 0.02M Na_2EDTA and 0.1M Imidazol) (Zaugg, 1982) and immediately frozen in liquid nitrogen. Gill Na^+/K^+-ATPase activity was measured as the production of inorganic phosphate (P_i), the assay being carried out as described by Zaugg (1982). Inorganic phosphate was determined by the method described by Fiske

and Subbarow (1925) as modified by Peterson (1978). Protein was determined using a Bio-Rad DC protein assay, with bovine serum albumin as the standard. Gill Na^+/K^+-ATPase activity is expressed as μmol P_i mg protein^{-1} h^{-1}. All fish sampled were dissected, and sex and maturation status determined. Gonadosomatic index (GSI) was calculated as gonad weight 100 W^{-1}. Condition factor was calculated for each fish as $[(W\ L^{-3}) \cdot 100]$.

A fully factorial two-way ANOVA was used to test for the effects of time and photoperiod treatment on GSI, gill Na^+/K^+-ATPase activity, weight change, fork length and condition factor, and a Tukey post-hoc test was used to detect the specific timing of temporal changes. Effects of fish size (fork length) on gill Na^+/K^+-ATPase activity, and plasma chloride concentrations and osmolalities following the SWCTs, were analysed for each sampling date within each group, using a Spearman rank test. Effects of time and treatment on plasma chloride concentrations and osmolalities, following the SWCTs, were analysed by a two-way ANCOVA, using fork length of the same fish as a covariate, and a Tukey post-hoc test was used to detect the specific timing of temporal changes. A probability level of 0.05 was applied in all tests. All computations were performed with SYSTAT version 5.2.1 (Wilkinson, 1990). Data that were normally distributed (Lilliefors, 1967), i.e. gill Na^+/K^+-ATPase activity, weight, fork length and GSI of the fresh water adapted fish, are presented as means ± SEM, whereas those that were found not to be normally distributed, i.e. plasma chloride concentrations and osmolalities following the SWCTs, are presented as medians ± 95% confidence limits.

3. Results

None of the fish held in fresh water (FW) died during the course of the study, and mortality during the SWCTs was low; 11 fish died in the first test on 13 January and one fish died on 2 May.

There were no significant differences in body weight or fork length between the groups at any time, and all groups increased significantly ($P < 0.001$) in weight and length from 142.9 ± 3.9 g and 23.5 ± 0.2 cm in mid-January to 287.7 ± 14 g and 28.9 ± 0.4 cm by early July (Fig. 1). Condition factor did not change significantly in any group, being on average 1.13 ± 0.01.

Gonadosomatic indices of the males subjected to 24L:0D and nLD rose significantly ($P < 0.05$) during the course of the study from 0.05 ± 0.01 and 0.03 ± 0.01 to 2.65 ± 0.84 and 1.22 ± 0.039, respectively. The increase in GSI was first evident amongst the males subjected to 24L:0D. No change in GSI was seen in males of the 4L:20D group. GSI of the females was unaffected by photoperiod treatment and remained stable around 0.15 throughout the study.

The development of gill Na^+/K^+-ATPase activity was significantly affected by photoperiod, resulting in different temporal patterns in the three groups. Gill Na^+/K^+-ATPase activity amongst charr of the nLD group changed little until late May–early June, when a significant increase ($P < 0.001$) from about 2.5 to about 6 μmol P_i mg protein^{-1} h^{-1} was recorded (Fig. 2). Enzyme activity then dropped to about 3 μmol P_i

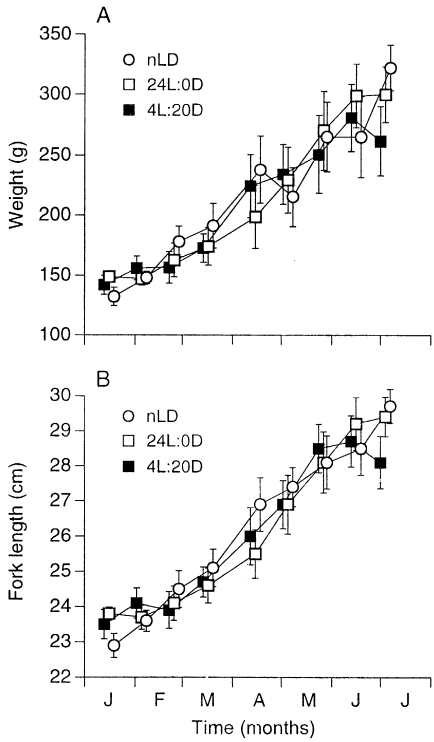

Fig. 1. Changes in weight (A) and fork length (B) of fresh water adapted Arctic charr subjected to simulated natural photoperiod (nLD), continuous light (24L:0D) or constant short photoperiod (4L:20D). Values given are means ± SEM of 10 individuals. (A) Significantly ($P < 0.05$) different from that of the other groups; (B) significantly ($P < 0.05$) different from other values within the same group.

mg protein^{-1} h^{-1} on 26 June and remained at this level until 2 July. Gill Na^+/K^+-ATPase activity of the charr held under 24L:0D changed over time ($P < 0.05$), but the Tukey test was not able to detect which pairs of values differed significantly from each other. Enzyme activity of the 24L:0D group was similar to that of the nLD group until mid-April. There was then an increase from about 2 to 4 μmol P_i mg protein^{-1} h^{-1} (Fig. 2), enzyme activity remained elevated during the next 3 weeks, and this was followed by a steady drop during the next 2 months. Levels in early July were similar to those recorded during January–March. The charr held under 4L:20D displayed no increase in gill Na^+/K^+-ATPase activity during the course of the study. There was no effect of fish size (fork length) on Na^+/K^+-ATPase activity on any sampling date within any group.

The tolerance to seawater increased significantly over time ($P < 0.001$) in all groups. When the data for plasma chloride and osmolality were related to the size of the sampled fish (fork length), the tolerance to seawater was found to be size-dependent on some sampling dates within each group (Table 1). Analysis of covariance of plasma chloride and osmolality, using fork length of the challenged fish as a covariate, revealed,

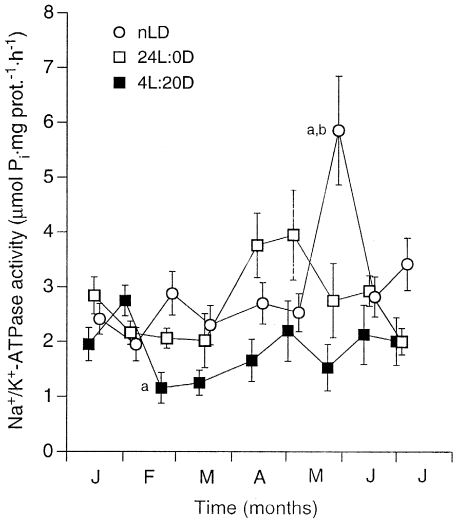

Fig. 2. Temporal changes in gill Na^+/K^+-ATPase activity in fresh water adapted Arctic charr subjected to simulated natural photoperiod (nLD), continuous light (24L:0D) or constant short photoperiod (4L:20D). Values given are means ± SEM of 10 individuals. (a) Significantly ($P < 0.05$) different from the other groups; (b) significantly ($P < 0.05$) different from other values within the same group.

however, that the temporal patterns of seawater tolerance differed significantly among the groups (chloride: time · photoperiod, $P < 0.001$, size: $P < 0.001$; osmolality: time · photoperiod, $P < 0.001$, size: $P < 0.001$), indicating an effect of photoperiod. The fish that survived the SWCTs conducted in January had elevated levels of plasma chloride and osmolality (Figs. 3 and 4), indicating that seawater tolerance was poor. Tolerance improved slightly in the next two tests (2 and 22 February) and no fish died during the SWCTs. From mid-April onwards, 6 weeks after the photoperiod regimes were established, the tolerance to seawater developed differently among the three groups. Charr of the 24L:0D group displayed a further increase ($P < 0.05$) in seawater tolerance during

Table 1
Significance in the effects of fish size (fork length) on plasma chloride concentrations and osmolalities, following seawater challenge tests. The data were analysed using a Spearman rank test. The numbers of observations are 20 or less; 13 January: 19 (nLD), 14 (24L:0D), 16 (4L:20D); 2 May: 19 (4L:20D)

		Sampling dates								
		10.01	30.01	20.02	11.03	09.04	30.04	21.05	11.06	02.07
Chloride vs. fork length	nLD	ns	*	ns	*	*	*	ns	*	ns
	24L:0D	ns	ns	*	ns	ns	*	*	ns	*
	4L:20D	*	ns	ns	*	*	*	*	ns	*
Osmolality vs. fork length	nLD	ns	*	ns	*	*	*	ns	ns	*
	24L:0D	ns	ns	ns	ns	ns	ns	ns	ns	*
	4L:20D	ns	ns	*	*	*	ns	*	*	*

* $P < 0.05$

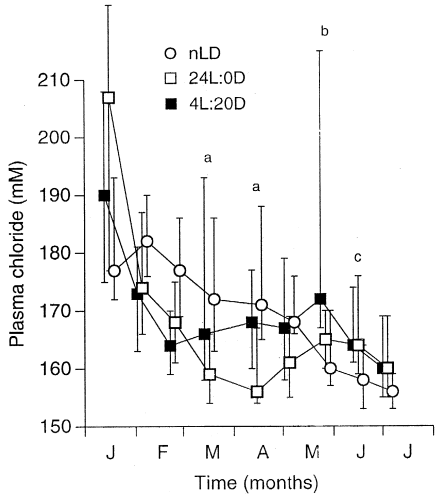

Fig. 3. Temporal changes in plasma chloride of Arctic charr held under either simulated natural photoperiod (nLD), continuous light (24L:0D) or constant short photoperiod (4L:20D) and subjected to 72-h seawater challenge tests. Values given are medians ± 95% confidence limits of 20 individuals, except on 13 January and 2 May when the number of fish were 19 (nLD), 11 (24L:0D), 16 (4L:20D), and 19 (4L:20D), respectively. The symbols are displaced slightly for clarity. (a) 24L:0D significantly ($P < 0.05$) different from nLD and 4L:20D; (b) 4L:20D significantly ($P < 0.05$) different from nLD and 24L:0D; (c) nLD significantly ($P < 0.05$) different from 24L:0D and 4L:20D.

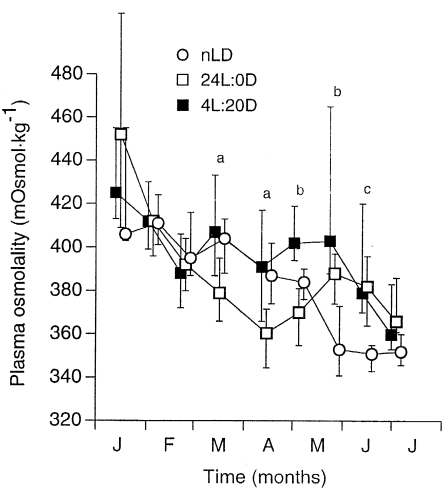

Fig. 4. Temporal changes in plasma osmolality of Arctic charr held under either simulated natural photoperiod (nLD), continuous light (24L:0D) or constant short photoperiod (4L:20D) and subjected to 72-h seawater challenge tests. Values given are medians ± 95% confidence limits of 20 individuals, except on 13 January and 2 May when the number of fish were 19 (nLD), 11 (24L:0D), 16 (4L:20D), and 19 (4L:20D), respectively. The symbols are displaced slightly for clarity. (a) 24L:0D significantly ($P < 0.05$) different from nLD and 4L:20D; (b) 4L:20D significantly ($P < 0.05$) different from nLD and 24L:0D; (c) nLD significantly ($P < 0.05$) different from 24L:0D and 4L:20D.

March–April (Figs. 3 and 4). In the other two groups, plasma chloride and osmolalities, following SWCTs, remained elevated, compared to that of the 24L:0D group, until 2 May (nLD) and 24 May (4L:20D), respectively. Seawater tolerance of the nLD group then (24 May) improved rapidly ($P < 0.05$), concurrent with the marked increase in gill Na^+/K^+-ATPase activity, and then remained high until the termination of the experiment. Fish of the 24L:0D group displayed decreased seawater tolerance during May, followed by an improvement during June–July. The different patterns of seawater tolerance during the late part of the experiment resulted in fish of the nLD having significantly ($P < 0.05$) lower levels of plasma chloride (24 May and 13 June) and osmolality (24 May, 13 June and 2 July) than those in the 4L:20D and 24L:0D groups. Although the differences were significant, plasma chloride and osmolalities in the 4L:20D and 24L:0D groups decreased in June and approached those of the nLD group by the termination of the study on 2 July. There were no significant effects of time or photoperiod treatment on the levels of plasma chloride (140 ± 1) and osmolality (328 ± 1) in the charr sampled from fresh water.

4. Discussion

Seawater tolerance of all groups improved significantly during the course of the study, but best performance was recorded in the nLD group between late May–early July, corresponding to the time of seawater residency of Arctic charr from north Norwegian river systems (Mathisen and Berg, 1968; Berg and Berg, 1989, 1993; Svenning et al., 1992, Rikardsen et al., 1997). The median levels of plasma chloride (about 155 mM) and osmolality (about 350 mOsm) of these fish were similar to those reported for Arctic charr subjected to a seawater challenge under similar conditions (Eliassen et al., 1998), but were significantly higher than those of the freshwater controls. This may indicate that the charr did not adapt completely during the challenge tests. It is possible that the degree of adaptation during the challenge tests depends, in part, on body size. For example, previous studies on Arctic charr (Arnesen et al., 1992; Halvorsen et al., 1993) and other salmonids (McCormick and Naiman, 1984; McCormick and Saunders, 1987; Hoar, 1988) have shown that body size affects seawater tolerance. The size-effect may be due to a more favourable surface-area-to volume ratio in large fish, and/or a gradually increasing osmo- and ionoregulatory capacity with size (McCormick and Saunders, 1987). It is possible, therefore, that the overall increase in seawater tolerance in all groups may be attributed, in part, to increases in body size, although the charr (22–32 cm) used in our tests were larger than those known to migrate from north Norwegian rivers (Svenning et al., 1992; Halvorsen et al., 1993).

Complete hypo-osmoregulatory performance in spring (May–June) may not be achieved until 1–2 weeks after transfer from fresh water to seawater (Staurnes et al., 1992; Halvorsen et al., 1993), even though elevations in both gill Na^+/K^+-ATPase activity and chloride cell numbers (Eliassen et al., 1998) suggest that the fish undergo preparatory changes prior to seawater entry. This may indicate that, in addition to the preparatory physiological changes that occur prior to the sea-run (Finstad et al., 1989; Damsgård, 1991), there is further adjustment of salt secretory mechanisms once the fish

come into contact with seawater. Further support for this view is the observation of an additional increase in gill Na^+/K^+-ATPase activity after transfer of anadromous Arctic charr to full strength seawater (Eliassen et al., 1998). A similar mechanism is likely to operate in sea-running brook charr (McCormick, 1994), and probably to some extent in *Salmo* and *Oncorhynchus* species as well (Hoar, 1988).

The relative differences in plasma chloride and osmolality concentrations observed in the different groups, following the seawater challenge, suggest that photoperiod treatment had a significant influence on the development of hypo-osmoregulatory performance. The seawater tolerance of the 24L:0D group improved significantly during March–April and peaked approximately 6 weeks in advance of fish held on the nLD. At that time the plasma chloride and osmolality levels of the 24L:0D group were significantly lower than those of fish in both the other groups. These data corroborate previous findings for other salmonids (Björnsson et al., 1989; Clarke et al., 1989; Okumoto et al., 1989; Duston and Saunders, 1990; Thrush et al., 1994; Sigholt et al., 1995) and suggest that extended daylength in spring may advance seawater tolerance of anadromous Arctic charr.

On the assumption of an endogenous smolting cycle (Hoar, 1976; Eriksson and Lundvist, 1982; Thorarensen and Clarke, 1989), Duston and Saunders (1990) proposed that photoperiodic entrainment of parr–smolt transformation may be dependent on the phase of the oscillator at the time of the perturbation. According to this hypothesis an accelerated photoperiod would lead to the perception that the clock was running ''behind time'', and result in a compensatory phase advance of the parr–smolt transformation (Clarke et al., 1985), as was seen in our 24L:0D group. The authors further suggested that the sensitivity of the response to an increase in photoperiod would increase over time, and there would eventually be a spontaneous response in the absence of a daylength cue. This view is consistent with the observation that the charr subjected to a 4L:20D also developed improved seawater tolerance during the course of the study, although this was not accompanied by changes in gill Na^+/K^+-ATPase activity. The increase in seawater tolerance was delayed in comparison with that of the nLD group, but by 2 July these fish performed almost as well in the SWCTs as the nLD fish. Similar observations have been made on Atlantic salmon (Duston and Saunders, 1990) and masu salmon (*O. masou*) (Okumoto et al., 1989), providing additional support to the contention that the seasonal cycle of seawater tolerance in salmonids is controlled by a circannual timing mechanism (Hoar, 1976; Eriksson and Lundvist, 1982; Thorarensen and Clarke, 1989).

Charr of both the nLD group and the 24L:0D group displayed consistent changes in gill Na^+/K^+-ATPase activity, with peak levels coinciding with elevated hypo-osmoregulatory performance. However, no such increase was recorded in the fish held under 4L:20D, even though these fish developed a tolerance to seawater during May–July (a change in median plasma osmolality from 400 to 358 mOsm). These findings, and the observation of a lack of correlation between seawater tolerance and gill Na^+/K^+-ATPase activity in Atlantic salmon (Saunders et al., 1989; Saunders and Harmon, 1990; Solbakken et al., 1994), suggest that the acquisition of seawater tolerance may not be entirely dependent on increased gill Na^+/K^+-ATPase activity. This questions the view that the development of seawater tolerance in salmonids is

causally linked to increased gill Na^+/K^+-ATPase activity (reviewed by McCormick and Saunders, 1987).

Acknowledgements

We thank Prof. Malcolm Jobling for constructive criticism of the manuscript.

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