

## Ionic and osmotic balance in the pike, *Esox lucius* L., in fresh and brackish water<sup>1</sup>

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Plasma  $\text{Na}^+$ ,  $\text{Cl}^-$  and  $\text{Mg}^{2+}$  concentrations in pike (*Esox lucius* L.) are maintained at higher levels in brackish than in fresh water. Plasma  $\text{Na}^+$ ,  $\text{Cl}^-$  and  $\text{Mg}^{2+}$  concentrations increased, and blood glucose and tissue water concentrations decreased during 4-day exposure to a slightly hyperosmotic medium. Blood mean corpuscular haemoglobin concentration (MCHC), a rough index of circulatory red cell volume, increased in pike exposed to slightly hyperosmotic and  $\text{Mg}^{2+}$ -supplemented brackish water. Transfer from brackish to fresh water caused a transitory increase in MCHC, but within 3 weeks the fish achieved levels typical of freshwater pike.

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### 1. Introduction

The northern pike (*Esox lucius* L.) occurs in Northern Europe both in inland fresh waters (FW) and in the brackish water (BW) of the Baltic Sea coast. Its geographical distribution shows it to be a stenohaline freshwater species. This stenohalinity must result from the inter-related physiological roles of gill, kidney and gut in the regulation of body hydromineral balance. It is not known whether pike populations from FW and BW differ physiologically in this respect. If they do, are the differences environmentally controlled by salinity, i.e. are they reversible? In this study some iono/osmoregulatory parameters have been measured in pike 1) caught in different waters (FW and BW), and 2) caught in BW and transferred to higher and lower salinities. The possible role of  $\text{Mg}^{2+}$  in acclimatization to BW is also considered.

### 2. Material and methods

The pike (*Esox lucius* L.) were caught with bag-nets, spinning tackle, and weir- or gill-nets in the waters

near Tvärminne Zoological Station (Gulf of Finland), and from two lakes, Päijänne-Vanhaselkä (central Finland) and Hirvijärvi (southern Finland). Only undamaged fish were used, and these were not fed after capture. Three sets of fish, each including both males and females, were investigated:

*Expt. I* (cf. Table 1). After capture in May—July these post-spawned fish were allowed to recover for 1—3 weeks in water of the original salinity before samples were taken. Maintenance methods, sampling procedures, test water qualities and some of the results have been reported previously (OIKARI 1975a, 1975b, OIKARI & SOIVIO 1977, OIKARI *et al.* 1978). The weight range of the fish was 240—1410 g (34—62 cm).

*Expt. II* (cf. Fig. 1). In June 1975, three test groups were arranged. The first group of fish was caught and kept continuously in BW, the second group was caught in BW and transferred to the FW of Peurunkajärvi (a lake in central Finland) for 3 weeks, and the third group was caught in Päijänne-Vanhaselkä and transferred to and kept for 2 weeks in the same FW as the second group. For the water quality of Peurunkajärvi see OIKARI & SOIVIO (1977). Before sampling, the fish were acclimated to  $10.5 \pm 0.5$  °C for at least 2 weeks. Their weights ranged from 240 to 1260 g (35—57) cm.

*Expt. III* (Table 2, cf. Figs. 2 and 3). The effects of salinity higher (12.8 ‰) and lower (FW) than the original BW and of water  $\text{Mg}^{2+}$  concentration were studied in five groups of pike: 1) "BW" consisted of fish captured and kept in BW, 2) "CBW" (concentrated BW) were caught in BW and exposed for 4 days to seasalt-enriched BW (salinity 12.8 ‰, i.e. slightly hyperosmotic in relation to the plasma of pike in BW),

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3) "MgBW" were caught in BW and exposed for 4 days to  $Mg^{2+}$ -supplemented BW (added as  $MgCl_2 \cdot 6H_2O$ , purum grade), 4) "FW exp." were caught in BW and exposed for 7 days to FW at Tvärminne Zoological Station, and 5) "FW" consisted of pike caught in Päijänne-Vanhaselkä and, after transportation, kept for 1 week in FW of Peurunkajärvi. For water qualities see Table 2. Water temperatures were kept at  $10.5 \pm 0.5$  °C. These experiments were conducted within one month during June-July (1974 and 1976), natural light-dark rhythm was maintained, and all samples were taken within 10–18 days after capture. The lengths of the pike caught in BW ranged from 43.5 to 66 cm (480–1600 g) and those of the FW controls from 35 to 57 cm.

To minimize the effects of stress during sampling, the pike were "caged" and stunned on the head before blood sampling (Soivio & OIKARI 1976), except the first and third groups in Table 1, which were free-swimming and anaesthetized with MS-222 (OIKARI 1975a). All blood samples were drawn from the exposed heart into a syringe with a hypodermic needle. Samples of white muscle tissue were taken from the mid-part of the epaxial lateral muscle. The methods for determining the concentrations of plasma electrolytes, blood glucose, tissue water, and the blood mean corpuscular haemoglobin concentration (MCHC), were as before (OIKARI 1975a, SOIVIO & OIKARI 1976).

### 3. Results

*Expt. I.* A hydromineral steady state presumably prevails in both FW and BW stocks of pike, but different plasma ionic levels are maintained (Table 1): mean concentrations were higher ( $P < 0.05$ ) in BW than in FW, by 19 % for  $Cl^-$ , by 15 % for  $Na^+$ , and by 55 % for  $Mg^{2+}$ .

Table 1. Concentrations (mmol/l) of plasma ions of *Esox lucius* in brackish (about 6.2 ‰ S) and in fresh water (Expt. I). Means  $\pm$  SEMs, N = number of animals.

Source	Water temp. °C	$Cl^-$	$Na^+$	$Mg^{2+}$	N
<b>Brackish-water groups:</b>					
Tvärminne	10	$121.5 \pm 0.76$	$142.7 \pm 1.87$	$1.38 \pm 0.127$	8
»	10	$122.7 \pm 1.07$	$143.1 \pm 1.48$	$1.23 \pm 0.121$	6
»	21	$121.6 \pm 0.51$	$140.4 \pm 1.22$	$0.97 \pm 0.034$	9
<b>Fresh-water groups:</b>					
Päijänne	11	ND <sup>1</sup>	$126.8 \pm 4.32$	$0.74 \pm 0.020$	11
»	7.5	$102.0 \pm 3.51$	$120.2 \pm 3.49$	$0.79 \pm 0.012$	11
Hirvijärvi	12–16 <sup>2</sup>	$103.1 \pm 2.51$	$123.6 \pm 2.75$	$0.78 \pm 0.031$	12

<sup>1</sup> ND = not determined

<sup>2</sup> daily variation

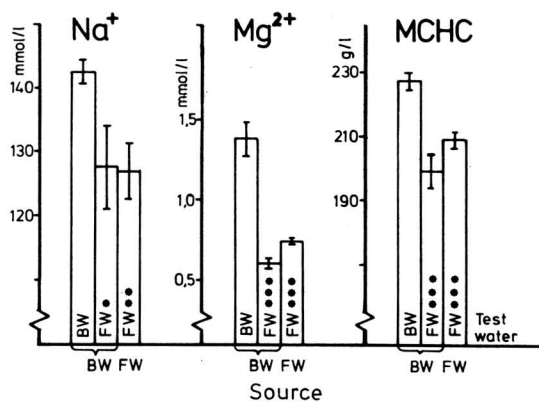


Fig. 1. Effects of ambient salinity on plasma  $Na^+$  and  $Mg^{2+}$ , and blood MCHC (mean corpuscular haemoglobin concentration) of pike (Expt. II). BW = brackish water of about 6.2 ‰ salinity, FW = fresh water; BW pike were acclimated to FW for 3 weeks. Asterisks indicate the statistical significance (Student's *t* test;  $P < 0.05$ , 0.01 and 0.001) of the differences between the BW and FW test groups (8–10 fish used for each analysis in each group; mean values  $\pm$  SEMs).

*Expt. II.* Three weeks' exposure of BW pike to FW reduces plasma concentrations of  $Na^+$  and  $Mg^{2+}$  to about the level typical of FW pike (Fig. 1). One week's exposure to FW led to a smaller reduction in  $Cl^-$  and  $Na^+$ , but about the same in  $Mg^{2+}$  (Fig. 2). Thus acclimation of the ionic balance to FW takes from 1 to 3 weeks.

*Expt. III.* Several statistical significances are compiled in Table 3. Short-term exposure of BW-acclimated pike to water of 12.8 ‰ salinity (CBW), which is slightly hyperosmotic as well as hyperionic in relation to plasma  $Na^+$ ,

Table 2. Groups of Expt. III, and mean water quality during exposures to different media (see Figs. 2 and 3). BW = brackish water, FW = fresh water, FW exp. = exposed to FW, S = water salinity ‰, ion concentrations in mmol/l. N = number of fish.

Group No.	Source and N	medium	Concentration in test water S	$Cl^-$	$Na^+$	K <sup>+</sup>	$Mg^{2+}$	$Ca^{2+}$
1. BW	8	Tvärminne BW	6.4	101	86	1.87	6.22	2.31
2. CBW	6	»	12.8	203	168	3.74	10.83	2.38
3. MgBW	5	»	7.4	116	84	1.89	10.75	2.26
4. FW exp.	6	»	0	ND <sup>1</sup>	0.34	0.02	0.18	0.06
5. FW	11	Päijänne FW	0	ND <sup>1</sup>	0.17	0.03	0.06	0.11

<sup>1</sup> ND = not determined

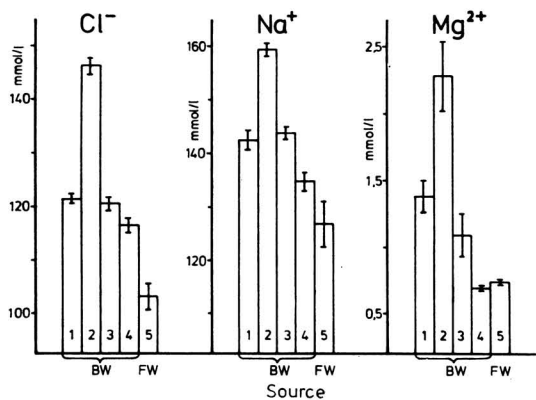


Fig. 2. Effects of water quality on plasma  $\text{Cl}^-$ ,  $\text{Na}^+$  and  $\text{Mg}^{2+}$  concentrations of pike (Expt. III, see Table 2). 1 = BW (controls), 2 = CBW (4 days), 3 = MgBW (4 days), 4 = FW exp. (7 days) and 5 = FW (controls). For further details see legend to Fig. 1.

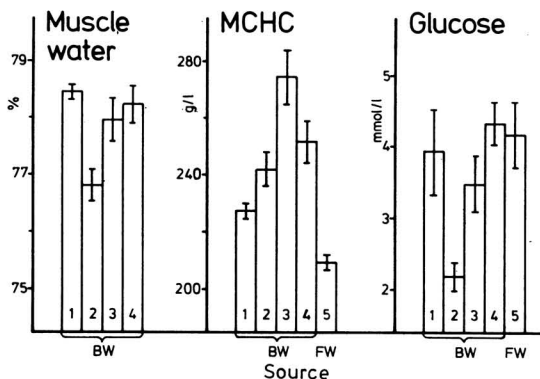


Fig. 3. Effects of water quality on muscle water, blood MCHC and blood glucose of pike (Expt. III). For details, see legends to Figs. 1 and 2.

$\text{Cl}^-$  and  $\text{Mg}^{2+}$ , caused significant increases in the plasma concentrations of these electrolytes. In these fish, the change in plasma  $\text{Mg}^{2+}$  was close to the change in external  $\text{Mg}^{2+}$  concentration, but in fish exposed to  $\text{Mg}^{2+}$ -supplemented BW (MgBW) the plasma  $\text{Mg}^{2+}$  concentration was unaffected. Nor were plasma  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations changed in MgBW. In CBW both muscle water ( $P < 0.001$ ) and blood glucose ( $P < 0.05$ ) concentrations were significantly reduced, whereas between the BW and FW groups there were no significant differences (Fig. 3). In MgBW muscle water concentration was only slightly lower ( $P < 0.05$ ) than in

Table 3. Significances (Student's  $t$  test) of differences in plasma ionic concentrations and blood MCHC between five test groups of *Esox lucius* (Expt. III). For groups, see Table 2.

	Group	1	2	3	4
Chloride	2	***			
	3	NS	***		
	4	**	***	*	
	5	***	***	***	***
Sodium	2	***			
	3	NS	***		
	4	*	***	**	
	5	**	***	**	NS
Magnesium	2	**			
	3	NS	**		
	4	***	***	*	
	5	***	***	*	*
MCHC	2	*			
	3	***	*		
	4	*	NS	NS	
	5	***	***	***	***

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$

NS = not significant

BW, but blood glucose concentration was unaffected.

As compared with BW-acclimated fish, blood MCHC increased significantly in CBW, and this was still more notable in MgBW (Fig. 3, Table 3). During 1-week exposure to FW, however, MCHC was also increased (Fig. 3), but after 3 weeks in FW the values had fallen to a lower level probably typical of FW specimens (Figs. 1 and 3).

#### 4. Discussion

Plasma concentrations of  $\text{Na}^+$ ,  $\text{Cl}^-$  and  $\text{Mg}^{2+}$  in pike are clearly dependent on ambient salinity, and fish living in BW and FW environments maintain these electrolytes at different levels. In stenohaline FW teleosts as a rule exposure to higher salinities is followed by a rapid increase in plasma osmotic pressure and concentrations of major ions (BLACK 1957, LAHLOU *et al.* 1969, LUTZ 1973, MÜLLER *et al.* 1974, DAVIS & SIMCO 1976, NORTON & DAVIS 1977). Accordingly, in pike plasma concentrations of  $\text{Na}^+$ ,  $\text{Cl}^-$  and  $\text{Mg}^{2+}$  increased significantly in slightly hyperosmotic BW (CBW), but did not exceed the levels in the

water tested, as they did in *Leuciscus rutilus* (MÜLLER *et al.* 1974). The increase of plasma  $Mg^{2+}$  in CBW cannot have been directly due to the water  $Mg^{2+}$  concentration itself, because in pike exposed to  $Mg^{2+}$ -supplemented BW (MgBW) the plasma concentration was unaffected. Probably,  $Mg^{2+}$  ions accumulate in the blood plasma because of alimentary ingestion and absorption of the medium (HICKMAN & TRUMP 1969). Factors which may induce pike to drink more are higher osmotic (SHARRAT *et al.* 1964, MOTAIS 1967) or  $Cl^{-}$  (HIRANO 1974) concentrations. In the stenohaline *Carassius auratus*, the drinking rate was augmented during adaptation to a slightly hyperosmotic medium (LAHLOU *et al.* 1969). In the eel *Anguilla japonica* water with an NaCl concentration as low as 10 mM was effective in enhancing the drinking rate in FW-adapted fish (HIRANO 1974). So possibly the higher plasma  $Mg^{2+}$  concentration in BW with salinity of about 6 ‰ is associated with drinking of minute amounts. The increased level of plasma  $Mg^{2+}$  in CBW may also indicate that pike are unable to maintain a steady state in the plasma by renal excretion of divalent ions, in contrast to migrating coho salmon (*Oncorhynchus kisutch*) transferred to 25 ‰ S (MILES & SMITH 1968).

Although more water is taken up by drinking and less is lost by renal excretion (HICKMAN & TRUMP 1969), significant dehydration of muscle tissue occurred in CBW (Fig. 3), indicating that in hyperosmotic salinities the maintenance of body water balance is impaired.

In CBW blood glucose concentration declined significantly (Fig. 3). This accords with the observations of MÜLLER *et al.* (1974) in another stenohaline FW teleost, *L. rutilus*, in which the decline was associated with a sharp reduction in liver glycogen concentration, i.e. by use of energy reserves.

Mean corpuscular haemoglobin concentration (MCHC) of blood, which is used here as a rough index of red blood cell volume (see OIKARI 1978), shows increased values both in hyperosmotic sea water, which may passively follow from increased osmotic concentration in blood plasma, and also in  $Mg^{2+}$ -supplemented BW. The changes in MCHC seen in FW-exposed pike may indicate an overshoot in the immediate volume regulation, but are followed by an increase in red cell volume to a level typical of FW-adapted pike.

In conclusion, the Finnish pike population studied shows considerable homogeneity in the physiology of its ionic and water regulation. The results point to failure of iono/osmoregulation in salinities moderately hyperosmotic in relation to the blood.

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