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Seasonal changes in the hypo-osmoregulatory ability of brook charr: the role of environmental factors

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In winter, brook charr *Salvelinus fontinalis* demonstrated poor osmoregulatory ability. Salt-water (SW) transfer resulted in sharp increases in plasma osmolality and chloride levels, a decrease in white muscle water content and high mortality. A clear improvement of the hypo-osmoregulatory mechanism efficiency was observed in spring; by May or June, fish achieved full acclimation within 1 week of SW transfer. In early fall, the time needed to restore osmotic and ionic balance increased once again. A high level of stress was detected only in winter and fall, as indicated by plasma cortisol and glucose concentrations. Great improvement in osmotic and ionic regulation was observed when brook charr were transferred to 8° C SW in February and April while impaired SW adaptability was recorded in individuals transferred to 2·5° C SW in June and August. Temperature itself was more important than a thermal gradient between fresh and salt water. Stress indicators substantiate this temperature-dependent improvement of osmotic and ionic regulation in brook charr. The results support the hypothesis of a minor contribution of cortisol to hypo-osmotic regulation in brook charr, as the cortisol response always seemed to be associated with stress in the experiments.

Key words: salinity; temperature; osmoregulation; ionoregulation; cortisol.

INTRODUCTION

Smolting is considered to be undeveloped in brook charr *Salvelinus fontinalis* (Mitchill) and estuarine residence has been suggested to be an important step for saltwater (SW) acclimation and eventual seaward migration (McCormick *et al.*, 1985; McCormick & Saunders, 1987). In natural populations of eastern Canada, both the initiation of the seaward migration and the duration of SW residence seem to be related to water temperature (White, 1941; Smith & Saunders, 1958; Dutil & Power, 1980). No evidence of physiological preparation before movement to SW was found in an anadromous brook charr population (McCormick & Naiman, 1984*a*), nor were such cues found by Audet & Claireaux (1992) in a study on a domestic strain. In contrast, major differences in the ability of brook charr to tolerate direct transfer to SW in spring and summer were demonstrated, these differences being related to different levels of gill Na^+-K^+-ATPase activity (Besner & Pelletier, 1991; Pelletier & Besner, 1992). Studies on related species, Arctic charr *Salvelinus alpinus* (L.) (Finstad *et al.*, 1989; Arnesen *et al.*, 1992;

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Schmitz, 1995) and Dolly Varden *Salvelinus malma* (Walbaum) (Johnson & Heifetz, 1988) also support the idea of a differential seasonal ability in charr to SW acclimation.

To understand better the physiological processes involved in the seaward movement of brook charr, and to determine if this species shows seasonal differences in its ability to live in SW environments, we performed a series of SW transfers on two different year cycles. These experiments involved natural variations of photoperiod, salinity, and temperature typical of an estuarine environment. Our second objective was to test the importance of temperature conditions in the ability of brook charr to acclimate to SW. In natural conditions, SW and FW temperatures are usually different. In the study area, SW is colder than FW. The present study considered both the SW temperature itself and the temperature gradient that is experienced by the fish when it is transferred from FW to SW. In such a case, moving from FW to SW results in both a thermal and an osmotic shock. To be able to clarify the relationship between osmoregulatory processes and water temperature, and to discriminate between the effects of salinity *per se* and those of the temperature gradient between the two environments, a series of experiments was conducted in which the temperature gradient between FW and SW was manipulated. In these experiments, the thermal gradient was either cancelled or reversed in comparison to the normal situation at the time of the transfer.

METHODS

Brook charr were purchased from a certified disease-free local hatchery that produces hybrids between two domestic strains, Nashua and Baldwin. They were brought to the INRS aquaculture station in Pointe-au-Père, Québec (48°31′N, 68°29′W), where they were raised under natural temperature and photoperiod conditions in running dechlorinated tap water. Fish were acclimated to laboratory conditions for at least 1 month before being used for experimental purposes. They were fed daily (0·4% w/w ration) with commercial dry pellets.

Saltwater transfer experiments involving no manipulation of water conditions were done twice: from October 1988 to August 1989 (experiment 1) and from November 1991 to August 1992 (experiment 2). The time lapse between these two series of experiments was due to the building of new wet laboratory facilities. Consequently, experimental conditions differ slightly between the two sets of experiments. In both cases, dechlorinated fresh tap water from the city of Pointe-au-Père was used. However, FW temperatures recorded during the late autumn and winter in experiment 1 were above those measured during the experiment 2 (Table I). Salt water was pumped from the St Lawrence estuary near the wet lab facilities. In experiment 1, the SW was pumped from a depth of 5 m and filtered through sand filters. In experiment 2, the SW pumping system had been relocated to a less exposed site *c.* 100 m away and 5 m deeper. This resulted in a reduction of the sediment charge in the incoming SW. The sand filtering system was the same. Salinity and SW temperature followed the natural seasonal changes occurring in the St Lawrence estuary in the area (Table I). While natural illumination was provided in experiment 1, the new lab facilities used in experiment 2 were equipped with an incandescent lighting system; changes in photoperiod were reproduced with timers.

During experiment 2, in November, February, April, June and August, two additional transfers were conducted. In the first, heating SW to FW temperature eliminated the temperature gradient between FW and SW. The second was done into SW heated to 8° C (in November, February, and April) or chilled to 2° C (in June and August) thus inverting the temperature gradient that normally occurs along with the transfer from FW to SW.

TABLE I. Temperature and salinity (mean ± S.D.) conditions in experiments involving no manipulation of natural conditions

	Freshwater temperature (° C)		Saltwater temperature (° C)		Saltwater salinity	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
February	3·9 ± 0·5	1·0 ± 0·0	− 1·2 ± 0·1	− 0·5 ± 0·0	31·1 ± 1·2	30·3 ± 0·8
April	6·8 ± 0·7	3·1 ± 0·8	2·4 ± 1·2	2·8 ± 0·9	28·3 ± 2·6	28·7 ± 1·2
May	8·7 ± 1·1	8·0 ± 0·7	4·7 ± 1·4	5·2 ± 0·6	27·3 ± 3·3	25·8 ± 1·1
June	9·7 ± 0·9	10·4 ± 6·5	8·1 ± 1·5	6·2 ± 0·4	27·1 ± 2·4	28·5 ± 1·4
August	14·3 ± 0·4	12·7 ± 0·4	9·4 ± 2·1	7·7 ± 0·3	28·3 ± 2·3	27·3 ± 1·6
October–November	10·3 ± 0·7	5·4 ± 0·6	5·6 ± 2·1	2·1 ± 1·4	27·0 ± 1·2	28·9 ± 0·4

Experimental transfers to SW were made on animals of both sexes (1+ year old); because of seasonal growth fish size could not be standardized between experiments (Table II). For each experiment, fish were transferred directly to SW in 500-l insulated tanks. Sixty fish were used for each transfer in experiment 1 and 80 fish per transfer in experiment 2. Samplings were done at day 0 (control FW fish sampled from the stock tank) and after 1, 3, 7, and 21 days in SW. Feeding was discontinued 48 h prior to any SW transfer or sampling, which means that experimental fish were fed on days 3, 4, 7, 8, 9, 10, 11, 14, 15, 16, 17, and 19. All samplings began at 1000 hours and the sampling of the eight fish was completed before 1130 hours.

For each sampling, eight fish were netted randomly from the experimental tanks. Special care was taken when sampling the fish. Fish were sampled one at a time. The sampler waited until a fish came close to the net to capture it to avoid chasing in the tanks. Fish were placed immediately in a dark-coloured bucket containing the anaesthetic solution and transported to an adjacent laboratory to perform measurements and caudal puncture. Each fish was anaesthetized in 0·02% MS-222 (3-aminobenzoic acid ethyl ester) neutralized with NaOH. After measurement of the body weight and length, blood was drawn by caudal puncture using ammonium-heparinized syringes. A small quantity of blood was kept for haematocrit and haemoglobin measurements and the remaining blood was centrifuged at 7000 ***g*** for 3 min. Then plasma aliquots were prepared, frozen on dry ice, and stored at -80° C for further analyses. A piece of epaxial muscle was excised, weighed, then dried at 80° C for 48 h. For each fish, blood haematocrit, haemoglobin, plasma osmolality, chloride, glucose, and cortisol was measured. Mean cellular haemoglobin concentration (MCHC) and percentage of water muscle content were calculated. In winter and spring, fish were small and the low blood volume prevented measurements of haemoglobin and osmolality for some groups. Cortisol was measured using a solid-phase (antibody-coated tube) technique (Kallestad procedure No. 825) requiring 20 µl of plasma per assay. The Quanticoat ^{125}I-Cortisol RIA kit used an antiserum directed against a cortisol-3-(O-carboxymethyl) oximino-bovine serum albumin conjugate. The analyte-specific rabbit antibody was bound to the walls of each tube. The per cent cross-reactivity (weight of analyte required to displace 50% of ^{125}I analyte per weight of unknown required to displace 50% of ^{125}I analyte) was reported to be 101·54 for cortisol, 0·41 for corticosterone, 0·01 for cortisone, 13·11 for deoxycorticosterone, 4·15 for 11-deoxycortisol, 14·22 for 21-deoxycortisol, 0·14 for dexamethasone, 1·24 for 6β-hydroxycortisol, 0·39 for prednisone, 45·00 for prednisolone, 0·01 for progesterone, 0·12 for 17-OH progesterone, and 0·05 for tetrahydrocortisone. Large brook charr were sampled and their plasma separated into aliquots for further determination of intra- and interassay coefficients of variation and to prepare serial dilutions of fish plasma for specificity checking. Serial dilutions of fish plasma were assayed and the slope of the displacement curve compared with the slope of the standard curve. Slopes were homogenous (ANCOVA, $\alpha = 0{\cdot}001$). Preliminary measurements showed that plasma cortisol was often below the average human plasma concentration for which the kit was designed (standard values: 1·0, 2·7, 10, 25, 60 µg 100 ml^{-1}). Then dilutions of the standards were prepared using the free hormone human serum in order to increase the range of the standard curve (0·25, 0·5, 0·75, 1·0, 2·7, 10, 25, 60 µg 100 ml^{-1}) (Audet & Claireaux, 1992). All assays were performed with 20 µl samples. With brook charr plasma, the intra- and interassay coefficients of variation were 8·7 and 8·3%. When plasma concentrations were too low to be measured adequately, they were assigned the lowest standard value of the modified assay (2·5 ng ml^{-1}). Calculation of the radioimmunoassay results was made with a LKB Clinigamma 3 counter utilising the spline-function program. Blood haemoglobin was measured by the cyanmethaemoglobin method (Sigma procedure No. 535A); plasma glucose was measured by enzymatic assay (Sigma No. 16UV); plasma osmolality was measured with an Advanced Micro-Osmometer 3MO; plasma chloride was measured with a Corning Chloride Analyzer. All assays were made in duplicate when sufficient plasma was available; a sample was re-assayed when the deviation from the mean was >5%.

Data were expressed routinely as mean ± S.E. (n), where n represents the number of fish. Normality and homogeneity of variances were checked by Kolmogorov–Smirvov and

TABLE II. Weight and length (mean ± S.D.) of brook charr transferred to salt water

Month	Experiment 1		Experiment 2		Natural thermal gradient		Absence of thermal gradient		Inverted thermal gradient	
	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)
February	63·2 ± 23·3	18·8 ± 1·9	62·5 ± 18·4	19·0 ± 1·8	62·6 ± 18·2	19·5 ± 2·1	55·3 ± 17·4	18·6 ± 1·8	49·6 ± 18·0	17·9 ± 1·9
April	73·1 ± 25·2	19·6 ± 2·2	69·2 ± 28·2	19·2 ± 2·2			64·3 ± 24·1	18·8 ± 2·0	58·7 ± 25·2	18·5 ± 2·3
May	78·7 ± 23·8	20·3 ± 1·9	74·4 ± 24·9	19·6 ± 2·0						
June	92·7 ± 18·9	21·2 ± 1·3	83·7 ± 21·6	20·5 ± 1·7	79·7 ± 28·2	20·1 ± 2·1	90·3 ± 25·6	24·4 ± 2·6	80·8 ± 20·6	20·5 ± 1·6
August	129·0 ± 36·5	23·1 ± 2·2	100·6 ± 19·6	22·0 ± 1·2	99·6 ± 19·8	22·0 ± 1·3	110·5 ± 21·2	22·5 ± 1·2	99·4 ± 17·9	22·1 ± 1·2
November	161·3 ± 42·8	25·2 ± 2·2	124·2 ± 48·6	23·3 ± 2·6	114·1 ± 40·3	23·0 ± 2·2	118·9 ± 39·8	23·0 ± 2·5	111·3 ± 33·6	23·1 ± 2·3

F_{max} tests respectively. Some sets of data had to be transformed prior to statistical analysis to get homogeneity of variances: fish weight, haematocrit, and MCHC were transformed as log (x); plasma chloride, osmolality, and glucose as x^{-1}. Multiway ANOVA analyses were done: years, months and days were used as factors when experiments 1 and 2 were compared; SW temperature conditions (normal, absence of thermal gradient, inverse thermal gradient), months and days were used when the effect of temperature was studied on the response to SW transfer. A covariate effect with fish size (weight and length) was also tested for all variables. *A posteriori T′* tests of comparison of means (Sokal & Rohlf, 1981) with $\alpha=0.05$ were applied following ANOVAs. For those variables for which transformations failed to give homogeneity of variances, the Games and Howell test was applied for comparison of means, designed for heterogeneous variances (Sokal & Rohlf, 1981). Cortisol data were not normally distributed; they were analysed using the nonparametric Kruskal–Wallis ANOVA. When significant effects were found, the Kruskal–Wallis test was followed by an *a posteriori* LSD test on ranks (Daniel, 1978).

RESULTS

SW TRANSFERS IN NATURAL TEMPERATURE AND SALINITY CONDITIONS (EXPERIMENTS 1 AND 2)

Following transfer to SW, year, month and day were significant determinants of both plasma osmolality and plasma chloride ($P \leq 0{\cdot}01$). Significant interactions between factors (year × month, year × day, and month × day, $P \leq 0{\cdot}05$) were also found. Despite this year effect, similarities occurred between experiments 1 and 2, such as rapid SW acclimation in spring and absence of SW acclimation in autumn and winter (Figs 1 and 2). These results were not related to fish length or weight as neither of these were significant covariate factors ($P > 0{\cdot}05$).

In February, no acclimation to SW occurred; plasma osmolality and chloride kept rising during the 21-day experimental period. Only one fish was alive after 21 days of exposure to SW in both experiments (Table III). In April, there was a clear improvement of the hypo-osmoregulatory performance characterized by both a decrease in the amplitude and duration of the osmotic imbalance and a full recovery to FW levels (day 0) by the end of the experimental period. The best osmotic performance was observed in different months for the two experimental series (June in experiment 1; May in experiment 2) and very low mortality was observed (Table III). For these two transfers, return to FW osmolality was achieved by 7 days. In experiment 2, this period of improved osmoregulatory performance was also characterized by the absence of a significant rise in plasma chloride. Later in the summer, a response similar to that in April was observed for plasma chloride while plasma osmolality remained higher than in FW fish (day 0) until the end of the experiment. A further increase in the osmotic imbalance and the absence of a return towards FW control values for either osmolality or chloride was seen in October–November while mortality increased substantially (Table III).

As expected, changes in the white muscle water content reflected the plasma osmotic response (Fig. 3). The most rapid return to the FW level was observed in June in experiment 1 and in May in experiment 2. The general results of the ANOVA were identical to those described for osmolality and chloride except that the overall year effect was not significant. In both experiments, the

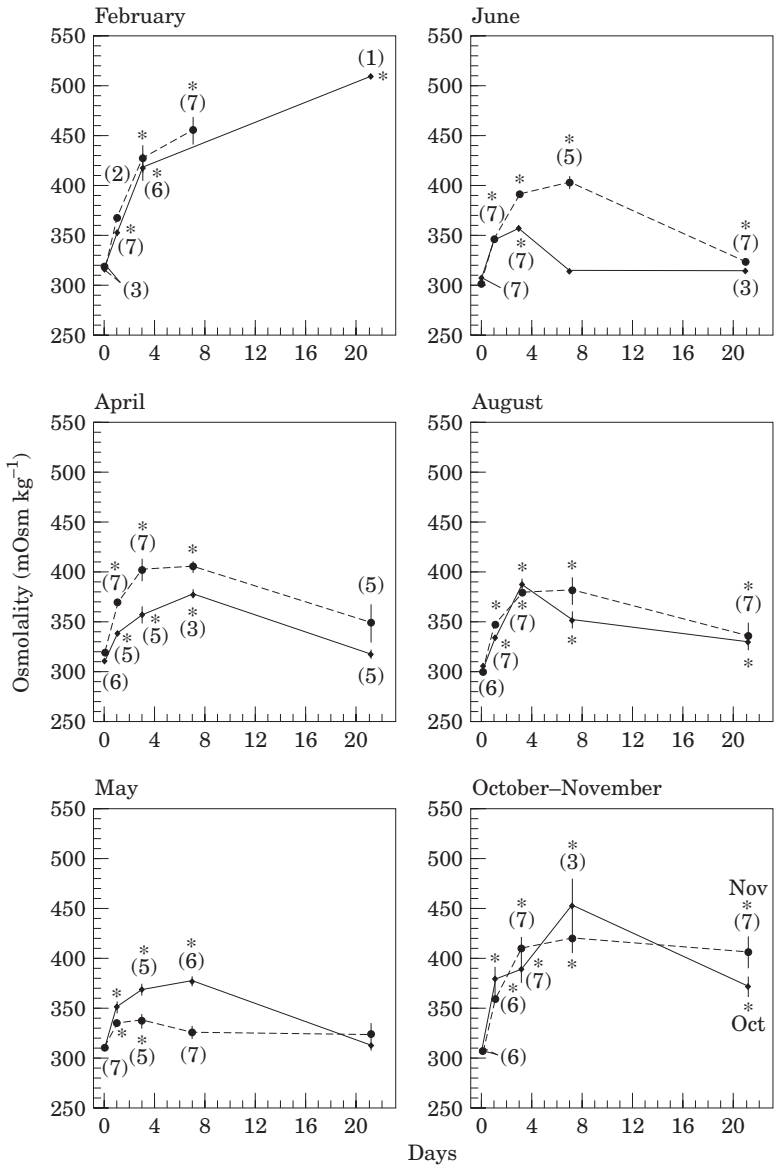

FIG. 1. Time course changes in plasma osmolality in brook charr directly transferred to SW at different time of the year. The asterisks (*) indicate that the mean in SW is different from the mean in FW on day 0. Means ± S.E. Number of fish (n) is indicated when different from 8. ——, Experiment 1; – – –, experiment 2.

regulation of muscle water content was efficient in all salinity trials except in February, when no signs of recovery were observed, and in October–November, when muscle water content was restored only partially. In experiment 1, a significant difference with FW fish was observed on day 21 in May, but this response can be ascribed to an over-compensation in the regulation of muscle water content, which then became higher in SW-acclimated fish than in

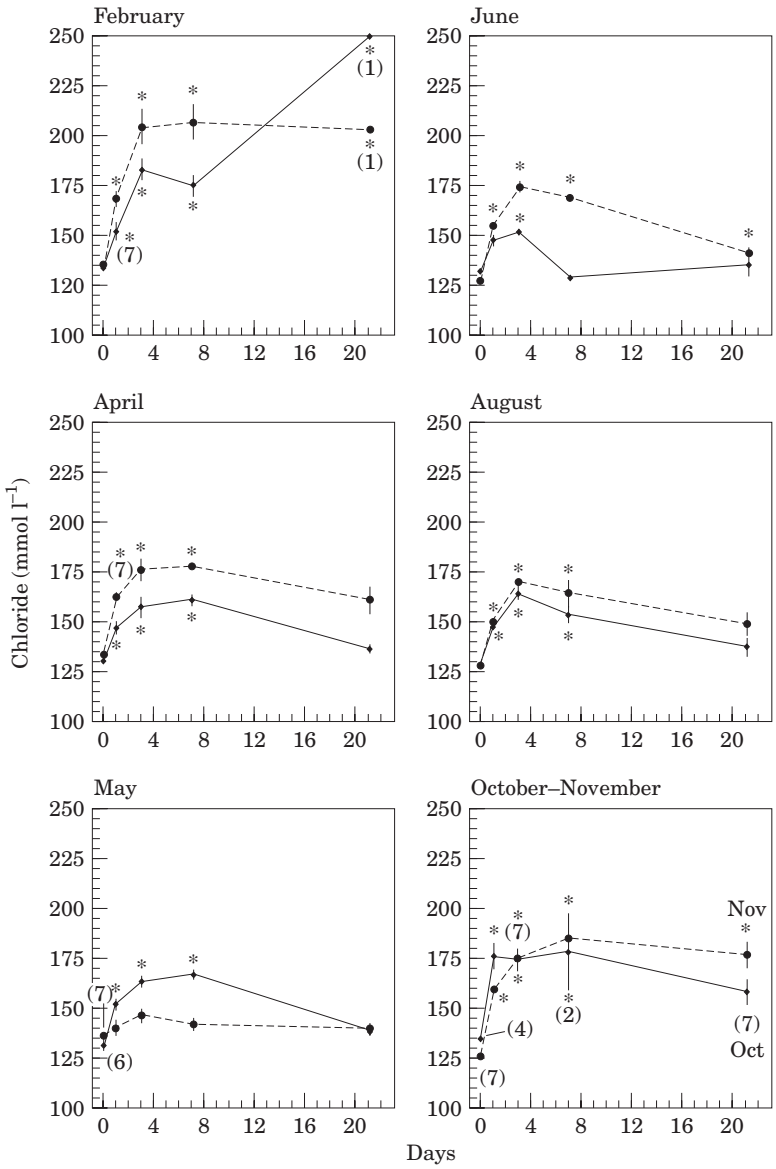

FIG. 2. Time course changes in plasma chloride concentration in brook charr directly transferred to SW. See Fig. 1 for more details.

FW control individuals. This result cannot be compared with the muscle dehydration observed in autumn and winter.

Other variables generally recognized as stress indicators were also measured. Cortisol measurements were not biased by a rank sampling effect ($\chi^2=12{\cdot}436$; d.f.=7; $P=0{\cdot}0871$). The highest plasma cortisol (Fig. 4) and glucose (Fig. 5) concentrations were observed in the transfers made in February; both parameters were still higher than in FW fish after 21 days. In October–November transfers, high plasma cortisol concentrations were also maintained throughout

TABLE III. Mortality in brook charr directly transferred to salt water

Experiment	Month	Days																					Total
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
Exp. 1	2						10		6	1	2	4	2	3		2		2				1		33
Exp. 2	2		1	1	2	5	9	4	5	3	6	1	1	3	1		1			2				45
Exp. 1	4						1		1		1	1												4
Exp. 2	4					4	1	5	4		2	1	1	2										20
Exp. 1	5							2			1				1						1			5
Exp. 2	5																			1				1
Exp. 1	6																					1		1
Exp. 2	6							1							1						1			3
Exp. 1	8				1	4	1	1														2		9
Exp. 2	8								1		1	2	1		1					1				7
Exp. 1	10					10	5	1	2	3	1	1			1			1		1				26
Exp. 2	11		1	2	3	10		1	2	3	1		2	3		3	1							31
NTG	2		1	1	2	5	9	4	5	3	6	1	1	3	1		1			2				45
ATG	2				5	5	4	2	5	2	1	1	2			1				2				30
ITG	2			1	8		2	1	1		1		1							1				16
NTG	4					4	1	5	4		2	1	1	2										20
ITG	4				1	1		5	1			1					1				1			11
NTG	6							1							1						1			3
ATG	6													2										2
ITG	6				1		2	3	3	3	2	1	4		1	1	1		1	2		2	2	29
NTG	8								1		1	2	1		1					1				7
ATG	8								3					1	1									5
ITG	8						1	2	4	4	3	2	3	5		1	2	4					1	32
NTG	11		1	1	3	10		1	2	3	1		2	3		3	1							31
ATG	11				4	3	5	2		4	1				1			1					2	23
ITG	11					2		1	4	3				1			3	1	1		1	1	1	19

Experiments 1 and 2 involved no manipulation of natural conditions. NTG, Natural temperature gradient; ATG, absence of thermal gradient; ITG, inverted thermal gradient.

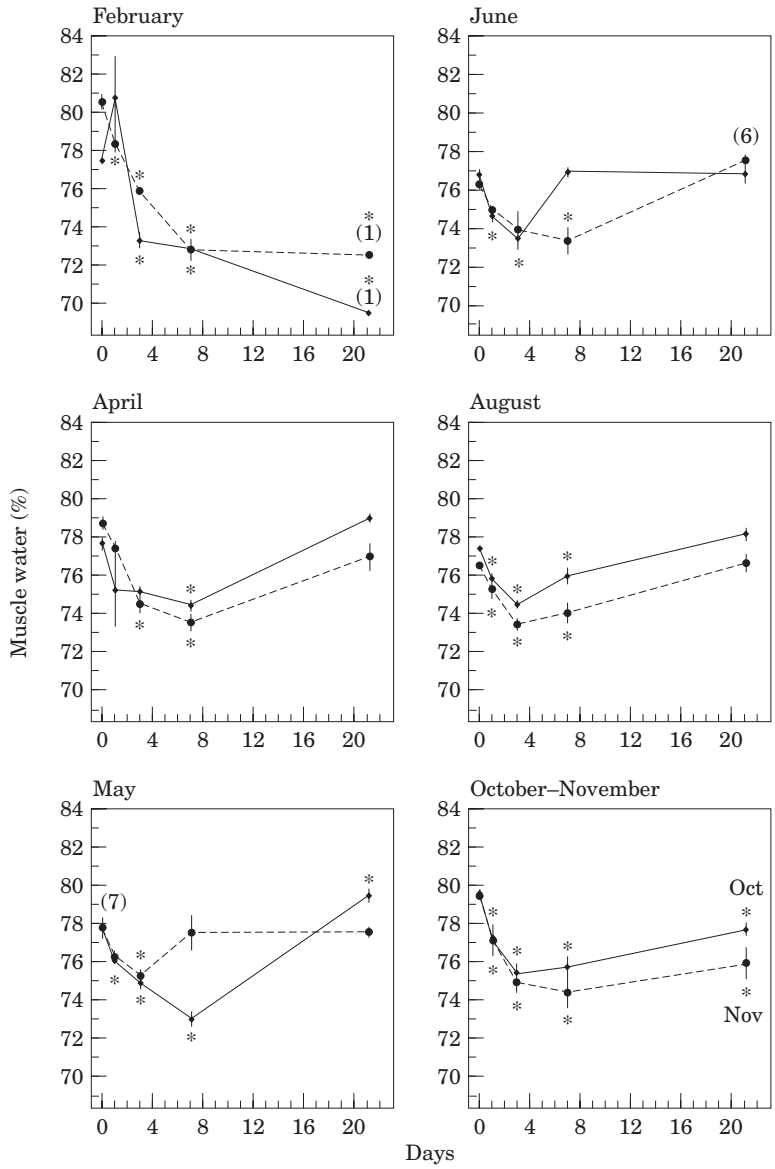

FIG. 3. Time course changes in muscle water content in brook charr directly transferred to SW. See Fig. 1 for more details.

the experimental period but this was more pronounced in experiment 1 than in experiment 2. In contrast to the February transfer, no significant change in glucose concentration occurred. A significant cortisol increase was generally observed following all transfers into SW. However, in contrast to autumn and winter, cortisol concentration was either back to FW levels or fell below 2 μg $100\ ml^{-1}$ 21 days after transfer. The pronounced variability of plasma glucose response did not allow the establishment of a clear relationship between the level of hyperglycaemia and the hypo-osmotic performance of brook charr. Even

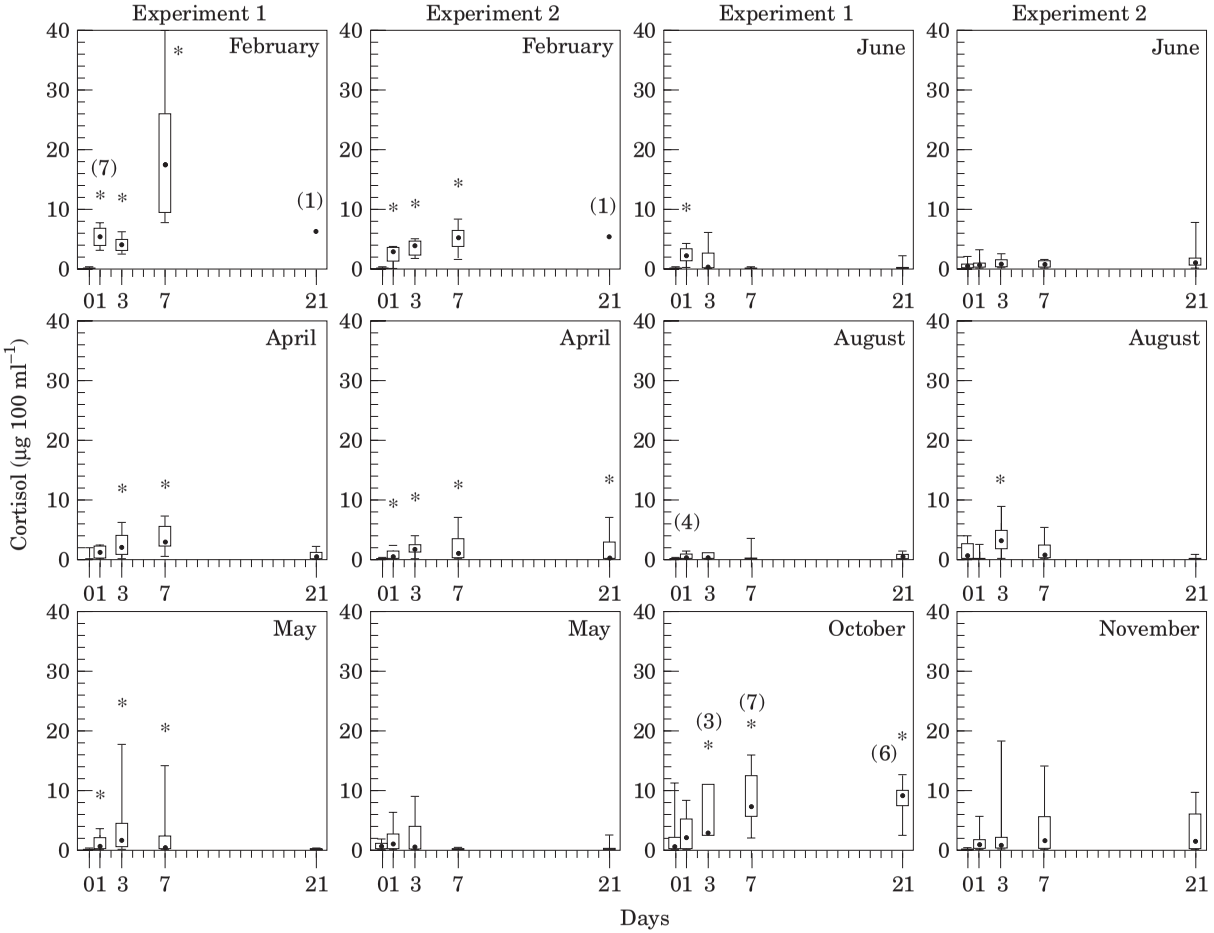

FIG. 4. Time course changes in plasma cortisol concentration in brook charr directly transferred to SW. Each box covers the middle 50% of the data values (between the lower and upper quartiles) and the whiskers extend out to the minimum and maximum values, while the central point (■, FW; ●, SW) is at the median. Number of fish (*n*) is indicated when different from 8.

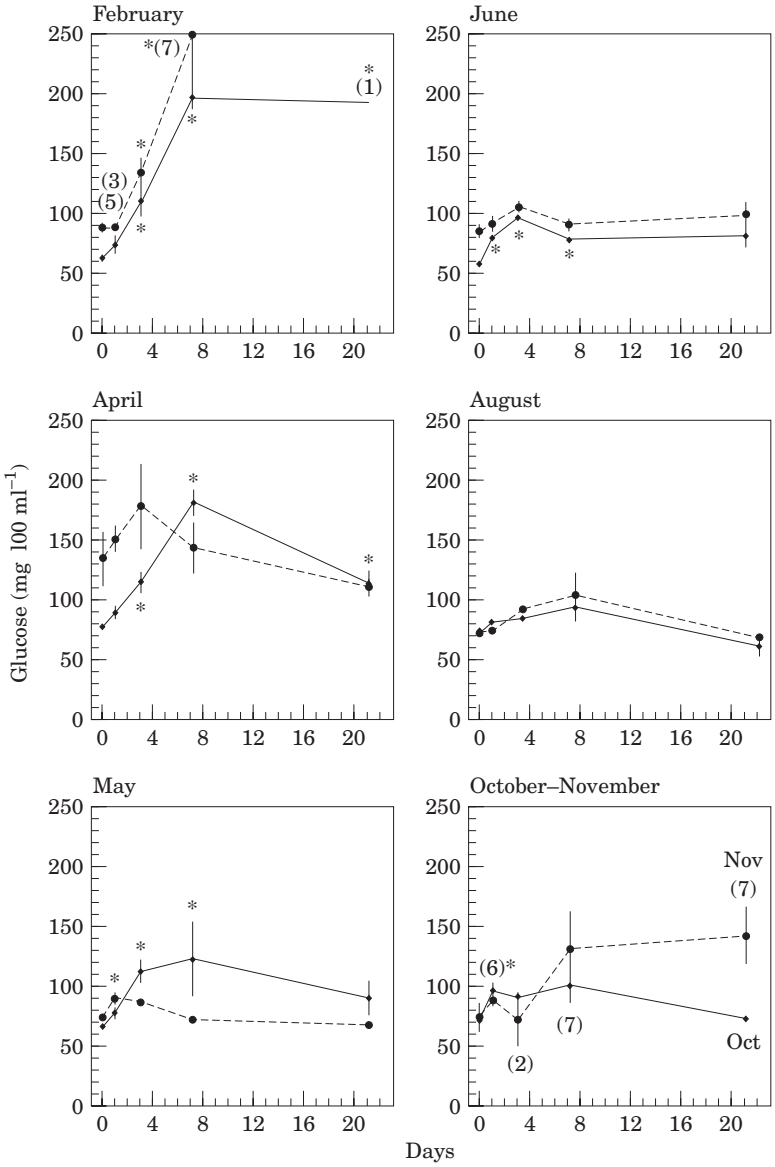

FIG. 5. Time course changes in plasma glucose concentration in brook charr directly transferred to SW. See Fig. 1 for more details.

though the nonparametric analyses of cortisol data did not allow the effect of fish size to be verified, length and weight were non-significant covariates for the glucose response ($P>0{\cdot}05$).

The general response of the haematological variables was characterised by the absence of a significant overall year effect, but again, interactions between factors were all significant ($P\leq 0{\cdot}05$). In May and August, no significant changes in any haematological variable were observed following transfer to SW except a transient 9% (experiment 1) and 20% (experiment 2) decrease in haematocrit on

day 3 in August. Only the haematocrit was measured in February and no significant change occurred then either. In April, a rise in blood and cell haemoglobin content was observed in both experimental series (Table IV). While the rise in blood haemoglobin concentration was still significant after 21 days, the MCHC was not significantly different from the FW level (day 0). In experiment 2, June SW exposure resulted in decreased haematocrit and unchanged haemoglobin concentration, leading to increased MCHC. No such changes were observed in the experiment 1. In fact, the main difference between the two sets of data lay in the lower haematocrit and haemoglobin levels reported in FW fish in June—experiment 1 compared with the control levels measured during the same month in experiment 2. In October–November, a significant rise in MCHC was observed during experiment 2 without significant concomitant changes for the two other variables. While haematocrit and MCHC response to SW transfers were also independent of fish size, fish weight was a significant covariate of the blood haemoglobin response ($P \leq 0{\cdot}05$).

SW TEMPERATURE MANIPULATION

The hypo-osmoregulatory ability was directly related to the SW temperature conditions in all experiments but the autumn. Except for haematological variables, the three factors considered (temperature condition, month, day) all had significant effects on the results ($P<0{\cdot}001$). In February, inverting the FW/SW thermal gradient by heating SW (8° C) allowed the surviving fish to regulate fully their plasma osmotic pressure and chloride concentration to FW control levels (day 0) (Figs 6 and 7). With no thermal gradient between SW and FW (SW heated to 2·5° C), fish were not able to acclimate fully, but the osmotic imbalance was reduced and more fish survived throughout the whole experimental period (Table III). In April, natural FW and SW temperatures were similar (no thermal gradient in natural conditions), so only a transfer to heated SW (8° C) was added. Then there were improved regulatory mechanisms, as indicated by shorter and less marked osmotic and ionic disturbances. The period of plasma osmotic imbalance was shorter and the intensity of the rise lower. In June and August, the removal of the temperature gradient was accompanied by a reduction in osmotic perturbations, although complete recovery did not occur within the 21 days of observations. However, plasma osmolality reached the FW level transiently on day 14 both in June and August. When the thermal gradient was inverted (water cooled to 2·5° C), the rise in both plasma osmolality and chloride was more pronounced and mortality increased substantially. In November, the trend first seen in August and leading to a temperature-independent loss in salinity adaptability, was confirmed. At this time of the year and in both experiments, none of the temperature gradient manipulations resulted in fully efficient regulation after 21 days in SW, although some improvements were noticed.

Under natural temperature conditions, changes in plasma cortisol and glucose concentrations were correlated with the seasonal changes in hypo-osmoregulatory ability. However, manipulation of SW temperature resulted in an uncoupling of the cortisol and glucose responses (Figs 8 and 9). In February, the absence of a thermal gradient between FW and SW reduced the amplitude of the rise in both plasma cortisol and glucose. On the other hand, the reversal of

TABLE IV. Time course changes in blood haematocrit, blood haemoglobin concentration and MCHC in brook charr directly transferred to salt water

Days	April		June		October–November	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
			Haematocrit (%)			
0	31·3 ± 1·7 (8)	29·3 ± 0·6 (8)	35·0 ± 1·0 (8)	44·7 ± 0·6 (8)	37·9 ± 2·6 (7)	36·5 ± 0·9 (8)
1	38·4 ± 3·1 (6)	26·6 ± 1·6 (8)	34·5 ± 1·1 (8)	44·0 ± 2·2 (7)	34·0 ± 1·5 (8)	35·2 ± 1·1 (8)
3	36·9 ± 2·3 (8)	29·5 ± 1·0 (8)	40·0 ± 2·4 (8)	38·5 ± 1·4 (5)	38·6 ± 4·0 (8)	31·1 ± 2·3 (8)
7	41·7 ± 1·6 (8)*	34·5 ± 2·1 (7)	37·7 ± 0·7 (8)	35·0 ± 1·3 (8)*	40·1 ± 2·7 (8)	34·1 ± 1·6 (8)
21	36·6 ± 1·2 (8)	36·2 ± 2·1 (7)*	36·2 ± 1·8 (8)	38·3 ± 1·3 (8)*	36·3 ± 2·3 (8)	38·3 ± 2·5 (8)
			Haemoglobin (g 100 ml^{-1})			
0	5·3 ± 0·2 (5)	5·9 ± 0·3 (7)	7·9 ± 0·2 (7)	8·8 ± 0·3 (8)	8·6 ± 0·5 (8)	6·7 ± 0·2 (8)
1	7·6 ± 0·5 (5)	6·3 ± 0·4 (8)	8·1 ± 0·2 (7)	8·4 ± 0·4 (8)	8·2 ± 0·4 (8)	7·4 ± 0·3 (8)
3	9·1 ± 1·5 (2)*	7·6 ± 0·5 (8)*	9·8 ± 0·5 (7)*	9·1 ± 0·3 (8)	9·4 ± 1·0 (8)	7·3 ± 0·9 (8)
7	9·3 ± 0·5 (3)*	8·3 ± 0·6 (8)*	6·7 ± 0·2 (7)	8·2 ± 0·4 (8)	9·8 ± 0·7 (8)	8·0 ± 0·5 (8)
21	7·8 ± 0·4 (5)*	7·2 ± 0·6 (8)*	8·6 ± 0·5 (7)	9·4 ± 0·4 (8)	8·5 ± 0·5 (8)	9·4 ± 0·6 (8)
			MCHC (g 100 ml^{-1})			
0	16·7 ± 1·5 (5)	19·9 ± 0·5 (7)	22·1 ± 0·6 (7)	19·6 ± 0·6 (8)	23·0 ± 1·3 (7)	18·5 ± 0·6 (8)
1	18·4 ± 3·3 (3)	24·2 ± 1·8 (8)	23·7 ± 0·9 (7)	19·4 ± 0·9 (7)	24·3 ± 1·0 (8)	20·9 ± 0·7 (8)
3	23·5 ± 1·3 (2)*	26·0 ± 2·1 (8)*	24·7 ± 0·7 (7)*	23·1 ± 0·4 (5)	24·7 ± 1·3 (8)	22·9 ± 1·3 (8)*
7	22·9 ± 1·7 (3)*	23·9 ± 1·6 (7)*	17·5 ± 0·5 (7)*	23·5 ± 1·2 (8)*	24·6 ± 1·2 (8)	23·5 ± 1·0 (8)*
21	21·1 ± 0·8 (5)	19·9 ± 0·8 (7)	24·3 ± 2·2 (7)	24·5 ± 0·5 (8)*	23·4 ± 0·5 (8)	24·8 ± 0·7 (8)*

Results are presented as mean ± S.E.; number of fish (*n*). The asterisks (*) indicate that the mean in SW is different from the mean in FW on day 0.

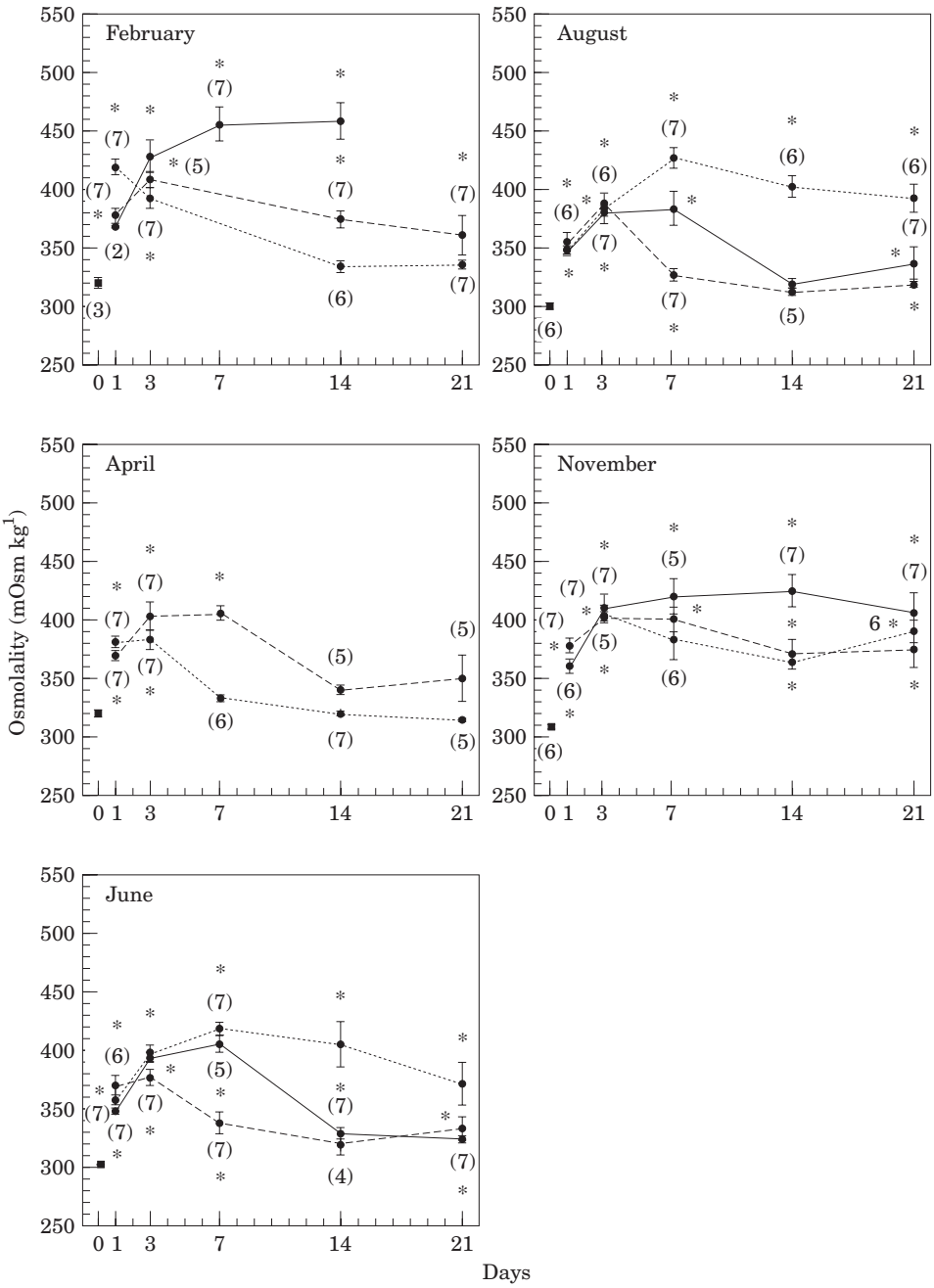

FIG. 6. Time course changes in plasma osmolality in brook charr directly transferred to SW in presence of natural temperature gradient (——), in absence of thermal gradient (– – –) or in inverted thermal gradient (· · ·). ■, FW; ●, SW. The asterisks (*) indicate that the mean in SW is different from the mean in FW on day 0.

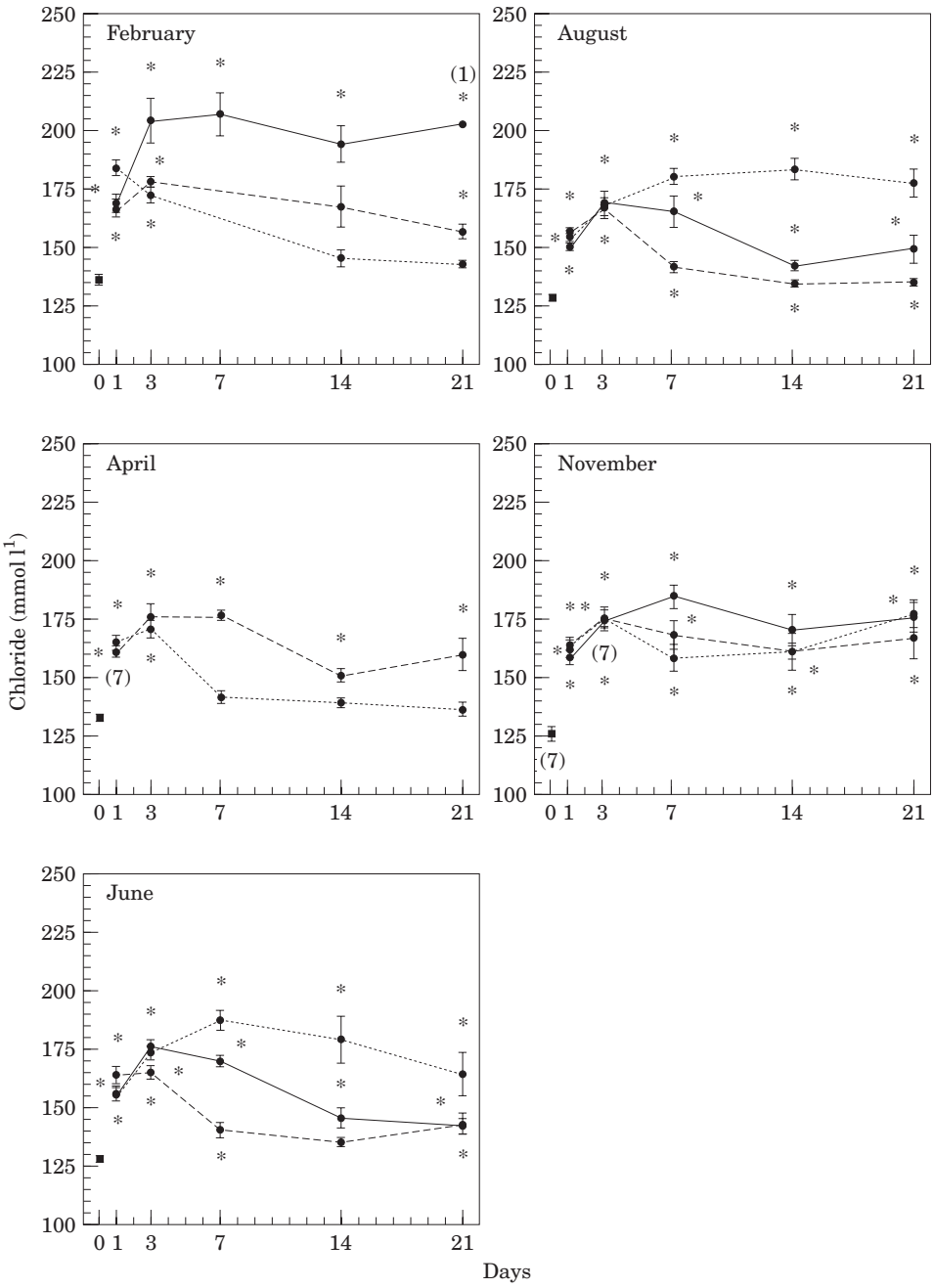

FIG. 7. Time course changes in plasma chloride concentration in brook charr directly transferred to SW in different SW temperature conditions. See Fig. 6 for more details.

the thermal gradient in both February and April was accompanied by less marked but still significant cortisol increases together with significant decreases in plasma glucose concentration. In June and August, plasma cortisol and

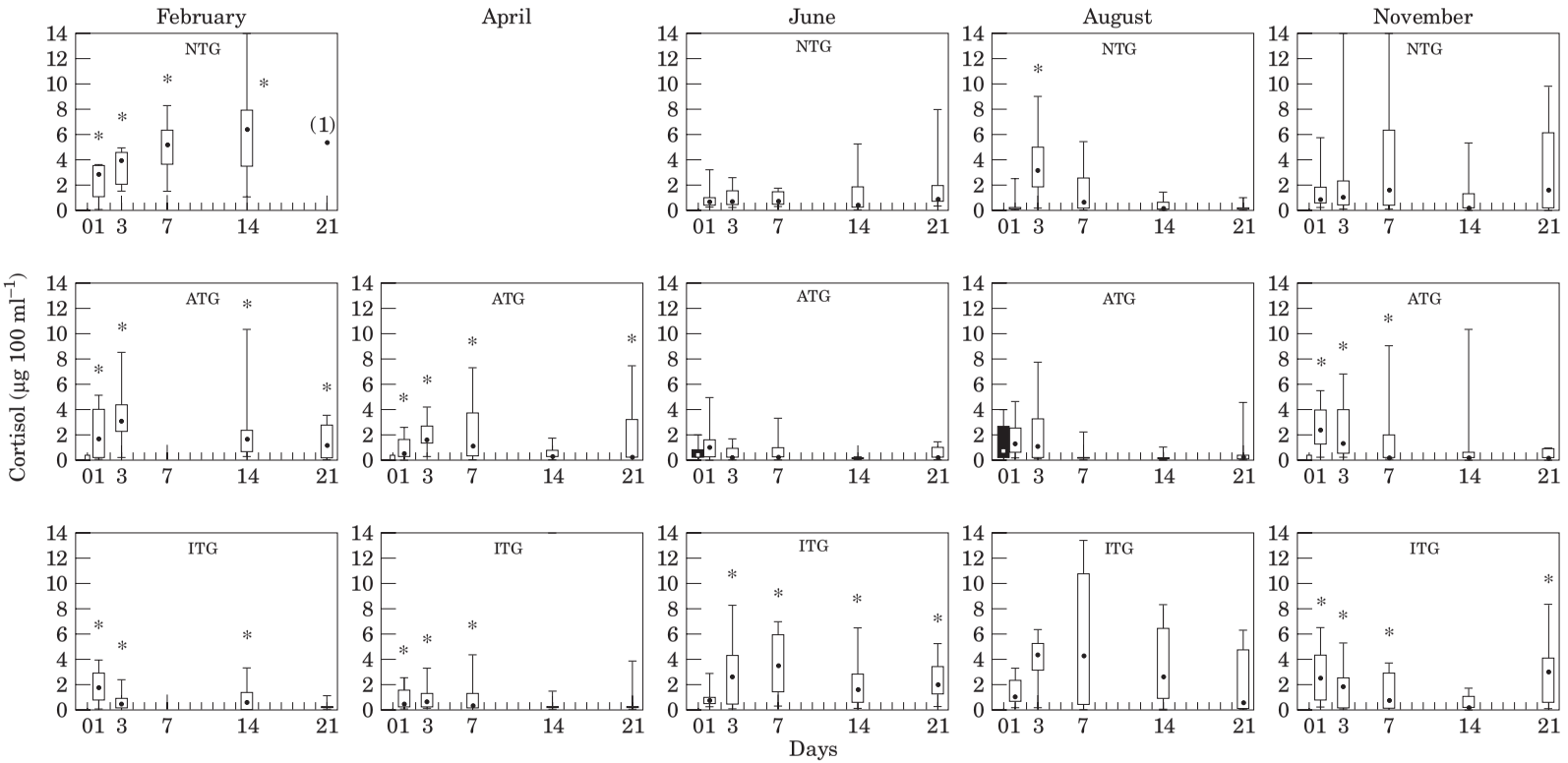

FIG. 8. Time course changes in plasma cortisol concentration in brook charr directly transferred to SW in different SW temperature conditions. NTG, Natural temperature gradient; ATG, absence of thermal gradient; ITG, inverted thermal gradient. For each month, FW data are presented only on the middle graph (ATG). See Fig. 4 for more details.

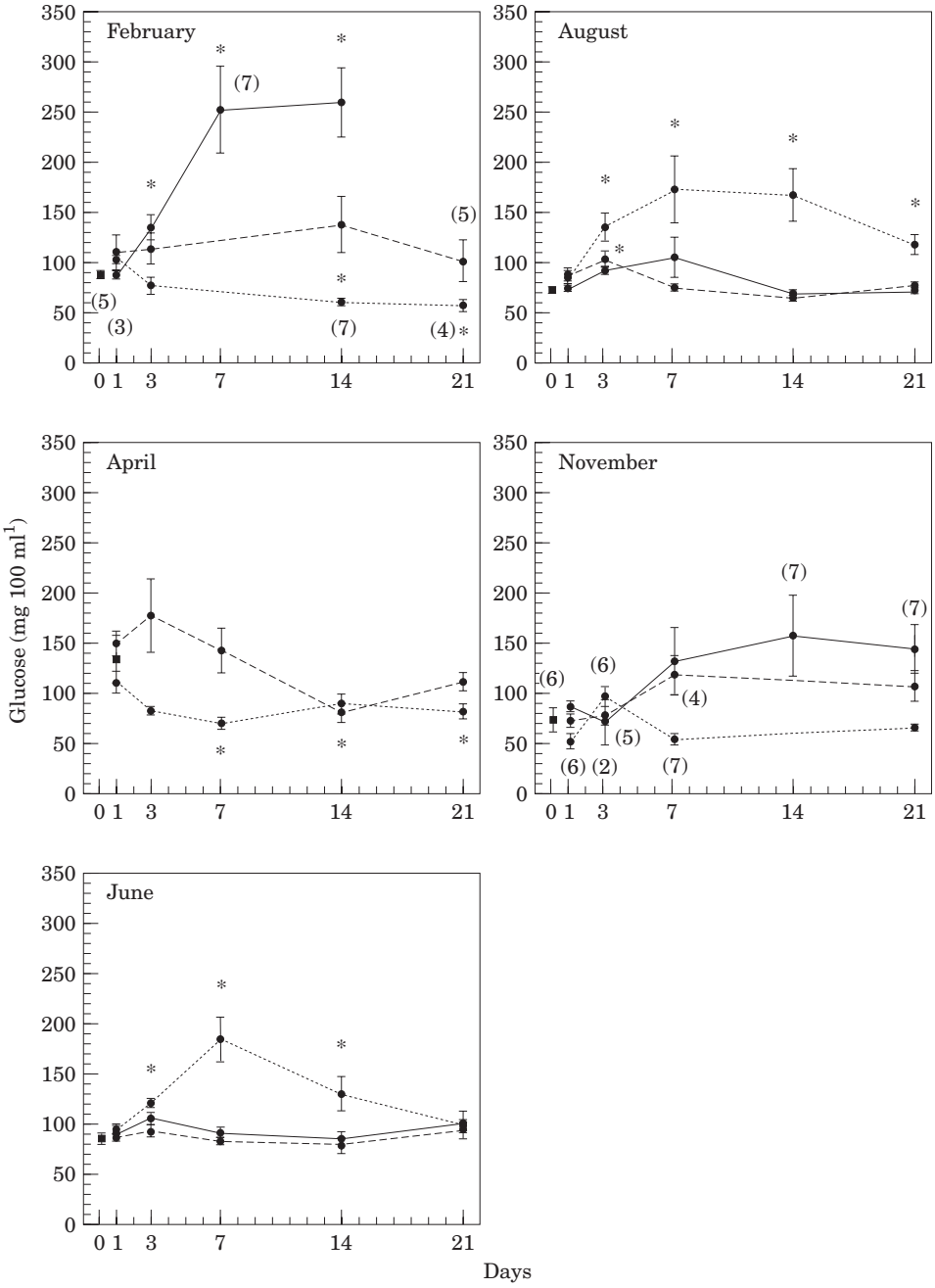

FIG. 9. Time course changes in plasma glucose concentration in brook charr directly transferred to SW in different SW temperature conditions. See Fig. 6 for more details.

glucose did not change following SW transfer under natural conditions or in the absence of a thermal gradient. However, decreasing SW temperature (inverted thermal gradient) produced significant rises in both variables for both months.

This rise was no longer significant for plasma cortisol in August in contrast to plasma glucose. In November, cortisol in SW-transferred fish remained above FW control levels whereas no significant differences in plasma glucose were found following SW exposure under the different temperature conditions ($P>0{\cdot}05$). The presence of a potential fish sampling rank effect on cortisol values was tested for each month and was nonsignificant except in August ($\chi^2=17{\cdot}5$, d.f.=7, $P=0{\cdot}0145$) where the seventh and eight sampled fish had higher values than the fourth first sampled fish. However, when the results from the three different temperature conditions were analysed separately, this rank effect was no longer significant (natural conditions: $\chi^2=9{\cdot}5$, d.f.=7, $P=0{\cdot}2172$; absence of thermal gradient: $\chi^2=8{\cdot}04$, d.f.=7, $P=0{\cdot}3289$; inverted thermal gradient: $\chi^2=12{\cdot}8$, d.f.=7, $P=0{\cdot}0772$) and comparisons between treatments remain unchanged.

No effect of SW temperature was found in the haematocrit, haemoglobin, or MCHC in response to SW transfer ($P>0{\cdot}05$). Interestingly, analysis for all the variables measured (except cortisol) showed significant covariate effect of fish weight in regard to this experimental design, indicating that the response to SW temperature was dependent on fish size.

DISCUSSION

For the last 50 years, field observation of migration patterns and the presence or absence of silvering have suggested the existence of seasonal variations in the hypo-osmoregulatory ability of brook charr (White, 1941; Wilder, 1952; Dutil & Power, 1980; Castonguay *et al.*, 1982; Montgomery *et al.*, 1990). Although the present study was carried out using a strictly FW-raised domestic strain of brook charr, the response to SW exposure displayed a strong and reproducible seasonal pattern. The highest hypo-osmoregulatory efficiency was observed in May or June, which is the period of the year when seaward migration takes place in natural anadromous populations. Only at this time of the year were fish able to regulate quickly their extra- (plasma) and intracellular (muscle) osmotic and ionic balance to the FW control level.

Freshwater controls were not sampled for each time point. Seasonal variations had been surveyed over a 12-month period in 1+ FW brook charr, including all variables measured in the present paper on the same age group (Audet & Claireaux, 1992). Seasonal changes were found for many of these variables, but not in ranges comparable with those that occur when a 1+ charr is transferred into SW. Moreover, all changes were significantly different on a seasonal basis and occurred rarely between two consecutive months (30- or 31-days interval).

When fish were transferred to SW during winter and early spring, plasma osmolality and chloride rose sharply, paralleled by a pronounced dehydration of the white muscle. The poor efficiency of the osmo-ionoregulatory mechanisms resulted in a high level of stress that was revealed by the long-term increase in plasma cortisol and/or glucose concentrations and by high mortality. These results corroborate those obtained for the closely related *Salvelinus malma*, whose ability for SW acclimation was correlated with water temperature (Johnson & Heifetz, 1988). Similarly, Saunders *et al.* (1975) showed that brook

charr exposed to cold SW in December experienced severe osmotic stress. These authors established that at 30‰, $-0{\cdot}7^{\circ}$ C would be a reasonable approximation of the lower lethal temperature for this species. The same depressive effect of low temperature on the osmoregulatory capacity in SW was shown in Atlantic salmon *Salmo salar* L., smolts (Sigholt & Finstad, 1990) and in rainbow trout *Oncorhynchus mykiss* (Walbaum) (Finstad *et al.*, 1988). The limiting effect of low SW temperature on osmoregulatory processes is substantiated further by the results of the temperature-manipulation experiments. In February, heating SW to summer temperature improved the efficiency of the regulatory mechanisms clearly even though it increased the temperature gradient between FW and SW. This greater influence of SW temperature over the magnitude of the FW to SW temperature gradient was found in all seasons except autumn.

The considerable but transient improvement in the hypo-osmoregulatory potential of charr in late spring (May/June) has been observed in both brook and Arctic charr. In brook charr, Besner & Pelletier (1991) examined responses to SW transfer monthly from May to August and observed an increase similar to the present one in the efficiency of the hypo-osmoregulatory mechanisms in June. In May and June, in addition to higher gill Na^{+}-K^{+}-ATPase activity in FW controls, they also observed faster activation of enzyme activity after SW transfer. This may underly the very rapid and complete regulation observed in May or June in the present study. Finstad *et al.* (1989) showed that the increased hypo-osmoregulatory ability of Arctic charr in June occurred in individuals acclimated to both 1 and 8° C, even though the performance was slightly better at 8° C. This rules out an exclusively temperature-related seasonal effect for this species, but indicates a possible role of photoperiod. The same authors also observed higher gill Na^{+}-K^{+}-ATPase activity in June compared with September and February. In landlocked Arctic charr, Schmitz (1992) found a very similar seasonal pattern of SW adaptability. Monthly SW challenge tests showed a slightly improved response in May and June. Present results show the important role of SW temperature in the hypo-osmoregulatory performance in the spring, as fish transferred to colder SW no longer performed better than those transferred at other times of the year. However, the numerous results showing improved SW adaptability in brook charr during spring also indicate a potential photoperiodic regulation.

In August, SW exposure resulted in osmotic and ionic disturbances of greater amplitude, suggesting that these fish had already lost some of their osmoregulatory potential. Similar results were obtained in both anadromous (Schmitz, 1995) and landlocked (Schimtz, 1992) Arctic charr. It was suggested that the decrease in SW adaptability at the end of the summer could be related to the increase in FW temperature. Besner & Pelletier (1991) showed that the decline in the hypo-osmoregulatory ability during summer was associated with a reduction in the gill Na^{+}-K^{+}-ATPase activity that could be related to the increase in FW temperature. In Atlantic salmon, the increase of FW temperature causes decreases in the Na^{+}-K^{+}-ATPase activity and salinity tolerance (e.g., Duston *et al.*, 1991).

During the autumn, a different situation was observed, as the amplitudes of osmotic and ionic perturbation following SW exposure were independent of SW temperature. Eliminating the temperature gradient or transferring fish to

warmer SW did not improve their osmoregulatory performance. Finstad *et al.* (1989) showed that the loss of hypo-osmoregulatory ability in Arctic charr in September and February was also independent of water temperature. In brook charr, McCormick & Naiman (1984*b*) reported that photoperiod affects SW survival only through its influence on male sexual maturation. However, this last study was done under a constant temperature of 10° C (FW holding and SW transfers); this could have masked or impaired complex interactions between environmental factors that regulate migratory movements. Lefrançois *et al.* (1997) studied the impact of gonad development alteration (ionizing radiation) on salinity tolerance in brook charr. They showed that following transfer to estuarine conditions in October, all sterile 1+ brook charr survived a prolonged stay in hyperosmotic environment during and beyond the spawning season while the maturing fish suffered high mortality. Based on these results and on those of McCormick & Naiman (1984*b*) discussed above, it could be hypothesized that in late summer–early autumn, the seasonal allocation of energy toward the gonads is, to a certain degree, incompatible with hypo-osmoregulation. Supporting this, Staurnes *et al.* (1994) showed that sexual maturation also severely impaired SW adaptability in anadromous Arctic charr kept under continuous daylight. Using hormonal implants, Lundqvist *et al.* (1989) showed that androgens inhibit SW adaptability in Atlantic salmon. In October–November, the degree of sexual maturation may override the effects of environmental factors on SW adaptability as SW temperature did not affect fish response. Moreover since 57% of the animals sampled in October were sexually immature, the question of the impact of photoperiod on the loss of efficient osmoregulatory ability in the fall remains open. The fact that the glucose response observed in the fall is different from the other seasons may be ascribed to the combined effect of the environmental conditions and sexual maturation cycle.

Fish used in the present study were obtained from an autumn-spawning brood stock. Because of the experimental design (no restraint imposed on growth), the 13-month-old animals tested in winter were much smaller than the 21-month-old individuals tested in autumn. The existence of a critical size influencing the timing of SW migration is generally recognized for salmonids (Folmar & Dickhoff, 1980); until they reach 14 cm, size was found to be the primary determinant of brook charr survival in SW (McCormick & Naiman, 1984*b*). In all our SW-exposure trials, fish were above that critical size, and neither weight nor length were significant covariates of the hypo-osmoregulatory responses when the various transfers were compared. The osmoregulatory ability of migrating Dolly Varden charr was not significantly correlated with fish length or weight (Johnson & Heifetz, 1988). These authors also hypothesized that the use of fish larger than the critical size for that particular species could explain this absence of correlation. Even though Besner & Pelletier (1991) and Pelletier & Besner (1992) selected their experimental fish for size (20 ± 1 cm), present results support completely their previous conclusions concerning the period from May to August under natural temperature conditions. In contrast to the present study, they observed a high mortality rate in summer, but these mortalities also could have been related to the occurrence of furunculosis in their stock (Pelletier, 1988). On the other hand, fish weight was a significant positive covariate for

osmo-ionic variables when the temperature treatments were compared, with cold SW being less tolerated than warmer SW by smaller fish.

Plasma cortisol concentrations in FW controls were low. Audet & Claireaux (1992) have already shown the presence of diel cycles in cortisol for 1+ FW brook charr that were different throughout the year. However, except in October, these cycles were all characterised by minimal values at 1000 hours for every month tested in the present study. In October, cortisol concentrations were consistently high but with no detectable diel cycle (Audet & Claireaux, 1992). Following SW transfers, changes in plasma cortisol concentration were reliable indicators of the intensity of the osmoregulatory stress faced by the animals. The decrease in the magnitude of the ionic and osmotic disturbances observed from February to June was accompanied by a marked decrease in the amplitude of the cortisol response. Conversely, the loss of hypo-osmoregulatory ability in October–November corresponded with higher and sustained rises in plasma cortisol concentrations. Plasma cortisol is generally recognized as a fairly good stress indicator in fish (Donaldson, 1981). Seawater-challenged coho salmon *Oncorhynchus kisutch* (Walbaum) also exhibit high cortisol concentrations associated with poor hypo-osmoregulatory ability; those high concentrations were also interpreted as a stress response (Avella *et al.*, 1990). Nichols *et al.* (1985) and Weisbart *et al.* (1987) reported rises in plasma cortisol levels and higher cortisol secretion rates (Nichols *et al.*, 1985) 48 h after the transfer of brook charr to SW. However these authors concluded that there was a direct role of cortisol in the adaptation of charr to the marine environment. It may still be argued that SW-acclimated fish in their experiment continued to display plasma chloride or osmolality values above FW control levels (e.g. 170 mEq l^{-1} in SW compared with $\approx$130 mEq l^{-1} in FW; 400 mOsm l^{-1} in SW compared with $\approx$300 mOsm l^{-1} in FW). In such circumstances, it can be suggested that the SW acclimation was not completed fully and that the measured cortisol concentrations were representative of the level of stress still experienced by the animals. Present data show that SW-acclimated charr can regulate their extracellular osmotic pressure to the FW level provided fish are in favourable environmental conditions.

Stress could also have been induced by the tank transfer itself or the reduced number of fish present in the tanks (sampling and mortality). Previous experiments in our laboratory have shown that, under these experimental conditions, the density variable alone had no significant effect on cortisol. In May and August, fish transferred at the same time, in similar tanks, and at similar densities were compared: control fish were transferred from FW to FW, experimental fish were transferred from FW to SW. There were no significant variations of cortisol concentrations in the FW controls over the 21-day sampling period (D. Hardy & C. Audet, unpublished data).

The possible role of cortisol in the proliferation or the differentiation of chloride cells has been suggested (Richman & Zaugg, 1987; Richman *et al.*, 1987; Laurent & Perry, 1990; Bisbal & Specker, 1991) but remains controversial. Indeed, in Atlantic salmon, studies have failed to establish a direct link between cortisol and the size or abundance of gill chloride cells or the direct stimulation of gill Na^+-K^+-ATPase (Langdon *et al.*, 1984; Langhorne & Simpson, 1986). An earlier experiment on FW fish did not allow identification of any change in

plasma cortisol that could indicate a link with the seasonal ability of brook charr to acclimate to SW that was shown in the present study (Audet & Claireaux, 1992). In recent years, the contribution of cortisol in facilitating survival in SW has been re-evaluated. Now its role is thought to be less prominent, at least in salmonids, while a predominant influence of the growth hormone has been suggested (Bolton *et al.*, 1987; Hasegawa *et al.*, 1987; Collie *et al.*, 1989; Yada & Hirano, 1991; Sakamoto *et al.*, 1993).

Elevated cortisol concentrations have been related to the necessity of maintaining high glucose concentrations in the blood, presumably to fuel increased energetic requirements during stress exposure (Leach & Taylor, 1980; Redding & Schreck, 1983; Virtanen & Oikari, 1984). Accordingly, the tremendous rise in plasma cortisol observed in February was accompanied by a 213% increase in glycaemia whereas the more subtle cortisol response measured at other times of the year was associated with less marked changes in plasma glucose. In cannulated brook charr transferred to SW, Nichols *et al.* (1985) found that plasma glucose did not vary as a function of cortisol. Leatherland (1985) suggested that a secondary stress response stage such as hyperglycaemia was not related necessarily to primary (catecholamines and corticosteroids) or tertiary stages (impaired growth, reduced reproductive success, and increased susceptibility to pathogens). Present results show that in experiments involving controlled SW temperature, the rise in plasma glucose was generally greater in the temperature treatment that brings the greater osmotic stress. Leatherland (1985) pointed out that the plasma glucose level may not be an adequate general and robust indicator of stress but may be rather more indicative of a specific type of stress in fish in a specific physiological state. Vijayan & Leatherland (1989), working on cortisol-implanted coho salmon, also suggested that the hyperglycaemic action of cortisol should be related to the presence of a certain cortisol threshold.

Haematological parameters have been used also as stress indicators, but the individual interpretation of each of the present experiments is not as straightforward in this respect. Under various experimental conditions, stress in fish results in the release of red blood cells from the spleen (Yamamoto *et al.*, 1980; Kita & Itazawa, 1989) and in the swelling of the erythrocytes (Baroin *et al.*, 1984; Cossins & Richardson, 1985; Borgese *et al.*, 1986). This double catecholamine-mediated process results in increased haematocrit and haemoglobin concentration and decreased mean cellular haemoglobin content (MCHC). Like other cellular compartments, blood is also subjected to dehydration (Bath & Eddy, 1979; Leray *et al.*, 1981). The loss of water from the plasma contributes to the increased haematocrit and haemoglobin concentration while red cell dehydration results in decreased haematocrit and MCHC. One might also speculate that haematocrit and blood haemoglobin should increase during SW transfer in response to a need for greater oxygen supply. Considering the above evidence, the perturbations reported in April may suggest a largely stress-related response, but the intricacy of the various processes involved makes a comprehensive interpretation of the haematological data difficult.

Taken together, these results substantiate the existence of a relatively small time-window during which brook charr are able to adapt efficiently to the estuarine environment. As is true for other charr species, seasonal differences in

SW adaptability are also present in brook charr, but annual SW temperature variation is clearly an important environmental factor restricting this ability to a brief period of the year, and possibly regulates anadromous migration. These experiments were made on domestic charr considered to be derived from exclusively FW populations. Currently our laboratory is conducting comparisons between this particular domestic stock and a wild anadromous strain from the Rivière Laval (Québec).

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