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THE EFFECTS OF SALINITY CHANGE ON THE EXERCISE PERFORMANCE OF TWO ATLANTIC COD (*GADUS MORHUA*) POPULATIONS INHABITING DIFFERENT ENVIRONMENTS

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Summary

The objective of this study was to determine whether differences in exercise physiology between Atlantic cod (*Gadus morhua*) populations from different salinity environments could be changed by acclimating individuals of each population to the natural salinity of the comparison population. The exercise-associated blood chemistry of cod from the brackish Bras d'Or lakes, which had previously been shown to be quite different from that of 'open-ocean' cod, changed to resemble the blood chemistry of their oceanic relatives after only 2 months of acclimation to full-strength salinity. In contrast, the blood chemistry of cod from the Scotian Shelf of the Northwest Atlantic Ocean showed little change after 2 months of acclimation to brackish water. These results demonstrate that the degree of osmoconformity to changes in environmental salinity is a population-specific not a species-specific trait. The blood chemistry differences between populations and salinities did not translate into differences in exercise performance: i.e. critical swimming speeds were statistically uniform across all combinations of population and salinity, although performance was more varied in fish swimming in 'non-native' waters. Other 'whole-animal' physiological characteristics, such as metabolic rate and the aerobic cost of transport, were dependent upon both population origin and the environmental salinity. Vigorous swimming was more energetically expensive at full-strength salinity than at 20‰ salinity, yet estimates of standard (i.e. resting) metabolic rate were lower for full-strength salinity. Environmental salinity also influenced the relative appearance of lactate and metabolic acid in the extracellular fluid compartment, with full-strength salinity favouring the relative appearance of lactate in the blood.

Multivariate statistical analyses of this data set showed that, in contrast to other fish species and studies, differences in blood oxygen transport appear to account for some of the swimming performance differences seen in Atlantic cod at 2 °C. The two experimental populations were cleanly separated by a principal components analysis, regardless of the salinity to which they were acclimated, confirming our earlier contention that these cod populations are physiologically distinct. A key feature of that distinctness is the greater phenotypic plasticity exhibited by the population from the more euryhaline, more eurythermal environment.

Key words: cod, *Gadus morhua*, exercise, physiology, multi-variate statistics, salinity, acclimation, aerobic and anaerobic metabolism, blood chemistry, fish, populations.

Introduction

To characterise exercise physiology of a species accurately, one must account for the environmental history and the population origin of the experimental animals (Taylor and McPhail, 1986; Huey *et al.* 1990; Giles, 1991; Randall and Brauner, 1991; reviewed by Garland and Adolph, 1991). For example, the external environment can alter the metabolic support of exercise and the transfer of metabolic byproducts of exercise in conspecifics exposed to different ambient pH levels (Nelson and Mitchell, 1992) or salinities (Tang and Boutilier, 1991; Randall and Brauner, 1991). Likewise, populations from diverse vertebrate taxa have been shown to be distinct for a variety of physiological characters (Garland and Adolph, 1991). While the ability to live at a wide range of environmental salinities (euryhalinity) is not thought to be common among teleost fish (Evans, 1979), many marine fish species previously thought to be stenohaline are actually quite tolerant of reduced salinities (Wu and Woo, 1983). The Atlantic cod (*Gadus morhua*) falls into this latter category;

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while it is considered a marine species, populations exist in areas where salinity routinely falls to 7‰, and cod are known to tolerate salinities as low as 3‰ (Odense *et al.* 1966). One of these low-salinity habitats utilised by cod is the Bras d'Or lakes in Cape Breton, Nova Scotia, Canada (Nelson *et al.* 1994).

Approximately 4000 years ago, rising tidewaters started to flow into the Bras d'Or lakes (R. Stea, personal communication). On the basis of their current global distribution and degree of euryhalinity, Atlantic cod probably did not colonise the Bras d'Or system until several hundred years later, when the salinity reached about 7‰. Nelson *et al.* (1994) reported substantial intraspecific differences in exercise physiology when Atlantic cod taken from the Bras d'Or lakes and cod from the Atlantic Ocean proper were swum at their native salinities. If the basis of these differences is genetic, then 3000 or so years is a short time for such changes to have emerged under normal selection intensities, especially considering the intermittent migration of cod from the Sydney Bight (Northwest Atlantic Fisheries Organisation region 4VN) into the Bras d'Or system (J. Fennel, personal communication). One explanation is that the differences in exercise physiology we reported earlier (Nelson *et al.* 1994) are the result of acclimatisation of Bras d'Or cod to their lower-salinity environment. One goal of the work described here was to test this idea. A second goal of this work was to describe how natural variations in ocean salinity influence whole-animal performance measures (e.g. critical swimming velocity, U_{crit}, or maximal rate of oxygen consumption, $\dot{M}_{O_2}max$) and to investigate mechanisms that may be driving any observed effects.

Euryhaline fish have attracted considerable attention from physiologists; there is an extensive literature on the physiology of diadromous fishes as they move between environments during their migrations (Randall and Brauner, 1991; Brauner *et al.* 1992) and on the 'cost of osmoregulation' of euryhaline fishes exposed to various salinities (e.g. Febry and Lutz, 1987; Morgan and Iwama, 1990). However, little is known about how natural salinity gradients affect the physiological performance of marine fishes, particularly at the low temperatures (i.e. 2 °C) that characterise their natural environment for much of the year. Considering the importance given to whole-animal physiology in determining organismal fitness and ecosystem dynamics (e.g. Endler, 1986; Huey, 1991; Watt, 1991), this type of information is needed for understanding the biology of these fishes leading towards the eventual goal of their effective management or culture.

Materials and methods

Population origins and handling

Atlantic cod (*Gadus morhua* L.) of both sexes were collected from two locations with distinctly different environmental characteristics: (1) Scotian Shelf cod (SSC) were captured by trawling off the Nova Scotian coast on 25 October 1991; (2) Bras d'Or cod (BDC) were captured by angling on 1–2 November 1991 from the brackish Bras d'Or lakes in Cape Breton, Nova Scotia (see Nelson *et al.* 1994, for the environmental characteristics of this system). The body masses and total lengths of the animals (means ±1 standard deviation) were as follows: SSC, 1.14±0.06 kg, 47.8±0.08 cm, N=6; BDC, 1.03±0.06 kg, 46.7±1.15 cm, N=5. Fish were transported immediately to Dalhousie University in 1500 l aerated tanks containing water from their native environment. Experimental fish were exposed gradually to the experimental temperature (2 °C) and salinity over a period of at least 30 days and then were acclimated to the experimental conditions for an additional 50 days. All animals were kept in 6000 l circular tanks containing temperature-controlled, flowing, filtered Atlantic Ocean water or a free-flowing 2:1 mixture of filtered Atlantic Ocean water and dechlorinated Halifax city tap water. Both groups of fish were held in a current maintained by a submersible pump and were fed a mixed diet of chopped squid (*Illex illecebrosus*) and mackerel (*Scomber scombrus*). Experiments took place during the late winter and early spring when bottom temperatures from both environments are in the vicinity of 2 °C (Drinkwater and Trites, 1987; Nelson *et al.* 1994). The photoperiod was maintained on a natural cycle.

Experimental protocol

The experimental protocol followed that described in Nelson *et al.* (1994). At least 24 h prior to surgery, animals were placed in a darkened tube designed to mimic the swimming chamber of our swim-tunnel respirometer. During this time, fish were exposed to a current flow of 15 cm s^{-1} and were trained to avoid the back end of the tube by the use of a light gradient. Cod were anaesthetised with a 1:10 000 solution of MS-222 (Sigma) and were then cannulated in the afferent branchial artery as described by Nelson *et al.* (1994). After the 20 min operation, animals were placed into the swim-tunnel respirometer containing experimental water of the appropriate salinity at 2 °C. The swim-tunnel respirometer and calibration procedures are described in detail by Nelson *et al.* (1994) and Tang *et al.* (1994). Animals were induced to swim by establishing a light gradient which required the fish to swim into the water current to remain at low light levels. This was sufficient for most fish at low to intermediate current speeds, but a 12 V electrified grid with a manually activated switch was located on the downstream retaining screen to maximally motivate the animals.

After at least 24 h of recovery from surgery, an experimental trial was initiated by taking measurements of the rate of oxygen consumption ($\dot{M}_{O_2}$), blood pressure and ventilation rate at the 15 cm s^{-1} acclimation speed. Immediately after these measurements, a 1 ml blood sample was drawn and the velocity of water in the swim tunnel was then increased to 20 cm s^{-1}. Cod were exhausted using a critical swimming speed protocol (U_{crit}; Brett, 1964); 10 cm s^{-1} increments every 30 min). Blood was sampled at the end of each 30 min swimming period while oxygen consumption, ventilation rate and blood pressure were measured over a 20 min interval between each velocity increment. Upon exhaustion, the flow in the swim-tunnel

respirometer was reduced to 12 cm s^{-1} and measurements were made at intervals for 8 h post-exhaustion.

Measurements and calculations

Critical swimming velocity (U_{crit}) was calculated according to Brett (1964). The water velocity was corrected for the acceleration of the water around the fish (solid blocking) according to equations 105 and 106 of Webb (1974; note the error in equation 105: the denominator should be $S_t^{3/2}$), using empirically measured cross-sectional areas to calculate the fish's mean diameter, the fish's mass and unit density to approximate volume, and interpolated body-shape factor values from Pope and Harper (1966). For clarity, some graphical and tabular data are presented as if the fish were swimming at the nominal, uncorrected water velocity.

Venous blood was sampled anaerobically into a chilled gas-tight Hamilton syringe. A 50 μl subsample was immediately dispensed into 500 μl of ice-cold 0.6 mol l^{-1} $HClO_4$ and centrifuged at 13 000 ***g*** for 0.5 min. The supernatant was decanted, neutralised with KOH, re-centrifuged, and frozen at −80 °C for later measurement of glucose and lactate. A second subsample was centrifuged, the plasma withdrawn and frozen at −80 °C in sealed humidified chambers for subsequent measurement of plasma protein and ion concentrations. Blood pressure, P_{O_2}, pH, haemoglobin, haematocrit, mean cellular haemoglobin, glucose, lactate, plasma protein and total CO_2 were measured and calculations of HCO_3^- and P_{CO_2} were made as described in Nelson *et al.* (1994). Environmental P_{O_2} was measured and oxygen consumption calculated as outlined in Nelson *et al.* (1994). Base-excess (and base-deficit) was calculated as described in Nelson and Mitchell (1992). Since no 'at rest' measurements were made, the 'pre-swim' blood pH and plasma [HCO_3^-] of Scotian Shelf cod in 31‰ salinity water at 2 °C were taken as reference values. The blood non-bicarbonate buffering value (β_{NB}) was estimated from the haemoglobin concentration using the relationship described by Wood *et al.* (1982). Fish age was estimated from the annuli on otolith bones and the known date of capture.

Statistics

Comparisons of the two populations for a given physiological variable throughout the swimming and recovery period were made with the general linear models (GLM) procedure of the Statistical Analysis System (SAS Institute, 1989). The model was an unbalanced, three-way, repeated-measures analysis of variance (ANOVA) with population, salinity and time (either at a nominal swimming speed or time post-exhaustion) as class variables. Results from animals swum at their native salinities (Nelson *et al.* 1994) were included in the statistical analysis. The hypotheses that either the populations were not distinct for a given physiological measurement or the environmental salinities did not affect a given physiological variable were tested with specific *F*-tests using individuals as the error term and also with a Tukey's multiple-comparison test. Significance of differences among interaction means (i.e. specific combinations of time, salinity and population) were determined by comparison of least-squares confidence intervals (SAS Institute, 1989).

Principal components were calculated for animals from both populations swum at both salinities using the PRINCOMP procedure of the Statistical Analysis System (SAS Institute, 1989). To reduce the redundancy inherent in using all sampling times for each measured variable (140 combinations), the data were compressed into 40 variables per individual. Data were compressed by selecting key time points for each measured parameter and constructing integrative variables for several factors. Examples of integrative variables include: maximum lactate concentration, change in pH with exhaustive exercise, change in heart rate with exhaustive exercise, etc. A complete list of the variables used in the principal components analysis can be found in the Appendix.

Results

General features

With the exception of age, most general measurements were homogeneous among the groups swum at the acclimation salinities (present experiments) and for those swum at their native salinities (Nelson *et al.* 1994; Table 1). Animal size was deliberately limited to between 45 and 50 cm total length so that scaling effects would be minimised. One consequence of this design was a different age structure between the two experimental populations. Cod from the Bras d'Or system averaged 6.5±1.2 years old (S.D.), whereas the Scotian Shelf cod averaged only 3.8±1.1 years old (Table 1). Although this age discrepancy should be considered when interpreting the experimental results, it is worth noting that cod have a normal life-span of 15 years and have been recorded to live as long as 27 years (Lear, 1984). Thus, senescence is an unlikely determinant for any of our results.

Table 1. *Comparison of variables between fish used in the present experiments (normal typeface) and those used by Nelson* et al. *(1994; in italics)*

Treatment	*N*	Length (cm)	Mass (kg)	Condition factor	Age (years)
Scotian Shelf (SSC)					
Full salinity (31‰)	*6*	*48.8*	*1.10*	*9.5*	*3.33*
		(1.8)	*(0.11)*	*(0.9)*	*(0.82)*
Low salinity (20‰)	6	47.8	1.14	10.1	4.67
		(2.1)	(0.16)	(0.7)	(1.03)
Bras d'Or (BDC)					
Full salinity (31‰)	5	46.7	1.03	10.2	6.60
		(2.6)	(0.14)	(1.2)	(1.67)
Low salinity (20‰)	*5*	*46.4*	*1.00*	*10.0*	*6.33*
		(1.8)	*(0.11)*	*(0.7)*	*(0.52)*

Condition factor is equal to mass/(length)^3, where mass is in kg and length in m.

Values are means (± S.D.).

Table 2. *Mass exponents at various points during a critical swimming speed protocol*

Swimming speed ($cm\,s^{-1}$)	Power function mass exponent, *b*	r^2	Recovery time (h)	Power function mass exponent, *b*	r^2
25	0.836	0.802	0	0.653	0.289
30	0.898	0.448	0.5	0.545	0.181
35	0.817	0.620	1	0.063	0.008
40	0.965	0.645	1.5	0.895	0.415
45	0.914	0.650	2	1.031	0.534
50	1.209	0.869			

The mass exponent *b* is for an equation of the general form $\dot{M}_{O_2} = aM^b$, where $\dot{M}_{O_2}$ is in $\mu mol\,kg^{-1}\,min^{-1}$, where *a* is a constant and *M* is animal mass in kilograms.

Values were calculated from a different group of cod, all from the same population, uncannulated, with a greater size range (0.82–1.68 kg), and swum at 5 °C and 31 ‰; *N*=12.

The importance of matching the experimental groups for size rather than age is aptly demonstrated by the exercise-associated metabolism of a different group of non-cannulated cod measured at 5 °C (Table 2). In this group of animals, the scaling coefficient *b* (Table 2), which would be used to correct oxygen consumption measurements for size, undergoes a modest change from about 0.8 at mild swimming speeds to around 1.0 at maximal swimming. This small change in *b*, which is similar to changes in *b* reported by Armstrong *et al.* (1992) for northern pike and Brett and Glass (1973) for sockeye salmon, would allow numerical corrections for mass differences during the swimming phase of the experiment. However, the extreme fluctuations in the scaling coefficient after exercise in cod (Table 2) suggest that any corrections of $\dot{M}_{O_2}$ for mass during this period would be tenuous. Since one of the main goals of this work was to compare metabolism across populations and environments, the most robust experimental design was to match groups for size, not for age.

Whole-animal performance

Critical swimming speeds did not differ significantly between the populations at either salinity (Fig. 1). However, cod swimming at 'non-native' salinities (present experiments) exhibited increased variance in their swimming performance compared with animals swum at their native salinities (Fig. 1). For SSC swimming at 20 ‰, the coefficient of variation increased from 4.1 to 12 % and for BDC swimming at 31 ‰ the coefficient of variation increased from 6.6 to 10.3 %. Correction of the U_{crit} value for each fish to a standard value with the equation relating fish size to U_{crit} at 2 °C (corrected for body shape differences; Nelson *et al.* 1994) did not alter our conclusions that U_{crit} was independent of population and salinity or that swimming in 'non-native' waters increased the variance in U_{crit}.

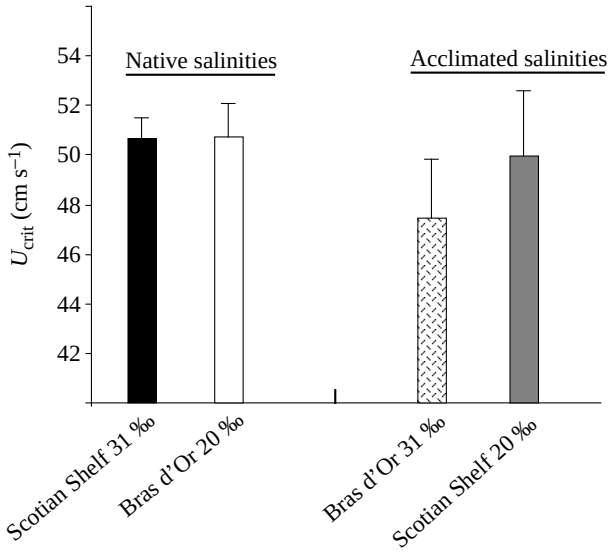

Fig. 1. Critical swimming speeds of Atlantic cod from the Scotian Shelf of the northwest Atlantic Ocean and the nearby brackish Bras d'Or lakes of Cape Breton Island, Nova Scotia. Means + standard errors of six (Scotian Shelf cod; SSC) or five (Bras d'Or cod; BDC) fish are presented. The animals are also grouped according to whether they swam in a salinity close to that found in their natural environment.

Cardiovascular and respiratory measurements

Heart rate was a function of both the animal's population origin and the salinity in which it swam, according to a significant interaction term between these variables in the ANOVA (P<0.0001; Fig. 2A). The low heart rates of Scotian Shelf cod at their native salinity (Nelson *et al.* 1994) increased substantially upon acclimation to reduced salinity. In fact, the highest heart rates recorded were for SSC recovering from exercise at 20 ‰ (Fig. 2A). Since cod from the Bras d'Or lakes also had a higher heart rate at 20 ‰ than at 31 ‰, especially while swimming, the specific test of the null hypothesis indicated that cardiac rate was not independent of salinity (P<0.001).

Ventilation frequency was also dependent upon an interaction between salinity and the population origin of the animal (P<0.01; Table 3). However, the lack of any statistical difference due to salinity alone suggests that the influence of salinity on oxygen consumption (Fig. 2B and see below) accrued largely from changes in ventilation volume. Claireaux and Dutil (1992) also reported that ventilation frequency in normoxic cod was independent of salinity. Cod from the Bras d'Or lakes had a significantly greater ventilatory frequency (Tukey's test, P<0.05), mirroring the greater oxygen consumption of this population (see below).

Rate of oxygen consumption

A plot of oxygen consumption for the entire experiment (Fig. 2B) yields a picture similar to heart rate. The ANOVA revealed a large significant interaction term between salinity and population, while the specific *F*-test indicated that lower

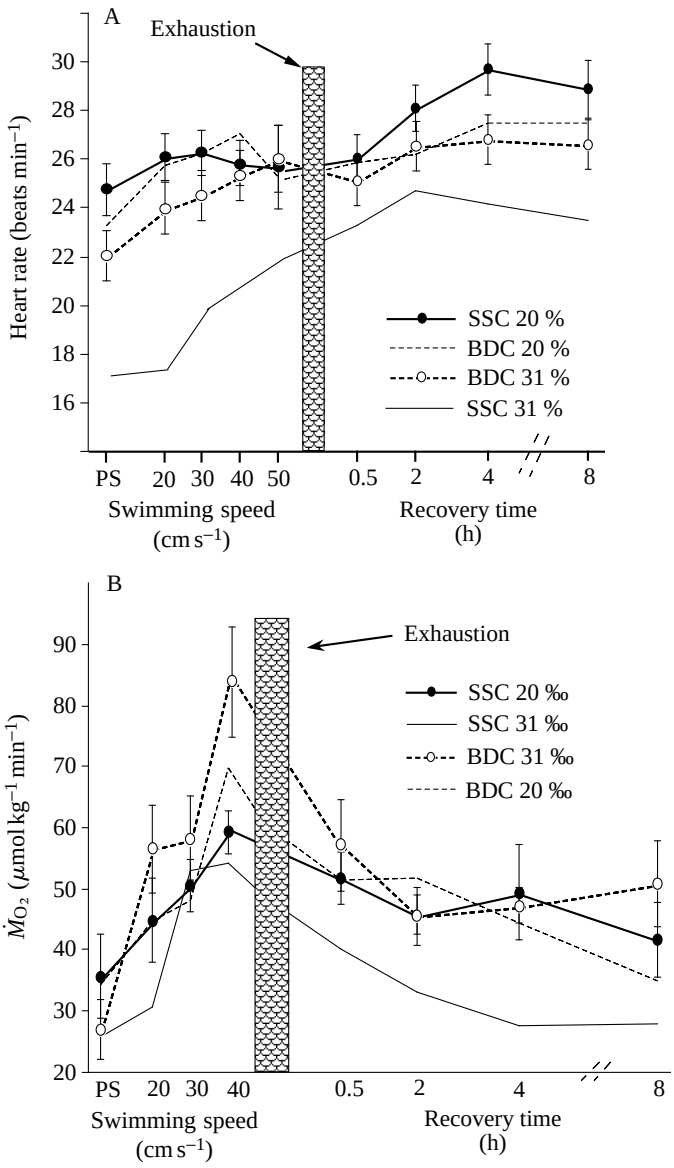

Fig. 2. (A) Heart rate in Scotian Shelf cod (filled circles) (SSC) swimming at a salinity of 20‰ and in Bras d'Or cod (open circles) (BDC) swimming at a salinity of 31‰ during swimming to their critical swimming velocity and at various times during recovery from exhaustion. Means and standard errors are presented for six (SSC) or five (BDC) animals. The pre-swim measurement (PS) was taken at 15 cm s^{-1} current velocity, but the animals were not necessarily swimming. Data from Nelson *et al.* (1994) for SSC (solid line) and BDC (dashed line) swimming at their native salinities are presented for comparative purposes. Note that during the recovery period the time increments are not strictly linear. (B) Whole-animal rate of oxygen consumption in Scotian Shelf cod (filled circles) (SSC) and Bras d'Or cod (open circles) (BDC) during swimming to their critical swimming velocity and at various times during recovery from exhaustion. Other details as in A.

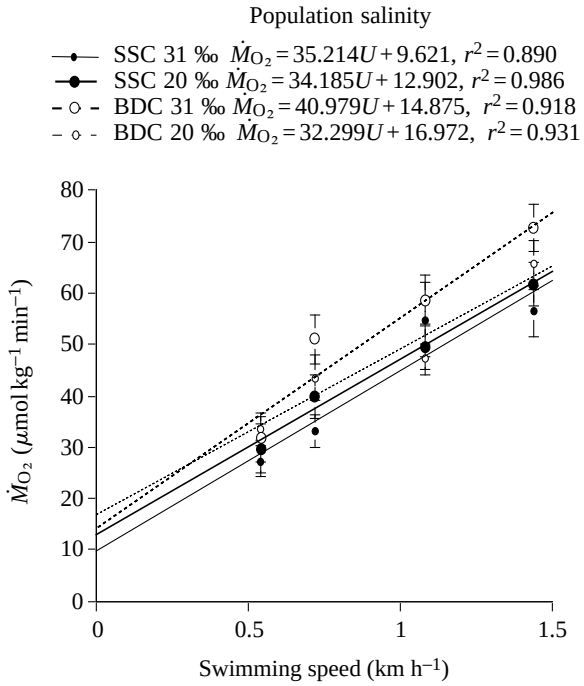

Fig. 3. Whole-animal rates of oxygen consumption in Scotian Shelf cod (filled circles) (SSC) and Bras d'Or cod (open circles) (BDC) during swimming to their critical swimming velocity at two salinities.

salinity caused increased O_2 consumption rate over the course of an entire experiment (P<0.05; Fig. 2B), largely because of the very low metabolic rate of SSC at their native salinity of 31‰. Populations were homogeneous for oxygen consumption by the specific test of that hypothesis, but BDC were considered to have a higher O_2 consumption by the less restrictive Tukey's test (P<0.05). When the rate of oxygen consumption of all four groups was plotted against swimming speed (in km h^{-1}), the resulting relationship was linear for all groups (Fig. 3).

If we use the slopes in Fig. 3 as estimates of the aerobic cost of swimming for these animals and the *y*-intercepts as crude estimates of basal metabolic rate (in fisheries research often called 'standard metabolic rate'; e.g. Cech, 1990), the interaction between environmental salinity and the population origin of the fish on metabolic rate is again apparent. For Scotian Shelf cod, acclimation to 20‰ caused little apparent change in the cost of locomotion, yet yielded an estimated 34% increase in basal metabolic rate; the efficacy of this estimate is supported by the relatively high heart and ventilatory rates of this group during the 'pre-swim' measurement (Fig. 2A; Table 3). In contrast, acclimation of fish from the Bras d'Or lakes to 31‰ caused a small 12.4% decrease in the apparent basal metabolic rate, yet produced a large 26.7% increase in the aerobic cost of swimming.

Blood ion measurements

Statistically significant interactions were found between both population origin and sampling time and environmental salinity and sampling time for the blood ion measurements. In short, BDC developed blood ion levels similar to or even greater than those of Scotian Shelf cod (SSC) at normal

Table 3. *Blood chemistry measurements and ventilation rates in Scotian Shelf cod (SSC) and Bras d'Or cod (BDC) prior to swimming (PS), at various swimming speeds and at various times during recovery from exhaustive exercise*

A

	PS				20 cm s^{-1}				30 cm s^{-1}				40 cm s^{-1}			
	BDC		SSC		BDC		SSC		BDC		SSC		BDC		SSC	
	20‰	31‰	20‰	31‰	20‰	31‰	20‰	31‰	20‰	31‰	20‰	31‰	20‰	31‰	20‰	31‰
Ventilation rate (breaths min^{-1})	*17.56* *(5.42)*	21.34 (3.24)	22.42 (4.78)	*16.35* *(4.18)*	*23.84* *(3.72)*	28.09 (4.96)	27.02 (3.77)	*19.71* *(5.51)*	*30.19* *(5.34)*	32.92 (5.18)	30.02 (3.36)	*29.24* *(3.11)*	*35.28* *(5.28)*	37.58 (3.13)	34.94 (2.69)	*35.72* *(3.81)*
Venous P_{O_2} (kPa)	*3.81* *(0.86)*	3.59 (0.40)	2.53 (0.83)	*3.11* *(0.85)*									*2.24* *(0.32)*	2.49 (0.34)	1.64 (0.16)	*2.08* *(0.22)*
Plasma [Na^+] (mmol l^{-1})	*180.2* *(5.54)*	186.8 (12.01)	185.8 (3.87)	*186.3* *(4.88)*	*181.8* *(6.72)*	191.0 (10.54)	185 (4.10)	*187* *(4.24)*	*182.6* *(6.66)*	191.4 (10.78)	189.3 (7.94)	*188.5* *(3.27)*	*189.5* *(6.46)*	193.7 (12.66)	192.0 (0.82)	*194.8* *(4.36)*
Plasma [Cl^-] (mmol l^{-1})	*159.2* *(5.02)*	165.6 (5.55)	170.5 (4.51)	*170.5* *(3.83)*	*159.6* *(4.67)*	167.0 (6.20)	172.3 (3.30)	*171.3* *(4.23)*	*161* *(7.31)*	168.6 (6.50)	174.5 (4.12)	*171.5* *(2.59)*	*167.2* *(3.40)*	174.7 (12.34)	173.0 (1.73)	*175.0* *(4.69)*
Plasma [HCO_3^-] (mmol l^{-1})	*8.37* *(0.83)*	8.95 (0.53)	9.26 (0.78)	*8.66* *(0.55)*	*8.26* *(0.78)*	9.57 (0.61)	9.44 (0.61)	*9.07* *(0.76)*	*8.72* *(0.72)*	10.12 (0.68)	9.77 (0.62)	*9.50* *(1.10)*	*8.52* *(1.18)*	10.65 (0.76)	9.78 (1.10)	*9.87* *(0.63)*
Plasma P_{CO_2} (kPa)	*0.42* *(0.06)*	0.48 (0.14)	0.46 (0.11)	*0.43* *(0.10)*	*0.42* *(0.04)*	0.60 (0.18)	0.43 (0.09)	*0.39* *(0.10)*	*0.52* *(0.11)*	0.74 (0.22)	0.51 (0.08)	*0.56* *(0.21)*	*0.82* *(0.13)*	0.96 (0.29)	0.83 (0.09)	*0.93* *(0.13)*
Plasma pH	*7.92* *(0.04)*	7.89 (0.09)	7.94 (0.05)	*7.95* *(0.05)*	*7.92* *(0.04)*	7.87 (0.14)	7.97 (0.06)	*7.99* *(0.08)*	*7.84* *(0.09)*	7.74 (0.07)	7.90 (0.05)	*7.86* *(0.15)*	*7.61* *(0.10)*	7.62 (0.07)	7.67 (0.07)	*7.65* *(0.09)*
Plasma [glucose] (mmol l^{-1})	*4.85* *(1.29)*	7.17 (1.77)	7.12 (2.81)	*6.31* *(2.18)*	*4.88* *(1.29)*	7.12 (2.30)	6.94 (2.28)	*5.94* *(1.71)*	*4.99* *(1.40)*	7.66 (2.11)	7.83 (3.26)	*6.07* *(1.58)*	*4.95* *(1.45)*	7.39 (2.18)	6.82 (3.24)	*6.52* *(1.55)*
Blood [lactate] (mmol l^{-1})	*0.43* *(0.14)*	0.54 (0.52)	0.61 (0.59)	*0.87* *(0.62)*	*0.47* *(0.32)*	0.90 (0.46)	0.62 (0.40)	*0.79* *(0.74)*	*0.92* *(0.31)*	1.20 (0.45)	0.63 (0.34)	*0.85* *(0.49)*	*2.30* *(0.54)*	2.82 (0.40)	2.12 (0.72)	*2.73* *(1.85)*
Plasma [protein] (mg dl^{-1})	*2.97* *(0.49)*	4.37 (0.59)	2.68 (0.58)	*6.22* *(0.76)*	*2.95* *(0.58)*	4.41 (0.53)	2.64 (0.60)	*6.23* *(0.93)*	*3.01* *(0.81)*	4.31 (0.94)	2.53 (0.72)	*6.37* *(0.98)*	*3.45* *(0.74)*	4.77 (0.75)	2.44 (0.73)	*6.20* *(1.00)*
Haematocrit (%)	*25.6* *(2.71)*	22.4 (3.14)	22.1 (8.40)	*23.2* *(3.94)*	*25.8* *(3.07)*	22.7 (4.21)	21.6 (8.03)	*23.5* *(4.57)*	*25.5* *(2.82)*	23.2 (3.29)	21.3 (7.86)	*22.8* *(3.41)*	*25.8* *(3.51)*	24.5 (3.71)	24.5 (9.73)	*23.8* *(2.88)*
Haemoglobin (mg dl^{-1})	*4.57* *(0.55)*	3.98 (0.02)	4.35 (0.61)	*4.57* *(0.95)*	*4.64* *(0.54)*	3.86 (0.02)	4.28 (0.61)	*4.53* *(1.05)*	*4.59* *(0.47)*	3.88 (0.02)	4.11 (0.62)	*4.30* *(0.87)*	*4.03* *(0.50)*	3.92 (0.03)	4.40 (0.65)	*4.34* *(0.79)*

Table 3. *Continued*

B

	0 h				0.5 h				2 h				4 h				8 h			
	BDC		SSC		BDC		SSC		BDC		SSC		BDC		SSC		BDC		SSC	
	20 ‰	31 ‰	20 ‰	31 ‰	20 ‰	31 ‰	20 ‰	31 ‰	20 ‰	31 ‰	20 ‰	31 ‰	20 ‰	31 ‰	20 ‰	31 ‰	20 ‰	31 ‰	20 ‰	31 ‰
Ventilation rate (breaths min^{-1})					*24.74* *(3.89)*	25.71 (5.04)	23.02 (3.79)	*24.13* *(6.64)*	*22.04* *(5.03)*	20.87 (1.20)	21.7 (0.40)	*19.34* *(2.20)*	*22.16* *(5.24)*	20.68 (1.36)	20.57 (2.48)	*17.92* *(1.45)*	*20.77* *(3.84)*	21.17 (2.30)	19.74 (2.73)	*16.13* *(1.38)*
Venous P_{O_2} (kPa)	*3.44* *(0.62)*	3.36 (0.93)	2.38 (0.41)	*2.70* *(0.35)*					*2.52* *(0.71)*	3.23 (0.38)	2.11 (0.41)	*2.93* *(1.06)*	*2.81* *(1.30)*	3.10 (0.46)	2.66 (1.05)	*2.97* *(0.81)*	*2.99* *(0.81)*	2.64 (0.39)	2.90 (0.58)	*3.41* *(0.54)*
Plasma [Na^+] ($mmol\,l^{-1}$)	*194.0* *(4.0)*	207 (9.06)	205 (5.73)	*202.5* *(1.52)*	*194.2* *(2.77)*	205 (7.18)	206 (7.98)	*205.7* *(6.89)*	*197.2* *(5.93)*	204.8 (6.10)	202.2 (8.50)	*203* *(5.06)*	*194.2* *(8.10)*	202.2 (5.07)	195.8 (8.07)	*203.5* *(10.33)*	*190.2* *(5.50)*	190.8 (9.96)	190.5 (4.43)	*197.6* *(10.78)*
Plasma [Cl^-] ($mmol\,l^{-1}$)	*171.2* *(4.32)*	176 (5.79)	180.6 (2.51)	*180.8* *(3.19)*	*171.3* *(4.72)*	176.6 (8.62)	182.8 (2.95)	*181.2* *(2.99)*	*169.8* *(6.18)*	176 (9.98)	179.8 (2.49)	*179* *(4.65)*	*168* *(2.83)*	173.2 (9.68)	177.6 (3.91)	*178.2* *(5.78)*	*168.3* *(1.53)*	172.6 (7.30)	179.8 (5.38)	*177.6* *(5.64)*
Plasma [HCO_3^-] ($mmol\,l^{-1}$)	*7.44* *(1.77)*	10.48 (1.36)	9.11 (0.93)	*10.32* *(1.01)*	*5.57* *(1.13)*	8.24 (1.27)	8.09 (1.34)	*8.67* *(0.94)*	*4.77* *(0.99)*	7.47 (1.44)	7.48 (1.38)	*8.18* *(1.12)*	*5.06* *(1.22)*	7.54 (0.81)	7.89 (1.50)	*7.56* *(0.74)*	*6.02* *(1.23)*	7.75 (0.83)	7.82 (0.66)	*7.39* *(0.41)*
Plasma P_{CO_2} (kPa)	*0.83* *(0.10)*	1.04 (0.69)	0.86 (0.06)	*1.07* *(0.20)*	*0.75* *(0.11)*	0.86 (0.57)	0.86 (0.12)	*0.93* *(0.18)*	*0.61* *(0.15)*	0.52 (0.35)	0.47 (0.08)	*0.48* *(0.04)*	*0.50* *(0.11)*	0.44 (0.29)	0.40 (0.10)	*0.33* *(0.06)*	*0.43* *(0.09)*	0.40 (0.24)	0.38 (0.02)	*0.31* *(0.07)*
Plasma pH	*7.58* *(0.09)*	7.60 (0.07)	7.62 (0.07)	*7.58* *(0.06)*	*7.46* *(0.04)*	7.58 (0.08)	7.55 (0.10)	*7.57* *(0.08)*	*7.49* *(0.07)*	7.74 (0.10)	7.81 (0.10)	*7.84* *(0.05)*	*7.60* *(0.09)*	7.84 (0.08)	7.91 (0.10)	*7.98* *(0.06)*	*7.74* *(0.10)*	7.9 (0.02)	7.93 (0.03)	*7.98* *(0.08)*
Plasma [glucose] ($mmol\,l^{-1}$)	*4.72* *(1.79)*	7.02 (2.07)	6.71 (1.94)	*6.10* *(1.66)*	*5.80* *(1.16)*	8.14 (2.44)	7.38 (1.39)	*7.31* *(1.60)*	*6.89* *(1.43)*	9.14 (2.03)	8.19 (1.13)	*9.24* *(1.89)*	*8.87* *(1.37)*	10.78 (2.82)	8.85 (1.50)	*8.57* *(1.92)*	*8.52* *(0.71)*	11.65 (2.99)	9.46 (2.23)	*10.6* *(2.75)*
Blood [lactate] ($mmol\,l^{-1}$)	*3.53* *(0.53)*	4.53 (0.82)	4.14 (0.95)	*4.01* *(1.40)*	*5.95* *(1.06)*	6.96 (1.46)	5.60 (1.16)	*6.42* *(1.35)*	*8.49* *(1.84)*	8.22 (1.98)	5.77 (1.99)	*7.52* *(0.92)*	*10.40* *(1.43)*	7.03 (3.36)	5.35 (2.77)	*6.75* *(2.22)*	*9.35* *(1.97)*	4.84 (2.70)	3.37 (1.66)	*5.25* *(2.28)*
Plasma [protein] ($mg\,dl^{-1}$)	*3.29* *(0.65)*	4.86 (1.22)	2.67 (0.58)	*6.45* *(0.94)*	*3.29* *(0.87)*	5.45 (1.01)	2.84 (0.64)	*6.97* *(1.00)*	*3.69* *(0.74)*	4.87 (0.79)	2.80 (0.73)	*6.92* *(1.21)*	*3.48* *(0.97)*	4.63 (0.76)	2.58 (0.73)	*6.38* *(1.46)*	*3.10* *(0.99)*	5.14 (0.59)	2.52 (0.50)	*5.72* *(0.41)*
Haematocrit (%)	*27.1* *(2.77)*	24.0 (4.12)	22.8 (8.11)	*24.9* *(3.14)*	*30.0* *(2.67)*	24.7 (4.21)	25.5 (10.29)	*26.9* *(4.08)*	*29.1* *(1.83)*	24.5 (3.63)	23.5 (8.93)	*27.0* *(3.87)*	*25.8* *(3.84)*	22.3 (2.92)	22.6 (7.20)	*23.5* *(3.52)*	*21.9* *(3.24)*	20.3 (3.06)	19.1 (6.97)	*20.7* *(2.20)*
Haemoglobin ($mg\,dl^{-1}$)	*4.22* *(0.55)*	3.58 (0.22)	4.0 (0.57)	*4.32* *(0.64)*	*4.45* *(0.44)*	3.75 (0.19)	4.38 (0.69)	*4.73* *(0.77)*	*4.62* *(0.40)*	3.96 (0.20)	4.27 (0.71)	*4.92* *(0.83)*	*4.15* *(0.51)*	3.82 (0.15)	4.29 (0.57)	*4.60* *(0.83)*	*3.71* *(0.28)*	3.61 (0.21)	3.78 (0.51)	*4.35* *(0.60)*

For SSC, N=6 animals; for BDC, N=5 animals.

Values are means (± S.D.).

Significance levels are not presented on the table for clarity but are, at the $P<0.05$ level for populations differing from each other and times differing from the pre-swim value, as follows. Plasma [protein]: populations, all points; time, BDC 2 and 4 h of recovery, SSC 0.5 and 2 h of recovery. Venous P_{O_2}: populations, exhaustion; time, BDC 40 $cm\,s^{-1}$, 2 and 8 h of recovery, SSC none. Haematocrit: populations, exhaustion, 0.5 and 4 h of recovery; time: SSC and BDC, 0.5, 2 and 8 h of recovery. Haemoglobin: populations, 0.5, 2 and 8 h of recovery; time, BDC 40 $cm\,s^{-1}$, exhaustion and 8 h of recovery, SSC 8 h of recovery. Plasma [glucose]: populations, all points except 4 h of recovery; time, SSC and BDC 2, 4 and 8 h of recovery.

Results for fish used in the present experiments are in normal typeface; results taken from Nelson *et al.* (1994) are in italic type.

salinity, after only 50 days of acclimation to full-strength salinity (31‰). In contrast, SSC acclimated for 50 days to reduced salinity (20‰) retained blood ion levels close to those of SSC at normal salinity. This is illustrated by both the plasma Na^+ and Cl^- results (Table 3). All groups of fish experienced similar changes in plasma [Na^+] and [Cl^-] with the exercise and recovery protocol (Table 3). The reason for the speedier decline of plasma Na^+ levels after 2 h of recovery from exhaustive exercise in fish swimming in their 'non-native' environment (present experiments) is unknown (Table 3).

Blood acid–base measurements

Plasma [HCO_3^-], as calculated from total plasma CO_2 and blood pH, responded to the acclimation and exercise protocols with a large population×salinity interaction (ANOVA, $P<0.0001$; Table 3). Cod from the Bras d'Or system had a plasma [HCO_3^-] virtually identical to that of SSC when both groups were swum at 31‰, yet BDC swum at their native salinity had a plasma [HCO_3^-] which was significantly lower than that of all other groups throughout the exercise and recovery protocol. In contrast, acclimation of SSC to low-salinity water produced only modest differences in plasma [HCO_3^-] during both the exercise and recovery protocols. Plasma [HCO_3^-] was significantly lower for SSC at 20‰ for three sampling periods: exhaustion, 0.5 h of recovery and 2 h of recovery from exhaustive exercise ($P<0.05$). The specific *F*-tests indicated that plasma [HCO_3^-] was significantly lower in 20‰ water and Bras d'Or cod ($P<0.01$ and $P<0.05$, respectively) for the entire experiment, primarily because of the much lower plasma [HCO_3^-] of BDC at 20‰ (Table 3).

The venous P_{CO_2} data also revealed significant interactions between salinity and population origin (ANOVA, $P<0.05$), as well as population and time (ANOVA, $P<0.05$) and salinity and time (ANOVA, $P=0.001$). During the early swimming period, BDC at 31‰ had a significantly higher venous blood P_{CO_2} (Table 3), which is consistent with the greater CO_2 production expected from their higher metabolic rate during this period (Fig. 2B). During the latter part of the swim as the cod approached and reached exhaustion, animals from both populations swimming at 20‰ had a significantly lower venous P_{CO_2}. During recovery from exercise, P_{CO_2} was statistically uniform among populations and salinities.

According to the ANOVA, venous plasma pH was also dependent upon interactions of population and time ($P<0.01$) and salinity and time ($P<0.0001$). Cod from the Bras d'Or lakes had lower plasma pH during swimming, particularly at 31‰ (Table 3). This situation was reversed during recovery, when the plasma pH of BDC at 31‰ was similar to SSC values. The plasma pH of BDC at 20‰ remained substantially lower, reflecting the greater metabolic acid load incurred by BDC at 20‰ (see below). According to the specific *F*-tests, BDC, on average, had a lower plasma pH ($P<0.0001$) and animals swum at 20‰ had a lower plasma pH ($P<0.05$).

Bras d'Or cod swimming at 20‰ incurred a relatively greater metabolic proton load in the extracellular fluid compartment and a relatively lower [lactate]/base-deficit ratio in the blood (Fig. 4). BDC had significantly greater blood base-deficit at 20‰ at exhaustion and all subsequent blood samples when compared with BDC at 31‰ ($P<0.05$). SSC had a significantly lower base-deficit (higher base-excess) than BDC during the late phases of recovery from exhaustive exercise ($P<0.05$; Fig. 4).

Blood metabolite measurements

Plasma glucose concentration exhibited a significant interaction between population and salinity in the ANOVA ($P<0.0001$; Table 3). The low plasma [glucose] in cod from the Bras d'Or system at 20‰ was identical to SSC values after acclimation to 31‰. In contrast, plasma [glucose] in SSC was similar regardless of acclimation salinity. Changes in plasma [glucose] during exercise and recovery were similar among treatments (Table 3).

Blood lactate concentration also revealed significant interactions of population and salinity (ANOVA, $P<0.001$) and population and time (ANOVA, $P<0.0001$; Table 3). Bras d'Or cod were considered to have greater blood [lactate] for the entire experiment according to Tukey's test ($P<0.05$). This statistical result was entirely due to the comparatively higher blood lactate levels which occurred in BDC during recovery from exhaustive exercise (Table 3).

Plasma [protein] was also dependent upon an interaction between population and salinity according to the ANOVA ($P<0.0001$) but, because the salinity term was so large ($F=1527$), we can be confident in attributing most of this interaction to a salinity effect (Table 3). The specific *F*-test of the hypothesis strongly indicated that reduced environmental salinity lowers plasma protein concentration ($P<0.0001$), whereas the populations were considered to have homogeneous plasma [protein] by this test. In contrast, Dutil

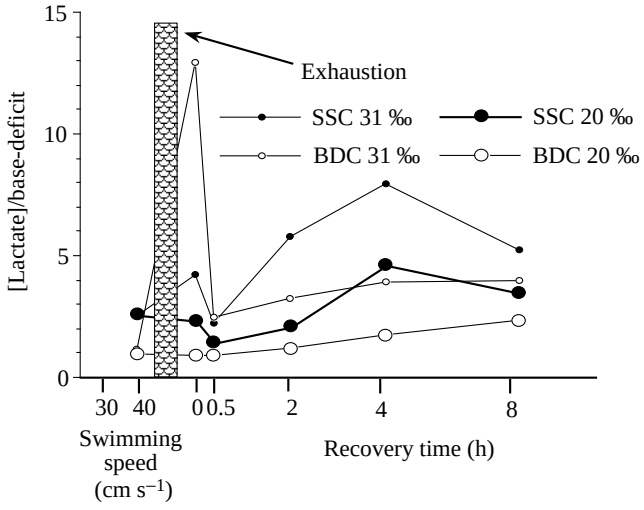

Fig. 4. The ratio of whole-blood lactate concentration to base-deficit in Scotian Shelf cod (filled circles) (SSC) and Bras d'Or cod (open circles) (BDC) during the end of their swim to critical velocity and at various times during recovery from exhaustion. Other details as in Fig. 2A.

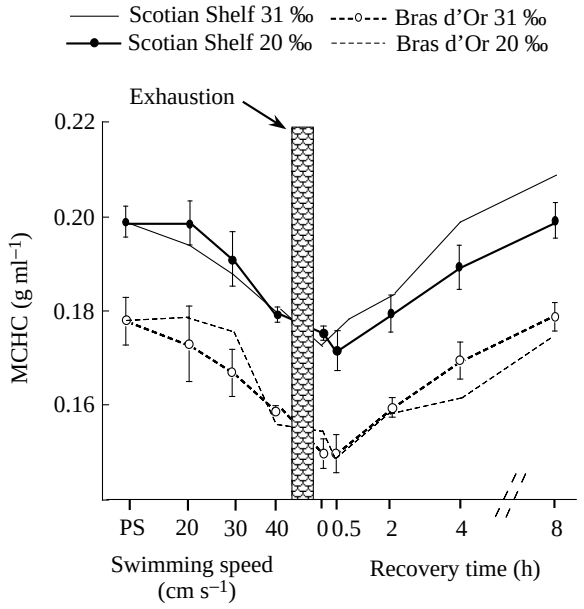

Fig. 5. Mean cellular haemoglobin concentration (MCHC) in Scotian Shelf cod (filled circles) (SSC) and Bras d'Or cod (open circles) (BDC) during swimming to their critical swimming velocity and at various times during recovery from exhaustion. Other details as in Fig. 2A; the populations were significantly different at all points.

et al. (1992) found no change in plasma protein concentration upon acclimating cod to a variety of reduced salinities. However, sampling differences between the two experiments (indwelling catheters in the present study *versus* stunning and venipuncture by Dutil *et al.* 1992) void a direct comparison.

Blood oxygen capacity indices

Blood haemoglobin concentration and haematocrit were statistically uniform across populations and salinity (Table 3). These measurements were characterised by a high degree of inter-fish variability. There was a small population×salinity interaction for haematocrit (ANOVA, $P<0.05$), but all population and salinity comparisons were insignificant.

The mean cellular haemoglobin concentration (MCHC) was largely intransigent to salinity change and was much lower in the Bras d'Or population (Fig. 5). In addition to there being a large population term in the ANOVA, the specific *F*-test indicated that the populations had different MCHCs ($P<0.05$). The potential physiological ramifications of the BDC having larger red blood cell volumes with less haemoglobin per cell are discussed in Nelson *et al.* (1994).

Discussion

Metabolic support of swimming performance

We found mean swimming performance to be independent of the salinity and population origin of cod, despite statistically significant differences in many of the physiological variables among the treatment groups. This result reinforces our earlier conclusion that intraspecific differences in the physiological support system need not translate into differences in overall exercise performance (Nelson *et al.* 1994). However, the larger variance in an individual's swimming performance in a 'non-native' environment (Fig. 1) suggests that the effect of a given environmental change on each individual's performance is poorly predictable from the mean population result (see also Nelson, 1989).

Changes in environmental salinity do not generally affect swimming performance of euryhaline fishes (Beamish, 1978). Despite elaborate experiments designed to uncover a mechanistic link between environmental salinity and U_{crit} (e.g. Glova and McInerney, 1977), most of the results from this type of experiment have, like ours, been negative. The exception to this generalisation occurs when animals are transferred to an alternative salinity shortly before the swimming test (e.g. Brauner *et al.* 1992). In this latter type of experiment, the observed reduction in swimming capacity probably has as much to do with the stress of transfer (Railo *et al.* 1985), with the immediate acid–base insult (Claiborne *et al.* 1994; Nonnette and Truchot, 1990) or with transient changes in plasma ion composition (Claiborne *et al.* 1994; Nonnette and Truchot, 1990; Brauner *et al.* 1992) as with any change in 'cost of osmoregulation' or cost of transport.

Despite there being little difference in exercise performance among cod populations and environmental salinities, there were population differences in the rate of aerobic energy expenditure and how that expenditure changed with exercise. Oxygen consumption tended to be higher in (1) fish acclimated to 20‰ water, (2) fish swimming and recovering in 'non-native' salinities and (3) fish from the brackish Bras d'Or system. These generalisations are complicated, however, by the differential response to exercise in the two populations. According to the slopes of the lines in Fig. 3 which relate rate of oxygen consumption to swimming speed, the energetic cost of swimming was greater at full-strength salinity, primarily for BDC. Febry and Lutz (1987) also found the energetic cost of swimming for a euryhaline tilapian fish to be greatest in the highest salinity water they tested (full-strength sea water, 35‰). Febry and Lutz (1987) did not see the apparent reduction in standard metabolic rate which we observed in animals acclimated to full-strength salinity. While much of the work in this area concludes that standard metabolic rate in fish follows the theoretical 'cost of osmoregulation' and is therefore greater at full-strength salinity than for salinities near iso-osmotic, the papers upon which this paradigm was developed utilised either anadromous fish or euryhaline fish from primarily freshwater taxonomic groups (e.g. Rao, 1968; Farmer and Beamish, 1969; Febry and Lutz, 1987; Job, 1969). As Morgan and Iwama (1991) have pointed out, it is quite possible that the paradigm for primarily marine fish which *tolerate* wide salinity zones, such as the Atlantic cod, is that basal metabolic costs are lowest in the most commonly experienced environment, i.e. full-strength sea water.

The linear nature of the relationship between oxygen consumption and swimming speed (Fig. 3) is somewhat problematical because the expected relationship is a power

function of the form $\dot{M}_{O_2}=aU^b$ (where U is swimming speed and a and b are empirically derived constants specific to species, animal size and temperature (Brett and Groves, 1979). However, linear relationships have been observed previously for cod (Tytler, 1978) and can be explained if recruitment of anaerobic muscle fibres occurs at intermediate swimming speeds, as has been observed electromyographically for other gadoids. Analysing the cost of transport in these animals, or its inverse which we term transport efficiency (i.e. how many kilometres a standard 1 kg animal can travel on 1 mmol of oxygen), supports the idea that these animals are recruiting anaerobic metabolism at swimming speeds as low as 30 cm s^{-1} (Fig. 6A,B). For example, the abrupt increases in transport efficiencies observed in Fig. 6 are consistent with increased contributions from anaerobic metabolism, although it is also possible that some undetected change in hydrodynamic efficiency could be involved.

The importance of both population origin and salinity in determining an animal's active metabolic rate is also apparent from our plots of transport efficiency (Fig. 6). The initially low metabolic rates of SSC at their native salinity translated into greater swimming efficiency and, most likely, a later entry into anaerobic metabolism (contrast the perceptible increase in efficiency at 30 cm s^{-1} in SSC at 20‰ with the line for SSC at 31‰, Fig. 6B). This apparent reduced efficiency at 20‰ did not produce a difference in critical swimming speeds because the average Scotian Shelf cod at 20‰ was able to elevate both its pre- and its post-exercise metabolic rate as well as its apparent use of anaerobic metabolism (Fig. 2B; Table 3). In contrast, differences in the rate of change of transport efficiency with swimming speed were not apparent for BDC swum at different salinities, which suggests that the large differences in the time of appearance of end-products of anaerobic metabolism between the two groups of BDC (Table 3) were due entirely to differences at the final (burst) phases of swimming and during recovery from swimming at U_{crit}.

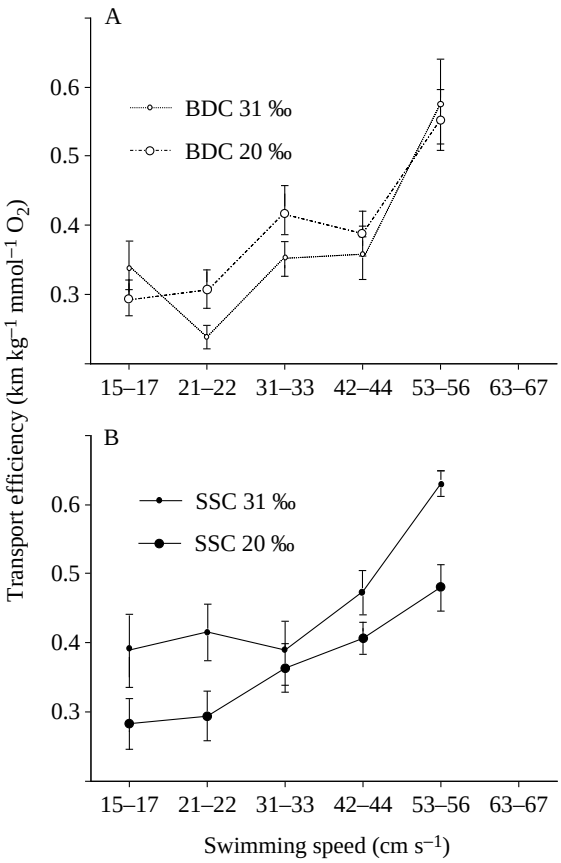

Fig. 6. Transport efficiency (distance of unit mass travel per millimole of oxygen consumed) in (A) Bras d'Or cod (BDC) and (B) Scotian Shelf cod (SSC) during swimming to their critical swimming velocity. Efficiencies were corrected for animal shape (see text) but have been grouped here for the purposes of clarity.

Ion and acid–base status

Acclimation and swimming of Scotian Shelf cod in low-salinity water produced virtually no change in plasma ion concentration or alteration in the way plasma ion concentrations changed with exercise. In contrast, acclimation and swimming of cod native to the Bras d'Or lakes in full-strength sea water produced significant elevations of plasma [Na^+] and [Cl^-] at virtually every sampling time. One could speculate that these different patterns of osmoconformity contributed to the previously discussed increases in metabolic costs observed either at rest (SSC) or during swimming (BDC). To our knowledge, intraspecific differences in degree of osmoconformity with salinity change have not been reported for fishes, although the significant differences in plasma [Cl^-] shown by Morgan and Iwama (1991) could be interpreted this way. Both Carter (1981) and Nordlie *et al.* (1992) report that populations of euryhaline poeciliids from disparate salinity environments had identical plasma osmolality upon acclimation to various salinities. Nordlie *et al.* (1992) addressed this question using brackish- and freshwater populations of the sailfin molly (*Poecilia latipinna*), while Carter (1981) utilised marine and freshwater populations of *Gambusia yucatana*. Both studies measured total plasma osmolality as opposed to the concentrations of individual ions. Our results resemble those seen when interspecific comparisons have been made between closely related species inhabiting different salinity environments. In these cases, the marine fishes usually maintain higher plasma osmolality than do the euryhaline species when both are acclimated to various salinities (e.g. Nordlie and Walsh, 1989). Interestingly, cod from a euryhaline environment, the Gulf of Saint Lawrence (Canada), exhibited their maximum plasma [Na^+] and [Cl^-] when acclimated to 21‰ salinity (Claireaux and Dutil, 1992); these fish had slightly lower plasma [Na^+] and [Cl^-] at 28‰ and larger reductions at 7‰. Taking the results of Claireaux and Dutil (1992) and ours together, we reach the conclusion that plasma [Na^+] and [Cl^-] are more flexible in cod native to euryhaline environments than in cod from the ocean proper. The presence of significant seasonal influences

on plasma ion levels and acclimation to salinity (Dutil *et al.* 1992) raises the spectre that this conclusion may be temperature- and/or time-specific. In contrast to the situation for more acute salinity changes (Brauner *et al.* 1992), we found no relationship between U_{crit} and plasma [Na^+] in the present study.

Since plasma [HCO_3^-] starts declining in animals from both populations swum at 20‰ while they approach exhaustion, but does not decline in animals swum at 31‰ until 0.5 h after exhaustion, it is tempting to speculate either (1) that there is an earlier recruitment of anaerobic muscle fibres at low salinity or (2) that acid excretion is disrupted at 20‰. The former does not appear to be true because salinity-based differences in the appearance of metabolic protons and lactate in the blood does not occur until after exhaustion (Fig. 4) and the expected salinity-based increase in transport efficiency is not observed for Bras d'Or cod (Fig. 6A). The second explanation is debatable because the blood base-excess is homogeneous within populations for the 40 cm s^{-1} blood sample and the expected rise in P_{CO_2} due to titration of plasma [HCO_3^-] by the extra protons (e.g. Tang *et al.* 1989) is not observed. Instead, the decline in venous plasma [HCO_3^-] in animals swimming at 20‰ appears to coincide with a low venous P_{CO_2} in these animals (Table 3). The mechanism by which low salinity reduces venous plasma P_{CO_2} in vigorously swimming fish, and therefore also plasma [HCO_3^-], is unknown. However, Claiborne *et al.* (1994) report increased rates of HCO_3^- loss in sculpins exposed to low salinity, suggesting that loss of HCO_3^- to the environmental water may be the mechanism involved.

The data presented here confirm an earlier hypothesis that the relative appearance of lactate or protons in the extracellular fluid (ECF) is under environmental control and is not homogeneous among members of a given fish species (Nelson, 1990; Nelson and Mitchell, 1992). Fig. 4 plots the ratio of lactate concentration to base-deficit (both in mmol l^{-1}) after lactate starts to appear in the blood. This analysis supports the finding of Tang *et al.* (1989) and Nelson and Mitchell (1992) that reducing the environmental ionic strength favours the appearance of metabolic protons over lactate in the ECF following exercise. Cod from the Bras d'Or lakes registered measurable changes in blood [lactate] after swimming for 0.5 h at 30 cm s^{-1}, whereas all cod had registered increases in blood [lactate] after 0.5 h of swimming at 40 cm s^{-1}, despite a continuation of the linear increase in the rate of oxygen consumption (Fig. 3; Table 3). The appearance of lactate after 0.5 h of swimming at 30 cm s^{-1} agrees with the appearance of increased [lactate] in the muscle of a different gadoid (*Gadus virens*) at 40 % of U_{crit} (Johnston and Goldspink, 1973).

The invariant nature of MCHC within a population, taken in conjunction with the many physiological characteristics of BDC which were identical to those of SSC at 31‰, means that our earlier speculation about MCHC differences between the populations being the root cause for some of the other physiological differences (Nelson *et al.* 1994) was naive. The lack of any definitive explanation for the large number of physiological differences between populations, and the reversion of these differences by acclimation in one population only, led us to seek alternative ways to analyse our data.

Multi-variate analysis

Since we were interested not only in whether the physiology of these populations differed under different environmental conditions, but in the consequences for whole-animal performance measures of possible ecological relevance, we searched for a technique that would allow us to analyse the data so that all physiological variables were considered simultaneously. Principal components analysis (PCA) finds combinations of variables that are not correlated and therefore measures different dimensions of the data. The principal components are ordered so that the first principal component explains the most variation in the data, the second the next most variation, etc. Results of a principal components analysis constructed from 40 variables are presented in Fig. 7; the variables used are listed in the Appendix. The inclusion of data points and the construction of new variables for this analysis were carried out *a priori*, without knowledge of results from similar analyses. In other words, this is a 'first-run' analysis and more dramatic results are possible with further refinement of variables. We present this first-level analysis here to illustrate the potential power that multi-variate analyses can

Table 4. *Three largest positive loadings and two largest negative loadings of the first three principal components*

	Loading	Variable	% Variation explained
First principal component	Positive	MCHC Blood [Cl^-] Blood pH after exhaustion	21
	Negative	Blood P_{CO_2} Age	
Second principal component	Positive	[Haemoglobin] Haematocrit Scope for heart rate	16
	Negative	Pre-exercise [glucose] Pre-exercise heart rate	
Third principal component	Positive	Post-exercise [HCO_3^-] Maximal oxygen consumption rate Post-exercise pH	13
	Negative	Pre-exercise pH Pre-exercise MCHC	

Principal components were constructed from 40 variables measured in 21 fish, 10 from Nelson *et al.* (1994) and 11 from the present study. MCHC, mean cellular haemoglobin content.

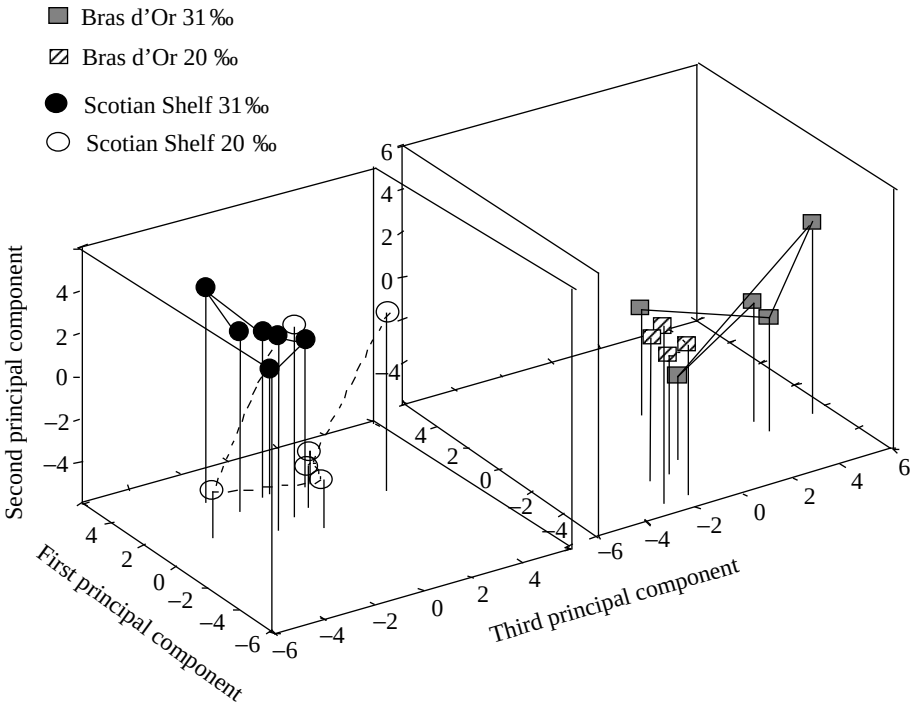

Fig. 7. The first three principal components constructed from 40 physiological variables. Means and standard errors for 12 Scotian Shelf cod (SSC) and nine Bras d'Or cod (BDC) with complete data sets. Data from Nelson *et al.* (1994) were included in this analysis. The variables considered are listed in the Appendix and Table 4.

bring to physiological data sets. While the principal components analysis itself was not overly robust (only 61 % of the cumulative variance was explained by the first four principal components), the contributions of individual physiological measurements to the magnitude of the principal component (eigenvector loadings) and subsequent correlations are instructive.

The first noteworthy result is that the two populations, Bras d'Or and Scotian Shelf cod, were 95 % separated on the first principal component axis (overlap of one individual; Fig. 7). The heaviest eigenvector loadings on this axis were many of the same factors we had previously identified as differing most among the populations (Table 4). This finding illustrates a possible advantage of multivariate statistics for examining similar data sets. When one performs an ANOVA on each measured variable and then searches for the mechanisms underlying interactions of treatments and interactions between measured variables, investigator bias and multi-collinearity (Slinker and Glantz, 1985) are potential pitfalls. In a principal components analysis, the algorithm determines the variable associations which are, by definition, not correlated, thereby avoiding these traps.

Our 'first-level' principal components analysis had a second principal component which correlated strongly with U_{crit} (F=13.5, P<0.01; Fig. 8). The eigenvector loadings (Table 4) indicate that this principal component is largely an amalgam of three things: (1) blood oxygen-carrying capacity; (2) cardiovascular capacity; and (3) the cod being minimally stressed before the experiment commenced. Since stress can cause large increases in oxygen consumption in cod (Saunders, 1963) and would therefore be expected to reduce the animal's scope for activity, principal component 2 appears to be largely a measure of the animal's ability to use oxygen for swimming at the critical speed. While this result seems logical, it contradicts recent conclusions that blood oxygen transport does not limit critical swimming performance (Gallaugher *et al.* 1992) and the univariate result that $\dot{M}_{O_2}$max does not correlate with U_{crit} (Kiceniuk and Jones, 1977). However, differences in species and experimental temperature need to be considered when comparing the two results. As swimming speed increases, Atlantic cod recruit their white muscle sooner than rainbow trout (Archer *et al.* 1990), and the recruitment of white muscle is predicted to be 'compressed' at our experimental temperature of 2 °C when compared with the temperature of

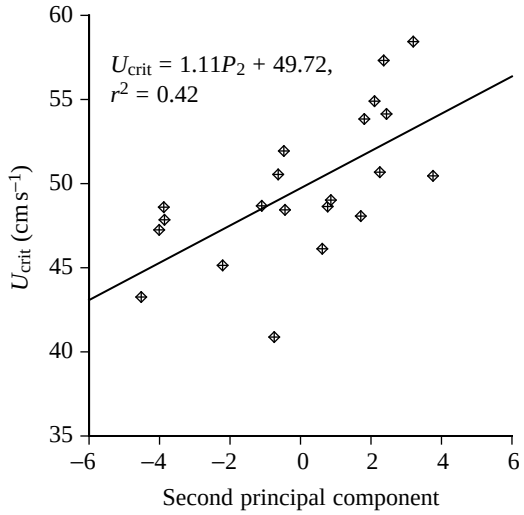

Fig. 8. Linear regression analysis of the second principal component (P_2) *versus* critical swimming speed (U_{crit}) for all 21 fish included in the principal components analysis.

'18–19 °C' used by Gallaugher *et al.* (1992) or 9–10.5 °C used by Kiceniuk and Jones (1977) (Rome *et al.* 1985). According to our analysis, routine variations in blood oxygen transport capability can contribute to differences in exercise performance for Atlantic cod at 2 °C. Interestingly, three animals with extreme U_{crit} performances contributed to the increased variance in swimming ability at 'non-native' salinities (see above) and were also found on extremes of the second principal component axis for their groups (two highest open circles and the highest shaded square on Fig. 7). If we consider the converse argument, the multi-variate analysis then implicates disruption in blood oxygen transport and/or stress as a reason for poor swimming performance. Further support for this argument can be obtained from the results of S. Reidy, J. A. Nelson and S. Kerr (in preparation), who found that gill parasitism by the copepod *Lernaeocera branchialis* lowered critical swimming speed in Atlantic cod by reducing oxygen uptake at the highest swimming speeds. This same parasite did not affect swimming performances powered primarily by anaerobic metabolism.

Finally, the third principal component is of interest because of the intuitive nature of the eigenvector loadings. The largest positive loadings (Table 4) are all measures of an animal's propensity to power it's swim aerobically as opposed to anaerobically. That this principal component did not correlate with U_{crit} supports our earlier contention (Nelson *et al.* 1994) that U_{crit} is a hybrid measure and that different animals use different levels of anaerobic metabolism in reaching their critical speed. Cod from the Bras d'Or lakes swimming at their native salinity of 20 ‰ appeared to use more anaerobic metabolism during the very last part of the swim and during recovery and had a lower metabolic rate near exhaustion than did BDC swimming at 31 ‰ (see above). Therefore, these groups are completely separated on the third principal component axis (Fig. 7). Fish which swam at 'non-native' salinities had greater variance in both the second and third principal components of this analysis, just as they had greater variance in critical swimming performance.

Appendix

List of variables used in the principal components analysis of physiological characters

Total fish length
Condition factor, [fish mass/(fish length)^3]×100
Fish age
Maximal rate of mass-specific oxygen consumption
Minimal rate of mass-specific oxygen consumption
Scope for activity (difference between the above two variables)
Rate of mass-specific oxygen consumption after 4 h of recovery from exercise
Blood [lactate] at exhaustion
Maximal blood [lactate]
Pre-exercise blood pH
Blood pH after 2 h of recovery from exercise
Minimum blood pH
Change in blood pH with exercise
Pre-exercise plasma [HCO_3^-]
Plasma [HCO_3^-] after 2 h of recovery from exercise
Minimum plasma [HCO_3^-]
Maximum plasma [HCO_3^-]
Change in plasma [HCO_3^-] with exercise
Partial pressure of CO_2 in fish swimming at 30 cm s^{-1}
Partial pressure of CO_2 at exhaustion
Blood [haemoglobin] in fish swimming at 30 cm s^{-1}
Blood [haemoglobin] after 2 h of recovery from exercise
Blood haematocrit in fish swimming at 30 cm s^{-1}
Blood haematocrit after 2 h of recovery from exercise
Pre-exercise MCHC
Blood MCHC at exhaustion
Change in MCHC with exercise
Pre-exercise plasma [Na^+]
Plasma [Na^+] after 2 h of recovery from exercise
Change in plasma [Na^+] with exercise
Pre-exercise plasma [Cl^-]
Plasma [Cl^-] after 2 h of recovery from exercise
Pre-exercise blood [glucose]
Blood [glucose] after 4 h of recovery from exercise
Pre-exercise plasma [protein]
Plasma [protein] after 2 h of recovery from exercise
Change in plasma [protein] with exercise
Pre-exercise heart rate
Heart rate after 2 h of recovery from exercise
Change in heart rate with exercise

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