Fish Physiology and Biochemistry vol. 3 no. 1 pp 7-16 (1987)
Kugler Publications, Amsterdam/Berkeley

Seasonal, reproductive, and nutritional influences on muscle "buffering capacity" in yellow perch (*Perca flavescens*)

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Keywords: "buffering capacity", environment, nutritional, perch, reproductive, seasonal, white-muscle

Abstract

"Effective non-bicarbonate" buffering capacity (or buffer value) was measured in white muscle of yellow perch (*Perca flavescens*) by titrations with mineral acid and base in a carbon-dioxide free, closed system. Yellow perch were collected at three month intervals throughout 1983 from an acidic lake (pH ~ 4.6) and two alkaline lakes (pH ~ 7.8) in northern Wisconsin. "Buffering capacity" was also determined for white muscle of perch kept in the laboratory under different regimes of temperature and ration. The mean "buffering capacity" of white muscle from yellow perch taken directly from natural environments ranged from 40.7 ± 3.1 (SD) slykes in March of 1983 to 53.7 ± 2.8 (SD) slykes in July of that year. These changes in "buffering capacity" were strongly correlated with water temperature. Egg production and thirty-day laboratory starvation produced significant decreases in "buffering capacity" and increases in the water content of yellow perch muscle. Fed perch in the laboratory had a temperature dependent "buffering capacity" similar to "field caught" fish. "Buffering capacity" of white muscle did not differ between yellow perch from acidic and alkaline lakes. Investigators using "buffering capacity" as a gauge of species differences in metabolic potential, should be wary of seasonal and reproductive factors that might alter their conclusions.

Introduction

Recent studies have focused on the "buffering capacity" or buffer value of muscle as an indicator of anaerobic potential in that tissue. "Buffering capacity" has been correlated with: locomotory activity of mammals (Castellini and Somero 1981), birds (Lykkeboe and Johansen 1975; Mill and Baldwin 1983), fish (Abe *et al.* 1985; Castellini and Somero 1981; Suyama 1958), and various invertebrates (England and Baldwin 1983; Morris and Baldwin 1984); diving performance in marine mammals (Castellini and Somero 1981; Marsh 1952) and birds (Mill and Baldwin 1983); and athletic condition in man (Sharp *et al.* 1983). Because the environment can affect acid-base regulatory systems of fishes (Heisler 1980), this study was undertaken to test whether environmental factors influence the "buffering capacity" of white muscle in an aquatic ectotherm, the yellow perch (*Perca flavescens*).

Because anaerobic production of ATP generates hydrogen ions (Hochachka and Mommsen 1983; Portner *et al.* 1984), metabolism in fish may be limited by intracellular acid-base status. The white muscle of fish is a poorly perfused, highly anaerobic tissue (Johnston *et al.* 1977), and constitutes the bulk of the yellow perch musculature. Use of white muscle by fish, increases intracellular free hydrogen ion (Heisler 1984a), suggesting that changes in the acid load might be mirrored in the

"buffering capacity". Influences on muscle "buffering capacity" in fish will likely be seen in the "non-bicarbonate" fraction, because fish regulate bicarbonate ion by relatively slow, ion-transport processes (Heisler 1982).

The apparent "buffering capacity" of a tissue depends on: the method employed to measure it (Burton 1978; Heisler and Piiper 1971), the pH range it is measured over (Bate-Smith 1938), and whether expressed in terms of hydrogen ion concentration or pH (Stewart 1981). We measured pH "buffering capacity" by titrating homogenates with strong acid and base in a bicarbonate free, closed system. Using this method, "buffering capacity" reflects the concentrations of various organic phosphate esters, low molecular weight imidazole-containing compounds, exposed side chains of protein-bound amino acids, primarily histidine, and the original concentration of inorganic phosphate plus phosphate liberated from adenosine tri-phosphate and creatine phosphate during fish capture and homogenate equilibration (Burton 1978; Somero 1981). These components are collectively referred to as the "effective non-bicarbonate", or "intrinsic buffering capacity".

Here we compare "white muscle-buffering capacity" of yellow perch captured at three month intervals during 1983 from acidic and neutral lakes in northern Wisconsin. Laboratory experiments were performed to determine whether temperature and ration influenced the "buffering capacity" of white muscle. We also analyzed how body size, sex, maturity, condition factor and water content of white muscle correlated with "buffering capacity".

Materials and methods

Field protocol

Yellow perch (6.5 g – 102 g; $\bar{x}$ = 27.5 ± 18.5 g (SD)) were collected from three lakes in Vilas County, Wisconsin (Table 1) on March (27–29), July (1–5), October (1), and December (29–31) 1983. All fish were captured by angling during daylight hours and secured as rapidly as possible (~10s). After capture, fish were weighed, measured for total length, sealed in an air-tight bag and placed between slabs of solid carbon dioxide (~60s). Fish were transported to the laboratory on dry ice and stored below −25°C until assayed.

Assay

Buffering capacity was measured by a modification of the procedure described by Van Slyke (1922). Approximately 0.5 g of white, epaxial musculature was excised from frozen fish. Skin, scales, and overlying red and pink muscle were removed prior to weighing and homogenization. Fillets were taken from the musculature dorsal to the transverse septum as far anterior as possible, to avoid intramuscle variability (Ironside and Love 1958). Homogenization was for 2′ at 500 rpm in a 15 ml Potter-Elvehjem tissue grinder, on ice, in 5 ml of carbon dioxide-free 0.15 N Na^+; Cl^-. The homogenate was added to a 25 ml flask, as were two 5 ml rinses of the tissue grinder.

Titrations were under "closed" conditions at constant temperature (25°C), with a nitrogen atmosphere. After temperature equilibration, the homogenate was acidified to approximately pH 6.5 with hydrochloric acid if necessary, and was titrated over the pH range of 6.5–7.5 (Fig. 1). Titration over this range produces a ten-fold change in the OH^-/H^+ on either side of the pN of pure water at 25°C (pH 7) and approximates ± 0.5 pH units of reported values for pH(i) in fish at 25°C (White and Somero 1982; Heisler 1980). For all titrations, the relationship between added titrant and the change in pH was linear ($|r| > .99$, Fig. 1).

Titrants were standardized 0.1 N sodium hydroxide and 0.2 N hydrochloric acid solutions and were added to the flask with a microburet. Sodium hydroxide was standardized every three weeks against potassium acid pthalate, and hydrochloric acid was standardized tri-monthly with freshly standardized sodium hydroxide. Hydrogen ion activity was measured on an Orion Research model 399A ion analyzer with expanded scale, using either a Corning model 476223 or Orion model 91-02 combination electrode.

Table 1. Selected physical and chemical characteristics of the three study lakes

Lake	Area (ha)	Maximum depth (m)	Mean annual pH	Conductivity μmhos/cm	Alkalinity mg $CaCo_3$/l
Wharton	13	6	4.6	9	0
Trout	1566	35	7.6	63	27
Allequash	164	10	7.9	79	39

(Data for Trout and Allequash Lakes taken from the Northern Lakes University of Wisconsin-Madison chemical data base. Data for Wharton Lake taken from Rahel (1983)).

White muscle water content was determined by drying approximate 0.25 g samples at 60°C for at least 24 h. Sex and maturity were determined visually.

Calculations

The pH change induced by titrants can be considered conventionally as a consequence of the direct addition of H^+ or OH^- to the homogenate, or as a consequence of changing the strong ion difference (SID) (SID = [strong base cations] − [strong acid anions]) of the solution (Stewart 1981). The absolute value of the inverse slope of a "best fit" line relating titrant added (ΔSID) versus ΔpH was used to compute β values (Van Slyke 1922; Stewart 1981) (Fig. 1). The symbol β has been used to describe buffer values or "capacities" determined many ways. In this report, β will refer to the "intrinsic" or "effective non-bicarbonate" buffering capacity and is expressed as "μequiv/g wet weight/pH unit". Water content is treated as a separate variable. The average value of β from consecutive titrations with sodium hydroxide and hydrochloric acid was used as the "buffering capacity" for a homogenate. Titration with acid and base produced similar values of β (Fig. 1). Two muscle samples were analyzed for each fish captured in March and July, 1983, but a single sample was prepared from the remaining fish.

Statistics

Comparison of individual means was with a reference "t" distribution (Box *et al.* 1978), after variance was determined by ANOVA. Significance of least squares regression lines was determined by the criteria in Draper and Smith (1981). A regression line was considered significant if the "F-statistic" exceeded the selected percentage point of the "F-distribution" and a predictor if it exceeded four times the selected percentage point. Stepwise multiple regressions were performed with a coefficient risk level of .05 (Draper and Smith 1981).

Laboratory experiment

"Buffering capacity" and water content were determined for white muscle of yellow perch kept in the laboratory at different temperatures, and either fed or starved. The fish in this experiment (mean 15.3 g; range 7–32.5 g) were captured in Vilas County, Wisconsin during Autumn, 1983. The fish were transported to Madison and acclimated for a minimum of thirty days at constant temperature in city well water. The fish were fed liberally during acclimation. At the beginning of an experiment, each fish was weighed, measured, and allocated randomly into one of nine treatment groups: fed at 2, 7, 15, and 24°C; not fed at these temperatures, and sacrificed at the start of the experiment. Two trials were run with identical conditions except the lowest temperature was changed from 7°C to 2°C and the acclimation temperature from 15°C to 8°C for the second trial. Fish were transferred to aquaria within 3°C of the acclimation temperature, and then brought to the test temperature over three days. In fed treatments, worms and minnows were provided in proportion to the expected metabolic

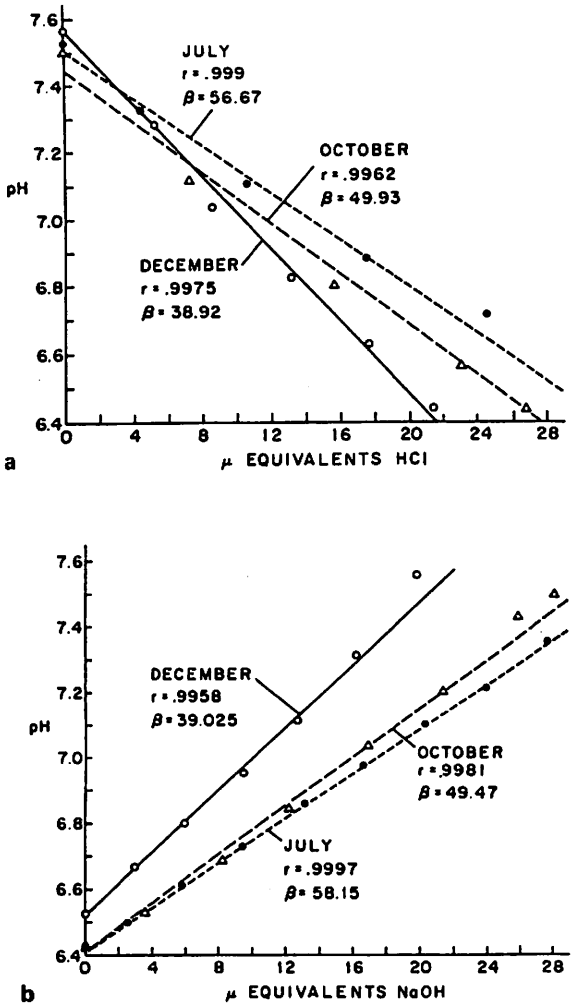

Fig. 1. Titration of homogenates from three individual fish captured at three different seasons in 1983 first with (a)NaOH and (b)HCl. The absolute value of the regression coefficient of a "best-fit" least squares line and the computed "buffering capacity" β in slykes are given. A "slyke" is the microequivalents of strong acid or base ($\triangle$SID) needed to change the pH of one gram of homogenate by one pH unit. The lines shown are hand drawn and not necessarily the same as the least square lines. The symbols and the mass of white muscle used in preparing the homogenate are as follows: July ● (.5192 g); October △ (.5144 g); December ○ (.5012 g).

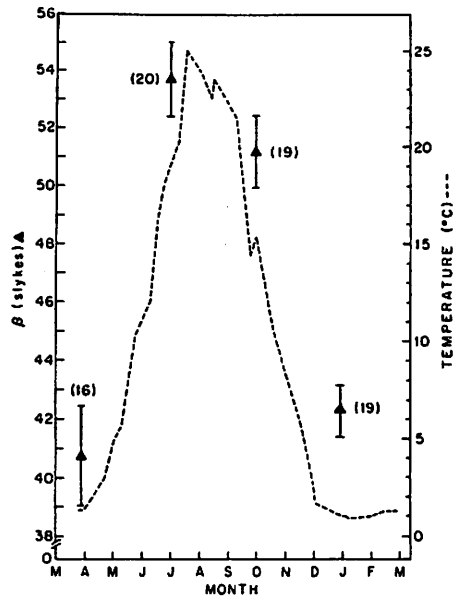

Fig. 2. Seasonal changes in "buffering capacity" of yellow perch white muscle from three lakes, and the water temperature of one of those lakes. Means and 95% confidence intervals of β are plotted with the sample size in parentheses. The temperature plot was constructed from 29 measurements at 1 meter depth in 1983 (Northern Lakes, University of Wisconsin-Madison Chemical Data Base and Dale Robertson unpublished results), and is for Trout Lake, Vilas County, Wisconsin.

rates of the fish (ration increased approximately 2.3 × per 10°C temperature rise). Sample sizes were constrained because some fish would not eat, and others died. The light cycle was 11:11 with one hour each of simulated dawn and dusk. After 28 days at a test temperature, each fish was weighed, measured, and quickly frozen between slabs of solid carbon dioxide. All assays were performed without knowledge of the treatment group the fish came from. Muscle "buffering capacity" and water content were determined as above. Total water soluble protein was measured in the white muscle of fish from the second trial. Approximate 0.25 g samples of white muscle were homogenized on ice in 0.15N Na^+; Cl^- and aliquots analyzed by the Bradford assay (Bradford 1976) on a Beckman Inst. DU-6 spectrophotometer at 556 nm. Bovine serum albumin was the standard.

Results

Determination of buffering capacity

Fig. 1 shows three individual titrations of homogenates made from three fish collected in 1983. Replicates from the same fish generally yield-

Table 2. Water content of white muscle from yellow perch collected at 3 month intervals during 1983

Month	n	Mean % water	Standard deviation
March	12	79.5	1.2
July	16	78.8	.79
October	17	78.3	.84
December	19	78.7	1.1

ed values within 2–3 slykes of each other, and the β for a homogenate was stable for one hour after homogenization, indicating no further hydrolysis of phosphate esters.

Seasonal effects

White muscle "buffering capacity" of "field-caught" yellow perch varied significantly with season (ANOVA $p < .001$) (Fig. 2). Perch from each collection were significantly different ($p < .05$ level or better), except fish collected in March and December 1983 were not different from each other.

Stepwise multiple regression analysis of "buffering capacity" with collection temperatures, muscle water content, condition factor, and mass revealed that environmental temperature was a significant predictor of "buffering capacity" ($r = .927$; $F = 185$; $p < .01$). Fish with more water in their white muscle tended to have lower "buffering capacities", but there was no significant change of water content with season (Table 2).

Reproductive condition

The buffering capacity of perch white muscle depended on fish size only during the winter (Fig. 3). Mass was significantly correlated with "buffering capacity" for the December ($r = -.721$; $F = 18.4$; $p < .01$) and March ($r = -.706$; $F = 13.9$; $p < .01$) groups. Removing points representing mature females from the regressions in Fig. 3 increased the slope of the December and March lines by 28% and 15%, respectively. Females with eggs have a reduced "buffering capacity" when compared with other fish collected at the same time (Table 3). The "buffering capacity" in mature females, was significantly lower in March than in December ($p < .001$). The reduced "buffering capacity" of mature females was reflected in a higher water content in their muscles (Table 3).

Environment

White muscle "buffering capacity" did not differ significantly among the lakes from which the fish were collected, despite large variations in morphometry and chemical composition (Table 1).

Laboratory experiment

Despite small sample sizes, different treatments of temperature and ration produced significant changes in both "buffering capacity" (ANOVA $p < .01$) (Fig. 4) and the water content (ANOVA $p < .05$) of white muscle. Comparison of means by a reference distribution (Box *et al.* 1978), indicated that perch fed at 24°C had a significantly higher "buffering capacity" ($p < .05$) than all except the 15°C fed treatment. This group had a correspondingly low muscle water content which was different ($p < .05$) from all but the 7°C fed treatment. No other paired differences were significant. Multiple regression analysis of all treatment groups indicated that differences in water content were the primary source of variations in "buffering capacity" followed by temperature and fish mass ($p < .01$). The predictive inverse relationship ($r = .74$; $F = 24.2$; $p < .01$) between water content and "buffering capacity" and the inverse correlation between water content and protein concentration ($r = -.90$) suggests that the starvation dependence of β is from the replacement of cellular constituents with water during starvation. Multiple regression analysis of fed treatments only (Fig. 4a) indicated that "buffering capacity" was significantly correlated with temperature ($r = .86$;

Table 3. Buffering capacity of yellow perch grouped by sex and maturity level

Month	Female		Male	
	Adult	Juvenile	Adult	Juvenile
December	41.2(8)[a]	41.8(2)[ab]	43.4(8)[b]	44.3(1)[b]
March	36.5(3)*[c]	43.2(4)[bd]	41.4(5)[abd]	40.7(4)[abd]

Letters indicate significance at $p < .05$ level for each date
* Muscle water significantly greater than other groups for that collection $p < .05$
Sample sizes in parentheses

$F = 19.4$; $p < .01$). The slope of the "best fit" line relating temperature to "buffering capacity" in the fed treatments was +.51, very similar to the slope (+.59) obtained from a plot of collection temperature versus "buffering capacity" for "field-caught" fish.

Discussion

Critique of methods

A theoretical justification for measuring "buffering capacity" is that classical acid-base treatments use this quantity for predicting the effect of acid-base perturbations on living systems. This interpretation is now controversial (Jennings and Reeves 1983; Stewart 1981). Although our study is founded in traditional acid-base chemistry, we recognize that little is known about acid-base regulation in the intracellular compartment and that the significance of the "buffer value" β is unsettled.

The method employed to determine β was similar to that described by Van Slyke (1922) and Castellini and Somero (1981). Unique to this study was the titration under a closed nitrogen atmosphere where the contribution of bicarbonate ion to the measured "buffering capacity" is negligible (Burton 1978). Measurement of the buffer value β at constant temperature avoids the added complexity of pK changes with temperature (Cameron 1984; Heisler 1984b). Castellini and Somero (1981) and Aickin and Thomas (1977) have reported that "buffering capacity" of muscle was independent of assay temperature, but recent evidence indicates that "buffering" is temperature sensitive in plasma (Bridges *et al.* 1984).

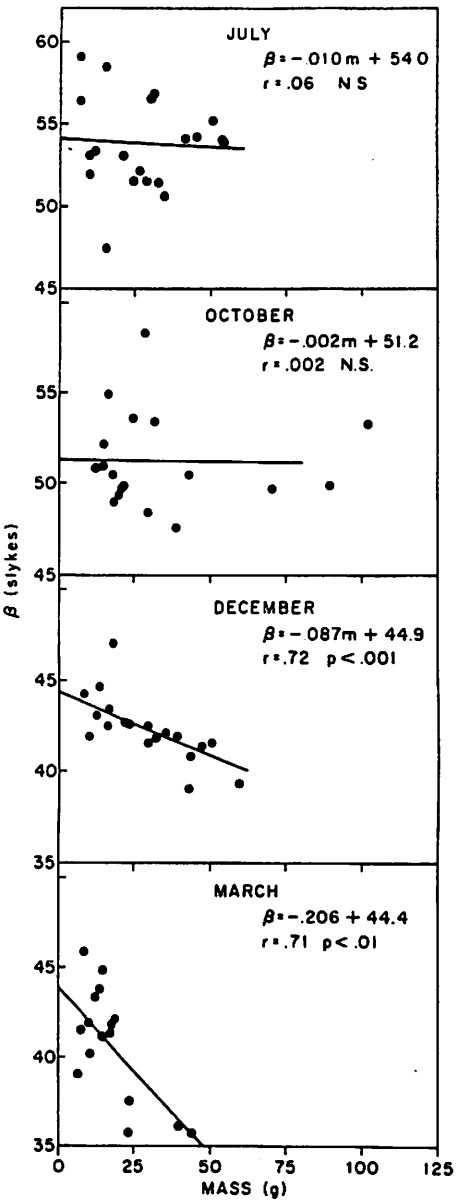

Fig. 3. Relation between body mass and the computed buffer value β of white muscle from yellow perch captured at three month intervals. Individual points and least squares "best fit" lines are plotted. The equation, regression coefficient and significance level of the F-test are given for each regression line.

Comparison of the β values for perch white

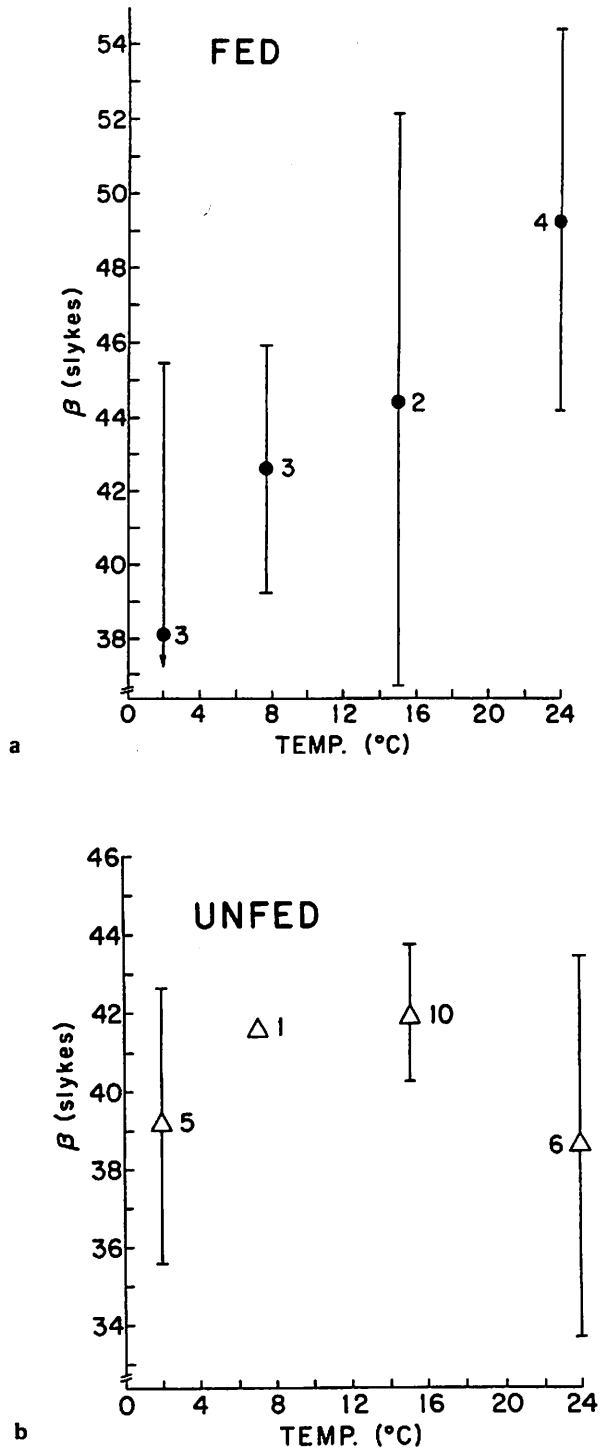

Fig. 4. Buffering capacity in yellow perch held in the laboratory for 28 days at various temperatures and either a) fed or b) starved. Means and 95% confidence intervals of β are plotted with the sample size adjacent. Fish sacrificed at the beginning of the experiments had a mean β of 43.13 ± S.D. 1.88 slykes (n = 5).

muscle with values from other fishes in Castellini and Somero (1981), suggests that our capture and assay procedures gave lower estimates of the "non-bicarbonate buffering capacity" then did their methodology. The many techniques used by investigators to determine "buffering capacity" (Burton 1978; Heisler and Piiper 1971), suggest that interstudy comparisons of absolute β values are difficult, unless the experimental protocol is duplicated precisely.

An important question is whether this measurement has physiological relevance. We used an homogenate technique despite problems in comparing homogenate conditions to those *in vivo*. In an homogenate, cellular compartments become destroyed, new sites on molecules may be exposed, and the mass balance of metabolic reactions change because the thermodynamic environment differs from that of the cell.

Seasonal effects

Large intraspecific changes in "buffering capacity" for the same muscle have been reported only for the horsehair crab (*Erimacrus isenbeckii*) at different molt stages (Motohiru and Inone 1971). Some authors have even commented on the lack of variation in "buffering capacity" for a given muscle and species (Bate-Smith 1938; Castellini and Somero 1981).

Among measured variables, collection temperature was the strongest predictor of "buffering capacity" in "field-caught" fish (Fig. 2). This relationship appears again in the increase in "buffering capacity" among fed fish in the laboratory (Fig. 4a). One interpretation of this correlation is that differences in reaction rates between proton producing and (consuming + excreting) reactions change with temperature, requiring the organism to have greater "buffering capacity" at higher temperatures. An alternative is that cellular constituents, capable of buffering under homogenate conditions, are changing in a temperature dependent manner.

It is unlikely that direct temperature effects on "buffering-active" compounds produced the

results shown in Fig. 2, since homogenates were equilibrated to the same temperature (25°C). Measuring the *in vitro* buffering at each collection temperature could change these results, depending on the method employed. Predicting this change requires knowledge of imidazole and phosphate buffer concentrations and their ratio (Cameron 1984; Heisler 1984b), information not available for perch. Abe *et al.* (1985) found a reduced imidazole/phosphate ratio in cold water (*Salmo gairdneri*) white muscle when compared with tropical (*Makaira nigricans*) muscle. In this case, titration of crude homogenates from the trout at the collection temperature ~ 10°C over the range pH 6.5–7.5 would cause relatively little change in the computed buffer value because of the smaller contribution of imidazole but titration of ± 0.5 units around the pN of water at 10°C (7.27) would cause large reductions in the computed β for trout because of the temperature insensitivity of phosphate pK.

Unmeasured factors that are temperature dependent could be responsible for the seasonal changes in "buffering capacity". Yellow perch (*Perca flavescens*) and European perch (*Perca fluviatilis*) have seasonal differences in swimming capacity and speed (Hergenrader and Hasler 1967; Sandstrom 1983), which parallel changes seen here. Fish use anaerobically produced ATP to fuel some of their swimming activity (Johnston *et al.* 1977), and use of white muscle during burst swimming can cause lactate and proton loads that require long periods to clear from the body (Black *et al.* 1962; Turner *et al.* 1983). The majority of protons generated during exercise are retained in white muscle until they are eliminated by branchial excretion or aerobic metabolism (Holeton *et al.* 1983; Wood *et al.* 1983). Without mechanisms to compensate for the ensuing depression of intracellular pH, the activities of enzymes could be reduced, or stopped (Hand and Somero 1983), impairing muscle function. Abe *et al.* (1985), Castellini and Somero (1981), and Sullivan and Somero (1980) have suggested that interspecific variations in swimming ability are reflected in white muscle "buffering capacity" and metabolic enzyme activities of fishes. The seasonal shift in swimming activity by yellow perch could be responsible for the changes in buffering capacity shown in Fig. 2.

Sullivan and Somero (1983) found seasonal variations in the activities of lactate dehydrogenase and pyruvate kinase, and proposed that reduced ration in the winter caused the reductions in enzyme activity. Ration certainly had an effect on the "buffering capacities" of yellow perch we kept in the laboratory, (Fig. 4), but the effect of ration was temperature dependent.

Based upon field (Fig. 2) and laboratory observations (Fig. 4) we suggest that environmental temperature is the controlling factor in determining seasonal changes in the "buffering capacity" of yellow perch, while ration and activity levels are potential limiting factors (Fry 1971). Further research is needed to discern the mechanism of temperature action and to relate these changes to actual *in vivo* β values at environmental temperature.

Reproductive and nutritive condition

Starvation significantly depressed "buffering capacity" at 24°C (Fig. 4). The increased water content of these fish suggests that metabolism of cellular materials from white muscle during starvation reduces *in vitro* "buffering capacity" of muscle. Johnston (1981) has documented the changes in white muscle characteristics that occur during starvation in plaice (*Pleuronectes platessa*), and found substantial proteolysis in white muscle. The reduced "buffering capacity" after starvation (Fig. 4b), was most likely caused by loss of proteins with amino acid side chains capable of buffering *in vitro*.

The reduced "buffering capacity" seen in larger fish in winter (Fig. 3) correlated with egg production by mature females (Table 3). Yellow perch in northern Wisconsin begin gonad proliferation in fall, and spawn shortly after ice-out in early May. The lack of a size dependence throughout the year, and the usual positive scaling of the activities of metabolic enzymes in fish (Somero and Childress 1980; Sullivan and Somero 1983) suggest that reduced "buffering capacity" in larger fish was not

caused by metabolic scaling.

The concomitant increase in water content in mature females (Table 3) suggests that reduced "buffering capacity" in these animals is from a reallocation of cellular material from white muscle to the maturing eggs. Mommsen *et al.* (1980) and Wood *et al.* (1960) have reported depleted histidine levels in the muscle of migrating sockeye salmon (*Oncorhynchus nerka*). Because the concentration of free imidazole compounds is low in perch (Lukton and Olcott 1958), it is unlikely that a similar depletion accounts for the low "buffering capacity" found in mature, female perch (Table 3). More likely, it is a decrease in the soluble protein fraction of the white muscle like that reported for various marine fishes (Ironside and Love 1958; Shulman 1974) prior to spawning, that is responsible. These results raise the possibility that female perch may be compromising swimming performance to maximize reproductive output.

Environment

"Buffering capacity" did not change with a thousandfold increase in environmental hydrogen ion. This suggests that environmental H^+ does not influence the acid load in white muscle of perch from Wharton Lake. Seasonal changes in "buffering capacity may help explain the seasonal differences in tolerance to low pH in brook trout (*Salvelinus fontinalis*) (Dively *et al.* 1977). These authors reported that brook trout caught in the summer maintained their acid-base status better when challenged with low environmental pH than did "winter-caught" fish.

Acknowledgments

We thank Drs. A.D. Hasler, G.S. Mitchell, P.A. Stewart, and K.M. Sullivan for criticizing the manuscript, and Charoen Nitithamyong and Carla Edwards for technical assistance. Supported by a Wisconsin Alumni Research Foundation Fellowship; Sigma Xi, Jefferson David Fund; and the Anna Grant Birge Fellowship.

References cited

Abe, H., Dobson, G.P., Hoeger, U. and Parkhouse, W.S. 1985. Role of histidine-related compounds to intracellular buffering in fish skeletal muscle. Am. J. Physiol. 249: R449–R454.

Aickin, C.C. and Thomas, R.C. 1977. Micro-electrode measurement of the intracellular pH and buffering power of mouse soleus muscle fibres. J. Physiol. 267: 791–810.

Bate Smith, E.C. 1938. The buffering of muscle in rigor; protein, phosphate, and carnosine. J. Physiol. 92: 336–343.

Black, E.C., Robertson, A., Conner, K., Lam, K.C. and Chui, W.G. 1962. Changes in glycogen, pyruvate, and lactate in rainbow trout (*Salmo gairdneri*) during and following muscular activity. J. Fish. Res. Board Can. 19: 409–436.

Box, G.E.P., Hunter, W.G. and Hunter, J.S. Statistics for Experiments: An Introduction to Design Data Analysis, and Model Building. New York, N.Y.: John Wiley and Sons, 1978.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analy. Biochem. 72: 248–254.

Bridges, C.R., Taylor, A.C., Morris, S.J. and Grieshaber, M.K. 1984. Ecophysiological adaptations in *Blennius pholis* (*L.*) blood to intertidal rockpool environments. J. Exp. Mar. Biol. and Ecology. 77: 151–167.

Burton, R.F. 1978. Intracellular Buffering. Respir. Physiol. 33: 51–58.

Cameron, J.N. 1984. Acid-base status of fish at different temperatures. Am. J. Physiol. 246: R452–R459.

Castellini, M.A. and Somero, G.N. 1981. Buffering capacity of vertebrate muscle: Correlations with potentials for anaerobic function. J. Comp. Physiol. 143: 191–198.

Dively, J.L., Mudge, J.E., Neff, W.H. and Anthony, A. 1977. Blood P_{O2}, P_{CO2}, and pH changes in brook trout (*S. fontinalis*) exposed to sublethal levels of acidity. Comp. Biochem. and Physiol. 57A: 347–351.

Draper, N. and Smith, H. Applied Regression Analysis, Second Edition. New York, N.Y.: John Wiley and Sons, 1981.

England, W.R. and Baldwin, J. 1983. Anaerobic energy metabolism in the tail musculature of the Australian Yabby *Cherax destructor* (Crustacea, Decapoda, Parastacidea): Role of phosphagens and anaerobic glycolysis during escape behavior. Physiol. Zool. 56(4): 614–622.

Fry, F.E.J. 1971. Effects of environmental factors on the physiology of fish. Pages 1–98 *In* W.S. Hoar and D.J. Randall, eds. Fish physiology. Vol. III. Locomotion. Academic Press, New York.

Hand, S.C. and Somero, G.N. 1983. Phosphofructokinase of the hibernator *Citellus beecheı*: Temperature and pH regulation of activity via influences on the tetramer-dimer equilibrium. Physiol. Zool. 56(3): 380–388.

Heisler, N. 1980. Regulation of the acid-base status in fishes. 1980. Pages 123–162. *In* M.A. Ali, ed. Environmental physiology of fishes. Plenum Press, New York.

Heisler, N. 1982. Transepithelial ion transfer processes as

mechanisms for fish acid-base regulation in hypercapnia and lactacidosis. Can. J. Zool. 60(5): 1108–1122.

Heisler, N. 1984(a). Acid-Base regulation in fishes. Pages 315–400 *In* W.S. Hoar and D.J. Randall, eds. Fish Physiology. Vol. Xa. Gills: Part A: Anatomy, gas transfer, and acid-base regulation. Academic Press, New York.

Heisler, N. 1984(b). Role of ion transfer processes in acid-base regulation with temperature changes in fish. Am. J. Physiol. 246: R441-R451.

Heisler, N. and Piiper, J. 1971. The buffer value of rat diaphragm muscle tissue determined by PCO_2 equilibration of homogenates. Respir. Physiol. 12: 169–178.

Hergenrader, G.L. and Hasler, A.D. 1967. Seasonal changes in swimming rates of yellow perch in Lake Mendota as measured by sonar. Trans. Am. Fish. Soc. 96(4): 373–382.

Hochachka, P.W. and Mommsen, T.P. 1983. Protons and Anaerobiosis. Sci. 219: 1391–1397.

Holeton, G.F., Neumann, P. and Heisler, N. 1983. Branchial ion exchange and acid-base regulation after strenuous exercise in rainbow trout (*Salmo gairdneri*). Respir. Physiol. 51: 303–318.

Ironside, J.I.M. and Love, R.M. 1958. Studies on protein denaturation in frozen fish. I. – Biological factors influencing the amounts of soluble and insoluble protein present in the muscle of the North Sea Cod. J. Sci. Food Agric. 9: 597–604.

Jennings, D.B. and Reeves, R.B. 1983. Commentary on the review article by Dr. Peter Stewart: Modern quantitative acid-base chemistry. Can. J. Physiol. and Pharmacol. 61: 1442–1443.

Johnston, I.A. 1981. Quantitative analysis of muscle breakdown during starvation in the marine flatfish *Pleuronectes platessa*. Cell Tissue Res. 214: 369–386.

Johnston, I.A., Davison, W. and Goldspink, G. 1977. Energy metabolism of carp swimming muscles. J. Comp. Physiol. 114: 203–216.

Lukton, A. and Olcott, H.S. 1958. Content of free imidazole compounds in the muscle tissue of aquatic animals. Food Res. 23: 611–618.

Lykkeboe, G. and Johansen, K. 1975. Comparative aspects of buffering capacity in muscle. Respir. Physiol. 25: 353–361.

Marsh, B.B. 1952. Observations on rigor mortis in whale muscle. Biochim. Biophys. Acta 9: 127–132.

Mill, G.K. and Baldwin, J. 1983. Biochemical correlates of swimming and diving behavior in the little Penguin *Eudyptula minor*. Physiol. Zool. 56 (2): 242–254.

Mommsen, T.P., French, C.J. and Hochachka, P.W. 1980. Sites and patterns of protein and amino acid utilization during the spawning migration of salmon. Can. J. Zool. 58: 1785–1799.

Morris, G.M. and Baldwin, J. 1984. pH Buffering Capacity of Invertebrate Muscle: Correlations with Anaerobic Muscle Work. Mol. Biol. 5: 61–70.

Motohira, T. and Inone, N. 1971. Effect of some chemical compounds on the buffering capacity of horsehair crab *Erimacrus isenbeckki* muscle during intermolt stages. J. Food Sci. 36: 51–54.

Portner, H.O., Heisler, N. and Grieshaber, M.K. 1984. Anaerobiosis and acid-base status in marine invertebrates: a theoretical analysis of proton generation by anaerobic metabolism. J. Comp. Physiol. B 155: 1–12.

Rahel, Frank J. 1983. Population differences in acid tolerance between yellow perch *Perca flavescens*, from naturally acidic and alkaline lakes. Can. J. Zool. 61(1): 147–152.

Sandstrom, O. 1983. Seasonal variations in the swimming performance of perch (*Perca fluviatilis* L.) measured with the rotatory-flow technique. Can. J. Zool. 61(7): 1475–1480.

Sharp, R.L., Costill, D.L., Fink, W.J. and King, D.S. 1983. The effects of eight weeks of sprint training on the buffer capacity of muscle in man. Med. Sci. Sports Exercise. 15(2): p. 116.

Shul'man, G.E. Life cycles of fish. Physiology and Biochemistry. New York, N.Y.: John Wiley and Sons, 1974.

Somero, G.N. 1981. pH-Temperature interactions on proteins: Principles of optimal pH and buffer system design. Mar. Biol. Lett. 2: 163–178.

Somero, G.N. and Childress, J.J. 1980. A violation of the metabolism-size scaling paradigm: Activities of glycolytic enzymes in muscle increase in larger-size fish. Physiol. Zool. 53(3): 322–337.

Stewart, P.A. 1981. How to understand acid-base. A quantitative acid-base primer for biology and medicine. Elsevier, New York.

Sullivan, K.M. and Somero, G.N. 1980. Enzyme activities of fish skeletal muscle and brain as influenced by depth of occurrence and habits of feeding and locomotion. Mar. Biol. 60: 91–99.

Sullivan, K.M. and Somero, G.N. 1983. Size and diet-related variations in enzymic activity and tissue composition in the Sablefish – *Anoplopoma fimbria*. Biol. Bull. 164: 315–326.

Suyama, M., Koike, J. and Suzuki, K. 1958. Studies on the buffering capacity of fish muscle-IV. Buffering capacity of muscles of some marine animals. Bull. Jpn. Soc. Sci. Fish. 24(4): 281–285.

Turner, J.D., Wood, C.M. and Clark, D. 1983. Lactate and proton dynamics in the Rainbow Trout (*Salmo gairdneri*). J. Exp. Biol. 104: 247–268.

Van Slyke, D.D. 1922. On the measurement of buffer values and on the relationship of buffer value to the dissociation constant of the buffer and the concentration and reaction of the buffer solution. J. Biol. Chem. 52: 525–570.

White, F.N. and Somero, G.N. 1982. Acid-base regulation and phospholipid adaptations to temperature: Time courses and physiological significance of modifying the milieu for protein function. Physiol. Rev. 62(1): 40–90.

Wood, C.M., Turner, J.D. and Graham, M.S. 1983. Why do fish die after severe exercise? J. Fish Biol. 22: 189–201.

Wood, J.D., Duncan, D.W. and Jackson, M. 1960. Biochemical studies on Sockeye Salmon during spawning migration XI. The free histidine content of the tissues. J. Fish. Res. Board Can. 17(3): 347–351.