Effects of acute temperature change, *in vivo* and *in vitro*, on the acid–base status of blood from yellowfin tuna (*Thunnus albacares*)

RICHARD W. BRILL

Southwest Fisheries Science Center Honolulu Laboratory, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2570 Dole Street, Honolulu, HI 96822-2396, U.S.A.

PETER G. BUSHNELL AND DAVID R. JONES

Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, B.C., Canada V6T 2A9

AND

MANABU SHIMIZU

Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92717, U.S.A.

Received January 14, 1991
Accepted October 9, 1991

BRILL, R. W., BUSHNELL, P. G., JONES, D. R., and SHIMIZU, M. 1992. Effects of acute temperature change, *in vivo* and *in vitro*, on the acid–base status of blood from yellowfin tuna (*Thunnus albacares*). Can. J. Zool. **70**: 654–662.

In most fishes, blood acid–base regulation following a temperature change involves active adjustments of gill ion-exchange rates which take hours or days to complete. Previous studies have shown that isolated blood from skipjack tuna, *Katsuwonus pelamis*, and albacore, *Thunnus alalunga*, had rates of pH change with temperature (in the open system) equivalent to those necessary to retain net protein charge *in vivo* ($\approx -0.016\ \Delta pH \cdot {}^{\circ}C^{-1}$). It was postulated that this is due to protons leaving the hemoglobin combining with plasma bicarbonate (HCO_3^-), which is removed as gaseous CO_2, and that this ability evolved so that tunas need not adjust gill ion-exchange rates to regulate blood pH appropriately following ambient temperature changes. We reexamined this phenomenon using blood and separated plasma from yellowfin tuna, *Thunnus albacares*. Unlike previous studies, our CO_2 levels (0.5 and 1.5% CO_2) span those seen in yellowfin tuna arterial and venous blood. Various bicarbonate concentrations ($[HCO_3^-]$) were obtained by collecting blood from fully rested as well as vigorously exercised fish. We use our *in vitro* data to calculate basic physiochemical parameters for yellowfin tuna blood: nonbicarbonate buffering (β), the apparent first dissociation constant of carbonic acid (pK_{app}), and CO_2 solubility (αCO_2). We also determined the effects of acute temperature change on arterial pH, $[HCO_3^-]$, and partial pressures of O_2 and CO_2 *in vivo*. The pH shift of yellowfin tuna blood subjected to a closed-system temperature change did not differ from previous studies of other teleosts ($\approx -0.016\ \Delta pH \cdot {}^{\circ}C^{-1}$). The pH shift in blood subjected to open-system temperature change was Pco_2 dependent and lower than that in skipjack tuna or albacore blood *in vitro*, but identical with that seen in yellowfin tuna blood *in vivo*. However, pH adjustments *in vivo* were caused by changes in both $[HCO_3^-]$ and Pco_2. The exact mechanisms responsible for these changes remain to be elucidated.

BRILL, R. W., BUSHNELL, P. G., JONES, D. R., et SHIMIZU, M. 1992. Effects of acute temperature change, *in vivo* and *in vitro*, on the acid–base status of blood from yellowfin tuna (*Thunnus albacares*). Can. J. Zool. **70** : 654–662.

Chez la plupart des poissons, le contrôle acide–base du sang après un changement de température nécessite des ajustements importants des taux d'échange d'ions dans les branchies durant plusieurs heures ou jours. Des études antérieures ont démontré que dans du sang isolé de la Thonine à ventre rayé, *Katsuwonus pelamis*, et du Germon atlantique, *Thunnus alalunga*, les taux de changement du pH en fonction de la température (dans un système ouvert) étaient équivalents aux taux nécessaires au maintien de la charge nette des protéines *in vivo* ($\approx -0{,}016\ pH \cdot {}^{\circ}C^{-1}$). Le phénomène a été expliqué de la façon suivante : les protons libérés par l'hémoglobine se combinent aux bicarbonates du plasma (HCO_3^-), qui sont libérés sous forme de CO_2 gazeux, et cette propriété permet aux thons de ne pas avoir à réajuster les taux d'échange d'ions dans leurs branchies pour maintenir le pH de leur sang après des changements de température ambiante. Nous avons réexaminé ce phénomène chez l'Albacore à nageoires jaunes, *Thunnus albacares*, en utilisant du sang et du plasma séparé. Contrairement aux résultats obtenus chez les autres poissons, les concentrations de CO_2 que nous avons observées (0,5% et 1,5% CO_2) se trouvent à l'intérieur des limites rencontrées normalement dans le sang artériel ou veineux de l'Albacore à nageoires jaunes. Des concentrations variées de bicarbonates ($[HCO_3^-]$) ont été obtenues dans des échantillons de sang prélevés chez des poissons parfaitement reposés et des poissons soumis préalablement à des exercices intenses. Nous utilisons ici nos données obtenues *in vitro* pour calculer les paramètres physico-chimiques de base dans le sang de l'Albacore à nageoires jaunes : capacité tampon due à des sels autres que les bicarbonates (β), constante de la première dissociation de l'acide carbonique apparente (pK_{app}) et solubilité du CO_2 (αCO_2). Nous avons également déterminé les effets d'un changement important de température sur le pH artériel, $[HCO_3^-]$, et les pressions partielles de O_2 et de CO_2 *in vivo*. Le changement de pH dans le sang d'Albacores à nagoires jaunes soumis à un changement de température en système fermé ne diffère pas de celui obtenu au cours d'études antérieures chez d'autres téléostéens ($\approx -0{,}016\ pH \cdot {}^{\circ}C^{-1}$). Le changement de pH dans du sang exposé à des changements de température en système ouvert est fonction de la pression pCO_2 et moins important que les changements obtenus *in vitro* dans du sang de Thonines à ventre rayé ou de Germons atlantiques, mais identique au changement observé dans le sang d'Albacores à nagoires jaunes *in vivo*. Cependant, les ajustements de pH *in vivo* sont réalisés grâce à des changements de la concentration de $[HCO_3^-]$ et de la pression pCO_2. Le mécanisme responsable de ces changements reste encore à déterminer.

[Traduit par la rédaction]

Introduction

Tunas (family Scombridae, tribe Thunnini) are unique among teleosts: they possess vascular countercurrent heat exchangers, can have deep red muscle temperatures as much as 20°C above ambient temperature (Carey *et al.* 1971), and have sustained muscle temperatures at least 2–3°C above ambient (Dizon and Brill 1979*a*, 1979*b*). The blood of tunas is therefore regularly subjected to closed-system (i.e., constant CO_2 content, variable CO_2 partial pressure (Pco_2)) temperature changes as it passes through the vascular countercurrent heat exchangers. In homeotherms, when the extremities are exposed to cold, blood returning to the core may be subjected to temperature changes of up to 30°C (Bazett *et al.* 1948; Gordon *et al.* 1982; Irving 1972). Because this is similar to the maximum temperature shifts to which the blood of tunas is exposed, there are no *a priori* reasons to suspect that tuna blood should show acid–base changes that are different from those exhibited by mammalian blood during *in vitro* closed-system temperature changes.

Unlike other fishes, which remain at preferred temperatures (Crawshaw 1975; Beitinger and Fitzpatrick 1979; Reynolds and Casterlin 1979), tunas regularly subject themselves to rapid ambient temperature changes of 10°C or more during the vertical migrations that are a regular part of their daytime activity (Dizon *et al.* 1978; Holland *et al.* 1990). Such behavior exposes the blood within the gills to rapid open-system (i.e., constant Pco_2, variable CO_2 content) temperature changes. This unusual situation provides *a priori* reasons to suspect that tuna blood should show acid–base changes that differ from those exhibited by the blood of other teleosts during *in vitro* open-system temperature changes. As discussed by Somero (1986) and Cameron (1989*a*), intracellular pH and net protein charge are important factors in enzyme function. Therefore, exceptional mechanisms for regulation of net protein charge could be present in tuna blood.

The effects of temperature changes on *in vivo* and *in vitro* blood acid–base balance have been recently reviewed by Heisler (1984*a*, 1986), Truchot (1987), and Cameron (1989*b*). In general, ectothermic air-breathing animals regulate blood acid–base status, to maintain constant net protein charge, by adjusting Pco_2 in the arterial blood via changes in ventilation. In contrast, water breathers maintain blood acid–base status in the face of temperature changes via changes in blood bicarbonate concentration ($[HCO_3^-]$). This involves regulation of gill ion-exchange rates (i.e., changes in the plasma strong ion difference (Stewart 1981)). Whereas changes in arterial Pco_2 in air-breathing animals can be accomplished within minutes, active adjustments of blood $[HCO_3^-]$ in water-breathing animals, via adjustment of gill ion-exchange rates, may take from several hours to 24 h (Heisler 1984*b*). These latter sorts of adjustments seem ill suited to tunas because of their regular and repeated exposure to rapid ambient temperature changes.

Perry *et al.* (1985*a*, 1985*b*) were the first to specifically investigate blood acid–base regulation in tunas, including the effects of *in vitro* temperature change. During closed-system temperature changes (15 – 30°C), they found that the true plasma pH[1] of skipjack tuna, *Katsuwonus pelamis*, exhibited the expected rate of change ($-0.0165\ \Delta pH \cdot °C^{-1}$) due to decreased solubility of CO_2 and reciprocal titration of plasma proteins and plasma HCO_3^-. Surprisingly, Perry *et al.* (1985*b*) also found that the true plasma pH of skipjack tuna had a remarkably high rate of change ($-0.0131\ \Delta pH \cdot °C^{-1}$) when subjected to open-system temperature changes (15 – 30°C). This large pH shift was due to a large change in plasma $[HCO_3^-]$ ($-0.34\ \Delta mM \cdot °C^{-1}$) which, in turn, was presumed to be due to the release of protons from hemoglobin as the temperature increased. (In the open system, the released protons combine with plasma bicarbonate and it is removed as gaseous CO_2.) In similar *in vitro* experiments using blood from sand trout, *Cynoscion arenarius*, Cameron (1978) found much lower changes in true plasma pH ($-0.0048\ \Delta pH \cdot °C^{-1}$) and $[HCO_3^-]$ ($-0.05\ \Delta mM \cdot °C^{-1}$) during open-system temperature changes. Perry *et al.* (1985*b*) concluded that the high rate of $\Delta pH \cdot °C^{-1}$ seen in tuna blood *in vitro* was needed to maintain net protein charge *in vivo* during rapid ambient temperature changes. Cech *et al.* (1984), investigating the effects of temperature on the oxygen-binding characteristics of isolated blood of albacore, *Thunnus alalunga*, found identical rates of true plasma pH change ($-0.016\ \Delta pH \cdot °C^{-1}$) during both open- and closed-system temperature changes (10 – 35°C). Therefore this tuna species also appears to be able to maintain net protein charge, in the face of rapid ambient temperature changes, without requiring adjustment of active gill ion pumps.

Perry *et al.* (1985*b*) used blood from captive fish immediately after they had been paralyzed with a neuromuscular blocking agent (gallamine triethiodide); no time was given for the fish to recover from the trauma of handling. Cech *et al.* (1984) used similar techniques, except that blood was taken from fish shortly after they had been boated, because, at that time, albacore had never been successfully returned to shoreside tanks. Techniques for the anaesthetization, catheterization, recovery, and maintenance of catheterized tunas have subsequently been developed (Jones *et al.* 1986; Bushnell 1988). Blood samples can now be taken for *in vivo* studies or large quantities collected for *in vitro* studies from tunas that are fully recovered (i.e., have normal blood acid–base status) and that can make appropriate cardiorespiratory adjustments to environmental changes (e.g., changes in ambient oxygen levels; Bushnell *et al.* 1990; Bushnell and Brill 1991).

The present study investigated the changes in arterial pH, $[HCO_3^-]$, Pco_2, and O_2 partial pressure (Po_2) in yellowfin tuna, *Thunnus albacares*, exposed to acute temperature changes. The time course of the temperature changes, and the temperatures used (25 – 20°C and 25 – 18°C), match those to which small yellowfin tuna near Hawaii normally subject themselves (Holland *et al.* 1990). Also, in an attempt to understand the mechanisms driving the changes observed *in vivo*, we determined the rates of pH, $[HCO_3^-]$, and Pco_2 change using whole blood and separated plasma subjected to open- and closed-system temperature changes *in vitro*.

For the *in vitro* studies, we used blood taken from fish that had been vigorously exercised, as well as from those that had fully recovered from handling and surgery. Using blood from exercised and fully recovered animals affords comparison with previous studies and provides a range of initial plasma $[HCO_3^-]$. Furthermore, unlike previous studies, we employed Pco_2 levels that bracket the arterial and venous Pco_2 levels observed in swimming tunas (Jones *et al.* 1986). We also

[1]The pH measured when whole blood is equilibrated to a given gas mixture, or withdrawn directly from an animal, and then injected into the pH electrode.

gathered data that allow the calculation of basic physiochemical parameters (nonbicarbonate buffering (β), the apparent first dissociation constant of carbonic acid (pK_{app}), and CO_2 solubility (αCO_2)) and the construction of $pH-HCO_3^-$ (i.e., Davenport) diagrams specific to yellowfin tuna blood. These are needed for interpreting data from this and future studies on the effects of temperature and exercise on the blood acid–base status of tunas *in vivo* and *in vitro*.

Materials and methods

Yellowfin tunas were purchased from local commercial fishermen and maintained in outdoor tanks (at 25 ± 2°C) at the Kewalo Research Facility (Southwest Fisheries Science Center Honolulu Laboratory, National Marine Fisheries Service, National Oceanic and Atmospheric Administration). Fish were held in captivity for a few days to several months before use. Food was presented daily, but fish were not fed for at least 20 h prior to use in an experiment to allow sufficient time for gut clearance (Magnuson 1969). A total of 24 animals, ranging in weight from 1.4 to 2.6 kg, were used.

In vitro *studies*

To obtain blood for *in vitro* studies from animals whose condition approximated that of fish used by Perry *et al.* (1985*a*) and Cech *et al.* (1984) (henceforth referred to as "exercised"), fish were vigorously chased in their holding tank, netted, and stunned by a blow to the head. Approximately 20–40 mL of blood was then withdrawn by direct cardiac puncture and immediately mixed with 2000 IU of sodium heparin (0.2 mL of 10 000 IU · mL^{-1}).

To obtain blood from rested animals (henceforth referred to as "normal"), fish were dip netted and immediately anaesthetized by being placed in a plastic bag containing tricaine methanesulfonate (1 g · L^{-1}) buffered with an equal molar concentration of $NaHCO_3$. After initial anaesthetization, individual fish were moved to the laboratory and suspended on a surgical table in a chamois sling. A hose with running seawater was placed in the mouth, and oxygenated seawater, containing 0.1 g · L^{-1} of $NaHCO_3$ buffered tricaine methanesulfonate and chilled to approximately 22°C, was pumped over the gills. Both anaesthetic solutions were bubbled vigorously for at least 1 h prior to use to remove excess CO_2. While the fish was still anaesthetized, an 18 or 20 gauge Teflon catheter was introduced into either the ventral or dorsal aorta under manometric guide as described in Jones *et al.* (1986). A length of PE 160 tubing was connected to the catheter and was led out of the mouth through a hole made in the snout (for dorsal aorta catheterization) or simply sutured to the skin (for ventral aorta catheterization). At the completion of surgery, a 22 gauge hypodermic needle was inserted through the dorsal body musculature and into the neural canal immediately behind the skull. The needle was used for injection of 2% lidocaine hydrochloride. This procedure paralyzes all of the spinal motor nerves but apparently leaves all cranial nerves intact (Bushnell *et al.* 1990). The fish was then fixed under water in front of a pipe delivering air-saturated (25°C) fresh seawater at approximately 35 L · min^{-1}. Tunas maintained like this recover from anaesthesia within minutes, are able to control their own ventilation volumes, and will survive in good condition for up to 12 h (Bushnell *et al.* 1990).

Beginning approximately 1 h after surgery, ≈0.3-mL blood samples were periodically taken to check the true plasma pH. It generally took 1–3 h following surgery for the true plasma pH to return to a value considered normal in swimming tunas (≈7.7–7.8; Jones *et al.* 1986). Once it had reached this level, approximately 20–40 mL of blood was withdrawn as quickly as possible and with minimal disturbance to the fish. Samples were immediately pooled and mixed with 2000 IU of sodium heparin (0.2 mL of 10 000 IU · mL^{-1}). The fish was then promptly sacrificed with a massive overdose of sodium pentobarbital.

Immediately after the blood was obtained, a 1-mL sample was centrifuged, and 0.2 mL of the resultant plasma mixed with cold 8% perchloric acid to precipitate plasma proteins. This mixture was centrifuged and the resultant supernatent stored frozen (−4°C) for a maximum of about 6 months before lactate analysis. Lactate concentrations were determined using standard techniques (Sigma Chemical Co., St. Louis, Mo., kit 825-UV). Samples of whole blood were also taken at this time for measurement of hematocrit.

Two-millilitre samples of blood were placed in four glass tonometers. Two of the tonometers were kept in a temperature-controlled water bath maintained at 20°C (±0.2°C) and two in a temperature-controlled water bath maintained at 30°C (±0.2°C). One tonometer in each bath was supplied with air (saturated with water vapor) containing 0.5% CO_2; the other tonometer in each water bath was supplied with air (saturated with water vapor) containing 1.5% CO_2. Precise CO_2 levels in the gas mixtures were obtained using two Wösthoff gas mixing pumps, connected in series, to add pure (99.5%) CO_2 to air. The resultant gas mixtures were stored in automobile tire inner tubes.

The remaining blood was centrifuged and the resultant plasma (i.e., separated plasma) either stored at ≈3°C in a refrigerator or placed in a second set of tonometers in the water baths. These tonometers were also supplied with water vapor saturated air containing 0.5 or 1.5% CO_2. In experiments in which the separated plasma was stored overnight, it was allowed to warm slowly to room temperature the next morning and was then placed in the tonometers.

All tonometers were equilibrated for at least 1 h with their gas mixtures, at the appropriate temperatures, before the blood or separated plasma samples were introduced. Approximately 1 h after the blood or separated plasma samples were placed in the tonometers, ≈0.2-mL subsamples were withdrawn, in random order, and the pH was measured using two Radiometer *MKS* Mark 2 blood gas analyzers whose pH (and P_{CO_2}) electrodes were maintained at either 20 or 30°C. The effects of closed-system temperature changes on pH were measured by injecting the blood or separated plasma equilibrated at 20°C into the pH electrode maintained at 30°C, and vice versa. The pH of the true plasma and separated plasma were measured 2–5 times, depending on the consistency of the readings, and the results averaged. The effect of closed-system temperature change on P_{CO_2} was measured by analyzing the blood and separated plasma equilibrated at 20°C in the P_{CO_2} electrode maintained at 30°C. The pH electrodes were calibrated with pH 7.00 and 8.00 buffers and the P_{CO_2} electrode at two points using precision gas mixtures.

At the end of the experiment, ≈0.4-mL samples were taken for measurement of the total CO_2 concentration. Blood samples were placed in 3-mL Eppendorf tubes, under mineral oil, and centrifuged at room temperature. The resultant anaerobically isolated plasma (i.e., true plasma) was analyzed using the technique described in Cameron (1971). The total CO_2 concentration of separated plasma was measured using the same technique, but samples to be analyzed were taken directly from the tonometers.

The solubility of CO_2 (αCO_2, mM · $mmHg^{-1}$) in acidified yellowfin tuna plasma (pH = 3.1) was measured at 15, 20, 25, and 30°C after being equilibrated with water vapor saturated 99.5% CO_2 as described by Boutilier *et al.* (1985). Mean measured αCO_2 for yellowfin tuna plasma was used in all calculations. True and separated plasma [HCO_3^-] (mM) were calculated by subtracting the quantity of dissolved CO_2 ($\alpha CO_2 \times P_{CO_2}$) from measured total CO_2. The pK_{app} for true and separated plasma was calculated using measured variables and the Henderson–Hasselbalch equation:

$$[1] \quad pK_{app} = pH - \frac{\log[HCO_3^-]}{\alpha CO_2}$$

To avoid false replicates and to minimize variability, all variables were calculated individually for each fish and then averaged. All mean values are given ± standard error of the mean (SEM). All pairwise comparisons used the two-tailed Student's *t*-test; all multiple comparisons used the nested factorial analysis of variance design. $P < 0.05$ was taken as the minimum level for significant differences.

TABLE 1. Effects of P_{CO_2}, vigorous exercise, and temperature on acid–base status and pK_{app} of isolated yellowfin tuna blood and plasma

	0.5% CO_2		1.5% CO_2	
	20°C	30°C	20°C	30°C
Normal fish				
pH_{tp}	7.923±0.032 (8)	7.842±0.024 (7)	7.643±0.029 (8)	7.585±0.022 (8)
pH_{sp}	7.999±0.025 (8)	8.002±0.021 (8)	7.602±0.020 (8)	7.634±0.019 (8)
$[HCO_3^-]_{tp}$	13.0±1.2 (8)	10.4±0.7 (7)	18.0±1.37 (8)	14.2±1.0 (8)
$[HCO_3^-]_{sp}$	13.0±0.7 (8)	12.4±0.9 (8)	15.0±0.8 (8)	14.0±0.8 (8)
$pK_{app,tp}$	6.06±0.02 (8)	5.96±0.02 (7)	6.11±0.01 (8)	6.05±0.02 (8)
$pK_{app,sp}$	6.11±0.01 (8)	6.05±0.02 (8)	6.14±0.01 (8)	6.10±0.01 (8)
Exercised fish				
pH_{tp}	7.493±0.030 (4)	7.395±0.030 (4)	7.333±0.020 (4)	7.286±0.026 (4)
pH_{sp}	7.770±0.038 (5)	7.752±0.036 (5)	7.451±0.049 (5)	7.452±0.040 (5)
$[HCO_3^-]_{tp}$	4.1±0.3 (4)	3.1±0.2 (4)	7.1±0.2 (4)	6.1±0.5 (4)
$[HCO_3^-]_{sp}$	6.8±0.51 (5)	6.5±0.6 (5)	9.3±0.7 (5)	7.5±0.4 (5)
$pK_{app,tp}$	6.11±0.03 (4)	6.03±0.02 (4)	6.19±0.01 (4)	6.15±0.02 (4)
$pK_{app,sp}$	6.18±0.02 (5)	6.08±0.02 (5)	6.19±0.02 (5)	6.19±0.03 (5)

NOTE: Numbers of observations are shown in parentheses; tp, true plasma; sp, separated plasma.

TABLE 2. Effects of acute temperature change on acid–base status and pK_{app} of yellowfin tuna blood *in vivo*

	25°C	20°C	18°C
pH_{tp}	7.800±0.010 (11)	7.890±0.015 (5)	7.850±0.013 (6)
P_{O_2}			
mmHg	52.1±2.7 (6)	—	79.2±4.8 (6)
kPa	6.95±0.36 (6)	—	10.6±0.64 (6)
P_{CO_2}			
mmHg	5.4±0.2 (11)	4.5±0.2 (5)	3.9±0.07 (6)
kPa	0.73±0.03 (11)	0.60±0.03 (5)	0.52±0.01 (6)
$[HCO_3^-]_{tp}$ (mM)	12.2±0.6 (6)	—	13.5±0.5 (6)
$pK_{app,tp}$	6.02±0.02 (6)	—	5.97±0.01 (6)

NOTE: Numbers of observations are shown in parentheses; tp, true plasma.

In vivo *studies*

Fish for *in vivo* studies were treated essentially as described above for obtaining blood from fully recovered animals. The only differences were that only the dorsal aorta was catheterized and that the fish were not injected with heparin. After the interval of several hours required for a fish to recover from surgery and for the true plasma pH to return to normal, samples were taken for measurement of lactate and hematocrit (as described above). Following this, 6 fish (mean weight 1.29 ± 0.16 kg) were subjected to a series of step changes in water temperature between 25 and 20°C and 6 fish (mean weight 1.24 ± 0.14 kg) from 25 to 18°C by cooling or warming the water presented to the fish's mouth. Individual fish were subjected to 4 or 5 temperature changes and were maintained at 18 or 20°C for a maximum of 25 min. Between cooling cycles fish were held at 25°C for 30 min.

Arterial blood samples were taken for analysis of true plasma pH, P_{CO_2}, HCO_3^- (as described above), and P_{O_2} a few minutes prior to, and 15 and 20 min after, the completion of each temperature change. The cooling cycle was effected in 3 min and the warming cycle took 5–10 min. Blood P_{O_2} was measured using two Radiometer MKS Mark 2 blood gas analyzers, one with the P_{O_2} electrode maintained at 18 or 20°C and the other with the electrode at 25°C. The P_{O_2} electrodes were calibrated at 18, 20, and 25°C with a standard zero P_{O_2} solution and air-equilibrated saline.

To minimize any effects of changes in blood acid–base parameters occurring over time, and the variability between animals, results were calculated separately for each temperature and each temperature change and then averaged.

Results

The pH, $[HCO_3^-]$, and pK_{app} values of true and separated plasma from *in vitro* samples are given in Table 1; those from *in vivo* samples (plus mean P_{O_2} values) are given in Table 2. Mean plasma lactate levels for normal and exercised fish (4.6 ± 1.6 and 8.8 ± 2.6 mM, respectively) were not significantly different. Blood samples taken from fish used in the *in vivo* studies had a mean plasma lactate level (3.0 ± 0.3 mM) not significantly different from blood of normal fish. However, when comparisons were made at the same P_{CO_2} values and equilibration temperatures, $[HCO_3^-]$ in true and separated plasma from exercised fish was significantly lower than in the other two groups. Mean hematocrits in blood from exercised and normal fish and fish used in the *in vivo* studies (40.6 ± 4.7, 33.4 ± 1.2, and 34.2 ± 1.2%, respectively) were not significantly different.

The effect of temperature on αCO_2 in acidified yellowfin tuna plasma is shown in Fig. 1. The αCO_2 curve for rainbow

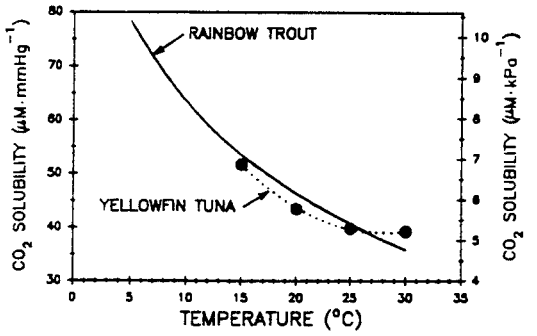

FIG. 1. Effects of temperature on CO_2 solubility in acidified yellowfin tuna plasma and acidified rainbow trout plasma. Data for rainbow trout are from Boutilier *et al.* (1985).

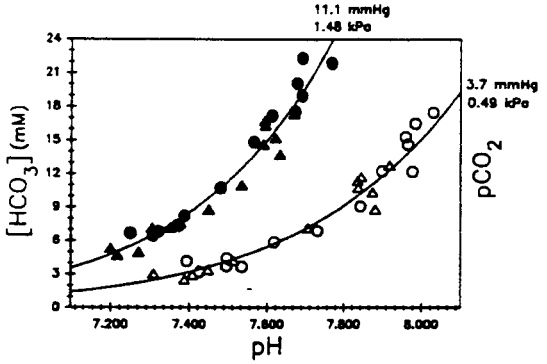

FIG. 2. A pH−HCO_3^- (i.e., Davenport) diagram for yellowfin tuna blood equilibrated to 0.5% CO_2 (Pco_2 = 3.7 mmHg or 0.49 kPa) at 20°C (○) and 30°C (△), and to 1.5% CO_2 (Pco_2 = 11.1 mmHg or 1.48 kPa) at 20°C (●) and 30°C (▲). [HCO_3^-] was measured in true plasma anaerobically separated at room temperature. Lines, added simply for illustrative purposes, were fitted using a nonlinear least-squares regression.

trout (*Oncorhynchus mykiss*) plasma (from Boutilier *et al.* 1985, extrapolated from 15 to 30°C) is plotted for comparison. The equation for a second-degree polynomial fit to the tuna data is

$$[2]\quad \alpha CO_2 = 98.855 \pm 2.827 - 4.2830 \pm 0.2629 \times T + T + 0.0770 \pm 0.0058 \times T^2,\ r^2 = 0.9996$$

(Regression parameters are given ± their standard deviation.)

Nonbicarbonate buffering values (β, $\Delta[HCO_3^-] \cdot \Delta pH^{-1}$, or Slykes) were calculated for true and separated plasma for each fish and then averaged. Mean β values for true or separated plasma from exercised and normal fish did not differ significantly, so data were combined. Mean β values were −20.9 ± 1.2 and −5.1 ± 0.7 $\Delta mM \cdot \Delta pH^{-1}$ for true and separated plasma, respectively.

All data on true plasma pH and [HCO_3^-] from exercised and normal fish are plotted as a pH−HCO_3^- diagram (Fig. 2). Data for blood equilibrated at 20 and 30°C have been combined and iso-Pco_2 lines (0.5% CO_2 = 3.7 mmHg or 0.49 kPa Pco_2 and 1.5 CO_2 = 11.1 mmHg or 1.48 kPa Pco_2) fitted to

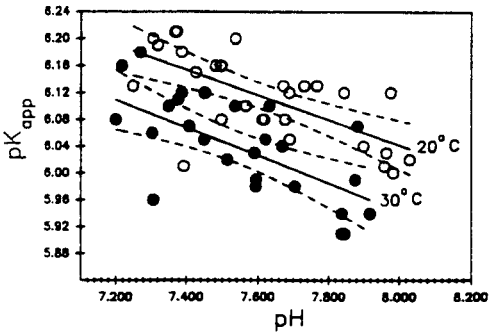

FIG. 3. Effects of temperature and pH on the pK_{app} of yellowfin tuna true plasma. The pK_{app} was calculated from measured variables and the Henderson−Hasselbalch equation. Open circles represent blood equilibrated to 20°C and solid circles blood equilibrated to 30°C. Regression lines were fitted separately for data collected at each temperature. Broken lines show the 95% confidence intervals of the regression lines.

the data, using least-squares nonlinear regression, simply for illustrative purposes.

The effects of pH and temperature on pK_{app} or yellowfin tuna true plasma are shown in Fig. 3. Linear regression equations 3 and 4 were fitted separately to the data collected at 20 and 30°C, respectively; regression lines (±95% confidence intervals) are also presented in Fig. 3.

$$[3]\quad pK_{app} = -0.187 \pm 0.040 \times pH + 7.534 \pm 0.307,\ r = 0.629$$

$$[4]\quad pK_{app} = -0.207 \pm 0.053 \times pH + 7.599 \pm 0.400,\ r = 0.615$$

(Regression parameters are given ± their standard deviation.)

In addition, a regression equation relating true plasma pH, pK_{app}, and temperature (°C) was calculated using an iterative procedure (Systat nonlinear estimation procedure). The data were fitted to a model similar to that used by Boutilier *et al.* (1985):

$$[5]\quad pK_{app} = 7.228 \times T^{-0.01546} + \log T \times (0.4513 - 0.04699 \times pH) - 0.04578 \times pH + 0.1582$$

Effects of temperature change in vitro

The effects of exercise and Pco_2 on $\Delta pH \cdot °C^{-1}$ of true and separated plasma during open- and closed-system temperature changes are shown in Fig. 4. Temperature affects the pH of true plasma and separated plasma very differently during open-system (i.e., constant Pco_2, variable CO_2 content) temperature changes. A nested factorial analysis of variance showed no significant differences in $\Delta pH \cdot °C^{-1}$ of samples taken from normal and exercised fish. There were, as expected, clearly significant differences in $\Delta pH \cdot °C^{-1}$ of true and separated plasma, and a significant effect of CO_2 on $\Delta pH \cdot °C^{-1}$.

The $\Delta pH \cdot °C^{-1}$ exhibited by both true and separated plasma was significantly greater during closed-system temperature changes (Fig. 4). The factorial analysis of variance performed on these data showed no significant differences in $\Delta pH \cdot °C^{-1}$ between samples from exercised and normal fish or between true and separated plasma, or between CO_2 levels.

1 and 2) is approximately 2–4 times that reported for other teleosts *in vivo* (≈4–7 mM; Cameron 1978) and is significantly higher than that measured in isolated (tonometered with 1% CO_2) skipjack tuna blood (≈8 mM at 20°C and ≈5 mM at 30°C; Perry *et al.* 1985*a*). This implies that the blood samples collected by Perry *et al.* (1985*a*) were from fish that had not fully recovered from the effects of transfer from their holding tank to the laboratory and the procedures required to implant the ventral aorta catheters.

The nonbicarbonate buffering capacity of yellowfin tuna blood ($-20.9\ \Delta[HCO_3^-] \cdot \Delta pH^{-1}$) is the highest yet recorded in any teleost, being approximately twice that measured in other fishes (e.g., rainbow trout; Wood and Jackson 1980), including skipjack tuna (Perry *et al.* 1985*a*). This could be explained by the high hemoglobin content of yellowfin tuna blood (Brill and Bushnell 1991), although skipjack tuna blood has equally elevated hemoglobin levels (Brill and Bushnell 1991) but lower nonbicarbonate buffering capacity. The large decrease in true plasma bicarbonate and pH seen in samples from exercised fish (Table 1) might therefore seem surprising at first, given the high nonbicarbonate buffering capacity of yellowfin tuna blood. However, tunas have a well-known capacity for extreme levels of white muscle anaerobic glycolysis and can achieve some of the highest white muscle lactate levels of any vertebrate species (Guppy *et al.* 1979).

The solubility of CO_2 in acidified tuna plasma appears to be slightly less than in acidified trout plasma. This may be due to the higher ion levels of the former (Saither and Rogers 1967; Boutilier *et al.* 1985; Bourke 1987). However, the CO_2 solubility data presented for trout plasma are extrapolated far outside the measurement temperature range, and direct comparison is probably not justified.

The effects of temperature and pH on pK_{app} can be clearly seen in Fig. 3. The effect of pH on pK_{app} is greater in yellowfin tuna true plasma than in the rainbow trout true plasma studied by Boutilier *et al.* (1985). The slope of the pH/pK_{app} regression line (at 15°C, the highest temperature used) is −0.080 in trout true plasma, but −0.17 (at 20°C, eq. 3) in yellowfin true plasma. However, the effect of temperature on pK_{app} ($\Delta pK_{app} \cdot °C^{-1}$) in tuna true plasma was less than that in rainbow trout true plasma. Using regression eqs. 3 and 4, $\Delta pK_{app} \cdot °C^{-1}$ was found to be −0.008 and −0.009 at pH 7.4 and 8.0, respectively. Similarly determined values for rainbow trout true plasma (Boutilier *et al.* 1985) were −0.016 and −0.021 at pH 7.4 and 8.0, respectively. The reasons for the differences in the effect of temperature on pK_{app} are unknown, but the differences are not due to methodology, since the techniques used by Boutilier *et al.* (1985) and in this study were essentially identical.

Table 1 also shows differences when pK_{app} is determined by tonometering whole blood and measuring the pH of true plasma versus tonometering and measuring pH of the separated plasma. These differences are most likely due to the effects of anaerobic centrifugation of blood at room temperature rather than at the equilibration temperature, and the "hemolysis effect" of red cells on true plasma pH measurements. The importance of these effects and the inherent errors in measuring P_{CO_2}, $[HCO_3^-]$, and pH have been reviewed recently by Boutilier *et al.* (1985) and will not be repeated here.

The effects of temperature on the pH of true plasma and separated plasma

That red blood cells are involved in the pH changes seen during open-system temperature change is obvious from Fig. 4. Clearly, the red cells are exchanging something with the plasma that causes a change in true plasma $[HCO_3^-]$ and, in turn, a change in pH. Perry *et al.* (1985*a*) assumed that the reduction in true plasma $[HCO_3^-]$ seen during open-system temperature change in skipjack tuna blood was due to protons leaving the hemoglobin, combining with plasma bicarbonate, and removing it as gaseous CO_2. However, this effect could also have been due to net bicarbonate uptake by the cell or net exchange of strong ions between the plasma and red cells, resulting in a change in plasma strong ion difference (Stewart 1981; Cameron and Iwama 1987; Cameron 1989*a*). What exactly is being exchanged between the red cells and plasma was not determined by Perry *et al.* (1985*a*) or by us.

The effect of open-system temperature change on the pH of yellowfin tuna true plasma was unusually great ($\approx -0.010\ \Delta pH \cdot °C^{-1}$) compared with similar observations made on the isolated blood of other teleosts ($-0.0048\ \Delta pH \cdot °C^{-1}$; Cameron 1978), but this occurred only when the blood was equilibrated to the lower CO_2 level. The smaller $\Delta pH \cdot °C^{-1}$ seen in yellowfin tuna true plasma equilibrated to 1.5% CO_2 is due to the steeper slope of the $pH/[HCO_3^-]$ line (Fig. 2) at this higher CO_2 level. In other words, when the blood is equilibrated to higher P_{CO_2}, equivalent changes in $[HCO_3^-]$ (Fig. 5) at constant P_{CO_2} result in smaller changes in true plasma pH (Fig. 4) because of the steeper slope of the $pH/[HCO_3^-]$ line (Fig. 2).

The $\Delta pH \cdot °C^{-1}$ of isolated yellowfin tuna true plasma equilibrated with 1.5% CO_2 is less than that observed in isolated skipjack tuna blood equilibrated to 1% CO_2. This difference is apparently due to the fact that Perry *et al.* (1985*a*) used blood taken from fish immediately after surgery. In skipjack tuna true plasma, $[HCO_3^-]$ was lower (total CO_2 was only ≈8 mM at 20°C), therefore the blood was operating on the flat portion of the $pH/[HCO_3^-]$ curve (Fig. 2), where relatively small changes in $[HCO_3^-]$ cause relatively large changes in pH.

Perry *et al.* (1985*a*) assumed that the large $\Delta pH \cdot °C^{-1}$ seen in isolated skipjack tuna true plasma was necessary to adjust pH appropriately *in vivo* in the face of rapid ambient temperature changes. They assumed that skipjack tuna must retain a constant relative blood alkalinity ($-0.016\ \Delta pH \cdot °C^{-1}$) during rapid temperature changes. Although some teleosts do appear to regulate pH to retain a constant relative alkalinity, others do not (Truchot 1987), and there are no *a priori* reasons to assume that skipjack tuna would do so. Unfortunately, no data are available on the effects of rapid ambient temperate changes on the blood pH of skipjack tuna *in vivo*, so Perry *et al.*'s (1985*a*) hypothesis that skipjack tuna adjust blood pH without adjusting gill ion-exchange rates remains untested.

Effects of temperature change in vivo

Our data show that arterial true plasma pH changes ($-0.0105 \pm 0.0010\ \Delta pH \cdot °C^{-1}$) *in vivo* match those found *in vitro* ($-0.0103 \pm 0.0008\ \Delta pH \cdot °C^{-1}$) at 0.5% CO_2 (i.e., at the P_{CO_2} approximating arterial P_{CO_2}). Therefore, at first glance, our data do appear to support the hypothesis of Perry *et al.* (1985*a*) that tunas need not actively adjust plasma $[HCO_3^-]$, via changes in gill ion-exchange rates, to regulate blood pH following ambient temperature changes. However, Fig. 5 shows that true plasma $\Delta[HCO_3^-] \cdot °C^{-1}$ values *in vivo* are significantly lower than those observed *in vitro* in blood from normal fish. In other words, red blood cells appear to respond somewhat differently to temperature changes under

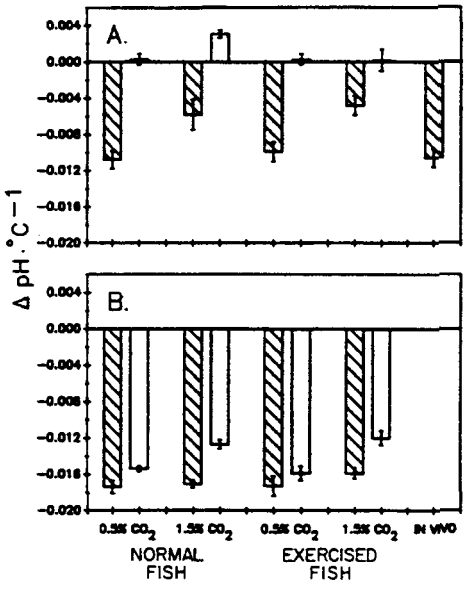

FIG. 4. Rates of pH change *in vitro* in true plasma (hatched bars) and separated plasma (open bars) for normal and exercised yellowfin tuna during (A) open-system (constant P_{CO_2}, variable CO_2 content) and (B) closed-system (constant CO_2 content, variable P_{CO_2}) temperature changes. True plasma pH changes (arterial blood samples) with temperature occurring *in vivo* are also shown in A.

The effects of open-system temperature changes on the $[HCO_3^-]$ of true plasma and separated plasma are shown in Fig. 5. The factorial analysis of variance performed on these data showed significant differences in $\Delta[HCO_3^-] \cdot °C^{-1}$ between samples from normal and exercised fish, and significant differences between true plasma and separated plasma. There was no significant effect of P_{CO_2}.

The effects of closed-system temperature changes on P_{CO_2} of blood and isolated plasma are shown in Fig. 6. The factorial analysis of variance performed on these data showed no significant differences in the behavior of samples taken from exercised and normal fish. There were, however, significant differences in $\Delta P_{CO_2} \cdot °C^{-1}$ between blood and separated plasma, and significant differences due to the P_{CO_2} to which the blood or separated plasma was originally equilibrated.

Effects of temperature change in vitro

The effects of rapid temperature changes on the arterial blood acid–base status are also shown in Table 2 and Figs. 4, 5, and 6. From the figures it is readily apparent that although the pH change *in vivo* matches that seen *in vitro* during open-system temperature changes at arterial (i.e., 0.5%) P_{CO_2} levels, the mechanisms driving these pH changes are different. Note that the change in $[HCO_3^-]$ observed *in vivo* is roughly half that observed *in vitro* in the true plasma obtained from normal fish. Surprisingly, the P_{CO_2} change observed *in vivo* matches that observed *in vitro* (closed system) at equivalent starting P_{CO_2} and $[HCO_3^-]$ levels (i.e., normal blood equilibrated with 0.5% CO_2). Also, arterial P_{CO_2} changes in an opposite direction to arterial P_{O_2}: at lower temperatures the former decreases and the latter increases.

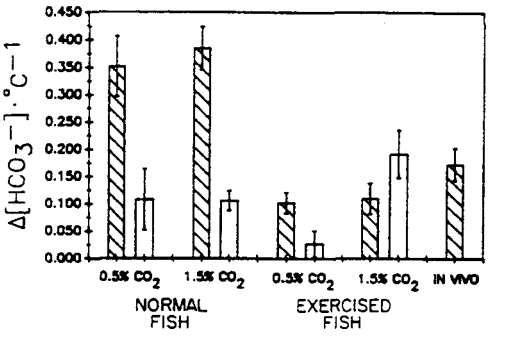

FIG. 5. Effects of *in vitro* open-system temperature changes on $[HCO_3^-]$ of true plasma (hatched bars) and separate plasma (open bars) from normal and exercised yellowfin tuna. The effects of *in vivo* temperature changes on true plasma $[HCO_3]$ (in arterial blood samples) are also shown.

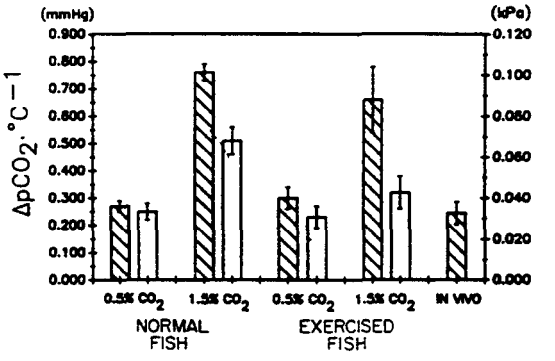

FIG. 6. Effects of closed-system temperature changes on P_{CO_2} of whole blood (hatched bars) and separated plasma (open bars) from normal and exercised yellowfin tuna. P_{CO_2} changes in arterial blood occurring *in vivo* are also shown.

Discussion

The pH values of both true and separated plasma at both equilibration temperatures and at both CO_2 levels are clearly lower in exercised fish than in normal fish. This is due to the plasma $[HCO_3^-]$ in the samples from exercised fish being approximately half that in samples from normal fish (Table 1 and Fig. 2). Note that the significant reductions in plasma $[HCO_3^-]$ occurred despite the absence of a significant elevation of plasma lactate. This suggests that protons may be released from the white muscle of yellowfin tuna unaccompanied by lactate, but proof of this phenomenon would require measurement of more parameters than was done for this study (Cameron and Cech 1990). White muscle lactate retention has been observed in other teleosts (Wardle 1978), including skipjack tuna (Perry *et al.* 1985*a*). The change in acid–base status of true plasma due to strenuous exercise ($\Delta pH \approx 0.4$ units) was similar to that observed in skipjack tuna (Perry *et al.* 1985*a*) and other teleosts following exhaustive activity (Wood *et al.* 1977; Turner *et al.* 1983).

The $[HCO_3^-]$ in true plasma from normal yellowfin tuna *in vitro* and in true plasma taken directly from live fish (Tables

these two conditions. Clearly, it is the increase in arterial P_{CO_2} observed *in vivo* that accounts for the observed equality of $\Delta pH \cdot °C^{-1}$ (Fig. 4) in spite of the differences in $\Delta[HCO_3^-] \cdot °C^{-1}$ (Fig. 5).

An increase in blood P_{CO_2} with increasing temperature is commonly observed in aquatic gill-breathing animals, although the magnitude of the increase is quite variable between species (Heisler 1986). As explained in detail by Randall and Cameron (1973), if fish were to increase arterial blood P_{CO_2} (and therefore decrease blood pH) by decreasing ventilation volume at higher temperatures, a concomitant decrease in arterial P_{O_2} would be expected. Because this is what we have observed in tunas *in vivo* (Table 2), it is possible that yellowfin tuna are indeed decreasing ventilation volume (or increasing gill resistance to O_2 and CO_2 transfer) as temperature increases, and are therefore compromising O_2 delivery to adjust blood pH. However, as explained in Truchot (1987), it is unlikely that gill-breathing animals can regulate P_{CO_2} via changes in ventilation.

In fishes, it appears that the dehydration of HCO_3^- (i.e., conversion of HCO_3^- to physically dissolved CO_2) is the rate-limiting step in CO_2 excretion (Perry 1986). As explained by Perry and Wood (1989), factors that reduce the rate of HCO_3^- dehydration (such as elevated circulating catecholamine levels post exercise) increase arterial blood P_{CO_2}. Because the rate of HCO_3^- dehydration is most likely directly proportional to temperature, increases in temperature would be expected to cause decreases in arterial P_{CO_2}, the exact opposite to what is observed *in vivo*.

In summary, the changes in arterial blood pH observed in yellowfin tuna *in vivo* resulting from rapid shifts in ambient temperature are due in part to changes in plasma $[HCO_3^-]$ caused by the red blood cells. They are also due in part to alterations of arterial blood P_{CO_2}. More research is clearly needed to determine the exact mechanisms responsible for both of these adjustments.

Acknowledgements

The authors thank Bob Boutilier, Dave Randall, George Iwama, and Steve Perry for reviewing early drafts of the manuscript. The editorial assistance of Leslie C. Williams is also gratefully recognized. Finally, we would like to thank the Natural Sciences and Engineering Research Council of Canada (NSERC) for operating, equipment, and travel grants supporting this research. P.G.B. was an NSERC International Fellow.

Bazett, H. C., Love, L., Newton, M., Eisenberg, L., Day, R., and Forster, R. 1948. Temperature changes in blood flowing in arteries and veins in man. J. Appl. Physiol. **1**: 3–19.

Beitinger, T. L., and Fitzpatrick, L. C. 1979. Physiological and ecological correlates of preferred temperature in fish. Am. Zool. **19**: 319–329.

Bourke, R. E. 1987. A study of delayed capture mortality syndrome in skipjack tuna, *Katsuwonus pelamis* (L). J. Fish Dis. **10**: 275–287.

Boutilier, R. G., Iwama, G. K., Hemming, T. A., and Randall, D. J. 1985. The apparent pK of carbonic acid in rainbow trout blood plasma between 5 and 15°C. Respir. Physiol. **61**: 237–254.

Brill, R. W., and Bushnell, P. G. 1991. Effects of open- and closed-system temperature changes on blood oxygen dissociation curves of skipjack tuna, *Katsuwonus pelamis*, and yellowfin tuna, *Thunnus albacares*. Can. J. Zool. **69**: 1814–1821.

Bushnell, P. G. 1988. Cardiovascular and respiratory responses to hypoxia in three species of obligate ram ventilating fishes, skipjack tuna (*Katsuwonus pelamis*), yellowfin tuna (*Thunnus albacares*) and bigeye tuna (*T. obesus*). Ph.D. dissertation, University of Hawaii, Honolulu.

Bushnell, P. G., and Brill, R. W. 1991. Responses of swimming skipjack (*Katsuwonus pelamis*) and yellowfin (*Thunnus albacares*) to acute hypoxia and a model of their cardio-respiratory function. Physiol. Zool. **64**: 787–811.

Bushnell, P. G., Brill, R. W., and Bourke, R. E. 1990. Cardio-respiratory responses of skipjack tuna (*Katsuwonus pelamis*), yellowfin tuna (*Thunnus albacares*), and bigeye tuna (*Thunnus obesus*) to acute reductions of ambient oxygen. Can. J. Zool. **68**: 1857–1865.

Cameron, J. N. 1971. Rapid method for determination of total carbon dioxide in small blood samples. J. Appl. Physiol. **31**: 632–634.

Cameron, J. N. 1978. Regulation of blood pH in teleost fish. Respir. Physiol. **33**: 129–144.

Cameron, J. N. 1989*a*. Acid–base homeostasis: past and present perspectives. Physiol. Zool. **62**: 845–865.

Cameron, J. N. 1989*b*. The respiratory physiology of animals. Oxford University Press, New York.

Cameron, J. N., and Cech, J. J. 1990. Lactate kinetics in exercised channel catfish, *Ictalurus punctatus*. Physiol. Zool. **63**: 909–920.

Cameron, J. N., and Iwama, G. K. 1987. Compensation of progressive hypercapnia in channel catfish and blue crabs. J. Exp. Biol. **133**: 183–197.

Carey, F. G., Teal, J. M., Kanwisher, J. W., and Beckett, K. S. 1971. Warm-bodied fish. Am. Zool. **11**: 137–145.

Cech, J. J., Jr., Laurs, R. M., and Graham, J. B. 1984. Temperature-induced changes in blood gas equilibria in the albacore, *Thunnus alalunga*, a warm-bodied tuna. J. Exp. Biol. **109**: 21–34.

Crawshaw, L. I. 1975. Twenty-four hour records of body temperature and activity in bluegill sunfish (*Lepomis macrochirus*) and brown bullheads (*Ictalurus nebulosus*). Comp. Biochem. Physiol. A, **51**: 11–14.

Dizon, A. E., and Brill, R. W. 1979*a*. Thermoregulation in tunas. Am. Zool. **19**: 249–265.

Dizon, A. E., and Brill, R. W. 1979*b*. Thermoregulation in yellowfin tuna, *Thunnus albacares*. Physiol. Zool. **52**: 581–593.

Dizon, A. E., Brill, R. W., and Yuen, H. S. H. 1978. Correlations between environment, physiology and activity and the effects on thermoregulation in skipjack tuna. *In* The physiological ecology of tunas. *Edited by* G. D. Sharp and A. E. Dizon. Academic Press, New York. pp. 233–359.

Gordon, M. S., Bartholomew, G. A., Grinnell, A. D., Jorgensen, C. B., and White, F. N. 1972. Animal physiology, principles and adaptations. Macmillan Publishing Co., New York.

Guppy, M., Hulbert, W. C., and Hochachka, P. W. 1979. Metabolic sources of heat and power in tuna muscles. II. Enzyme and metabolic profiles. J. Exp. Biol. **82**: 303–319.

Heisler, N. 1984*a*. Acid–base regulation in fishes. *In* Fish physiology. Vol. 10. Part A. *Edited by* W. S. Hoar and D. J. Randall. Academic Press, New York. pp. 315–401.

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. 1986. Acid–base regulation in fishes. *In* Acid–base regulation in animals. *Edited by* N. Heisler. Elsevier Scientific Publishing Co., Amsterdam. pp. 309–356.

Holland, K. N., Brill, R. W., and Chang, R. K. C. 1990. Horizontal and vertical movements of yellowfin and bigeye tuna associated with fish aggregating devices. Fish. Bull. **88**: 493–507.

Irving, L. 1972. Arctic life of birds and mammals. Springer-Verlag, New York.

Jones, D. R., Brill, R. W., and Mense, D. C. 1986. The influence of blood gas properties and pH of ventral and dorsal aortic blood in free-swimming tuna, *Euthynnus affinis*. J. Exp. Biol. **120**: 201–213.

Magnuson, J. J. 1969. Digestion and food consumption by skipjack tuna (*Katsuwonus pelamis*). Trans. Am. Fish. Soc. **98**: 370–392.

Perry, S. F. 1986. Carbon dioxide excretion in fishes. Can. J. Zool. **64**: 565–572.

Perry, S. F., and Wood, C. M. 1989. Control and coordination of gas transfer in fishes. Can. J. Zool. **67**: 2961–2970.

Perry, S. F., Daxboeck, C., Emmett, B., Hochachka, P. W., and Brill, R. W. 1985*a*. Effects of temperature change on acid–base regulation in skipjack tuna (*Katsuwonus pelamis*) blood. Comp. Biochem. Physiol. A, **81**: 49–53.

Perry, S. F., Daxboeck, C., Emmett, B., Hochachka, P. W., and Brill, R. W. 1985*b*. Effects of exhausting exercise on acid–base regulation in skipjack tuna (*Katsuwonus pelamis*) blood. Physiol. Zool. **58**: 421–429.

Randall, D. J., and Cameron, J. N. 1973. Respiratory control of arterial pH as temperature changes in rainbow trout *Salmo gairdneri*. Am. J. Physiol. **225**: 997–1002.

Reynolds, W. W., and Casterlin, M. E. 1979. Behavioral thermoregulation and the "final preferendum" paradigm. Am. Zool. **19**: 211–224.

Saither, B. T., and Rodgers, T. A. 1967. Some inorganic constituents of the muscle and blood of the oceanic skipjack, *Katsuwonus pelamis*. Pac. Sci. **21**: 404–413.

Somero, G. N. 1986. Protons, osmolytes, and fitness of internal milieu for protein function. Am. J. Physiol. **251**: R197–R213.

Stewart, P. A. 1981. How to understand acid–base. Elsevier/North-Holland, New York.

Truchot, J.-P. 1987. Comparative aspects of extracellular acid–base balance. Springer-Verlag, New York.

Turner, J. D., Wood, C. M., and Hobe, H. 1983. Physiological consequences of sever exercise in the inactive benthic flathead sole (*Hippoglossoides elassodon*): a comparison with the active pelagic rainbow trout (*Salmo gairdneri*). J. Exp. Biol. **104**: 269–288.

Wardle, C. S. 1978. Non-release of lactic acid from anaerobic swimming muscle of plaice *Pleuronectes platessa* L.: a stress reaction. J. Exp. Biol. **77**: 141–155.

Wood, C. M., and Jackson, E. B. 1980. Blood acid–base regulation during environmental hyperoxia in the rainbow trout. Respir. Physiol. **42**: 351–372.

Wood, C. M., McMahon, B. R., and McDonald, D. G. 1977. An analysis of changes in blood pH following exhausting activity in the starry flounder, *Platichthys stellatus*. J. Exp. Biol. **69**: 173–185.