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ORIGINAL PAPER

# Effects of open- and closed-system temperature changes on blood $O_2$-binding characteristics of Atlantic bluefin tuna (*Thunnus thynnus*)

**R. W. Brill · P. G. Bushnell**

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**Abstract** We investigated the effects of open- and closed-system temperature changes on the $O_2$ affinity of Atlantic bluefin tuna (*Thunnus thynnus*) blood using in vitro methods essentially identical to those previously employed on tropical tuna species. Bluefin tuna blood has a general $O_2$ affinity ($P_{50}$ = 2.6–3.1 kPa or 19–23 mm Hg at 0.5% $CO_2$) similar to that of skipjack tuna, yellowfin tuna, and kawakawa blood ($P_{50}$ = 2.8–3.1 kPa at 0.5% $CO_2$) but significantly above that of bigeye tuna blood ($P_{50}$ = 1.6–2.0 kPa at 0.5% $CO_2$). We therefore hypothesize that bluefin tuna are less tolerant of hypoxia than bigeye tuna. Further, we found the $P_{50}$ of bluefin tuna blood to be slightly reduced by a 10°C open-system temperature increase (e.g., from 4.83 kPa at 15°C to 3.95 kPa at 25°C) and to be completely unaffected by a 10°C closed-system temperature change. Bluefin tuna blood, therefore, had a significantly reduced Bohr effect when subjected to the inevitable changes in $PCO_2$ and plasma pH that accompany closed-system temperature shifts (0.04–0.09 $\Delta\log P_{50}\Delta pH^{-1}$) compared with the effects of changes in plasma pH accomplished by changing $PCO_2$ alone (0.81–0.94 $\Delta\log P_{50}\,\Delta\,pH^{-1}$). This response is similar to that of skipjack tuna blood, but different from yellowfin or bigeye tuna blood. During closed-system temperature changes at oxygen levels above $P_{50}$, however, bluefin tuna blood showed a reversed temperature effect (i.e., $PO_2$ decreased in response to an increase in temperature). Unlike in other tuna species, temperature effects on $O_2$ affinity of bluefin tuna whole blood were similar to those previously reported for hemoglobin solutions, suggesting that red cell-mediated ligand changes are not involved.

**Keywords** Cardiorespiratory · Fish · Hemoglobin · Hypoxia · Pelagic · Metabolic rate · Oxygen affinity · Scrombridae

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## Introduction

As a result of their almost continuous vertical movements and extensive migrations from tropical to temperate waters, Atlantic bluefin tuna (*Thunnus thynnus*) subject themselves to ambient temperature changes of 15°C or more over time periods ranging from tens of minutes (or fewer) to

several months (Lutcavage et al. 2000; Brill et al. 2002; Block et al. 2001a, 2001b; Gunn and Block 2001; Boustany et al. 2001; Stokesbudy et al. 2004). Blood passing through their gills must, therefore, maintain its functional properties while being subjected to a wide range of temperatures but where it is free to exchange gases and proton equivalents with the water passing over the gills. This is referred to as on ''open system'' temperature change.

Bluefin tuna, like other members of the family *Scombridae* (tribe *Thunnini*), are also regional endotherms (Graham and Dickson 2001). Their vascular counter-current heat exchangers enable them to sustain deep red-muscle fibers and some internal organs well above ambient temperature (Carey and Teal 1966; Carey et al. 1984). Vascular counter-current heat exchangers also reduce rates of heat transfer to or from the environment following abrupt changes in ambient temperature (Carey 1973; Neill et al. 1976). As a result, body temperature changes lag significantly behind rapid alternations in water temperature, and deep red muscle temperature of even relatively small tuna routinely differs from water temperature by >15°C (Gunn and Block 2001). Bluefin tuna blood is, therefore, also subjected to significant ''closed-system'' temperature changes (i.e., alterations in temperatures where the blood is not able to exchange gases or proton equivalents with another medium) as it passes from the gills (where it is at ambient temperature) through the vascular counter-current heat exchangers. During this situation, blood $O_2$ content ($[O_2]$) remains constant, whereas the partial pressures of $O_2$ and $CO_2$ ($PO_2$ and $PCO_2$, respectively) are variable. Moreover, whereas open-system temperature changes occur at a constant $PCO_2$ and are thus accompanied by relatively minor changes in plasma pH ($pH_e$) ($\approx 0.004\Delta\ pH_e°C^{-1}$), closed-system temperature shifts are accompanied by substantial alterations in $PCO_2$ and $pH_e$ ($\approx 0.016\Delta pH_e°C^{-1}$), due to changes in $CO_2$ solubility in the plasma and the reciprocal titration of plasma proteins and plasma bicarbonate (Cech et al. 1984; Perry et al. 1985; Truchot 1987; Brill and Bushnell 1991; Brill et al. 1992; Lowe et al. 2000).

Carey and Gibson (1983) found bluefin tuna blood to be relatively insensitive to open-system temperature change. Interestingly, while some studies have likewise reported the $O_2$ affinity of hemoglobin (Hb) solutions prepared from bluefin tuna blood to be independent of temperature, others have reported Hb $O_2$ affinity to increase with increasing temperature (reverse temperature effect) and to decrease with increasing temperature (normal temperature effect) above $P_{50}$ while simultaneously decreasing below $P_{50}$ (Rossi-Fanelli and Antonini 1960; Carey and Gibson 1977, 1983; Ikeda-Saito et al. 1983). Despite these seemingly contradictory results, it has become generally accepted that bluefin tuna blood has a reverse temperature effect. This unusual characteristic has been assumed to be necessary to prevent premature off-loading of $O_2$ as arterial blood is warmed in the vascular counter-current heat exchangers (Hochachka and Somero 1984; Carey and Gibson 1983; Cech et al. 1984).

It should be pointed out, however, that a reverse temperature response is not necessarily beneficial, as the increase in blood $O_2$ affinity on warming would reduce the $PO_2$ gradient from the blood to the mitochondria of any organs with elevated temperatures, and thus possibly reduce rates of $O_2$ delivery (Bushnell and Jones 1994). A reverse temperature response would also potentially compromise $O_2$ loading at the gills, especially in hypoxic conditions. The latter occurs because the $PO_2$ of venous blood would increase as the blood is cooled back towards ambient temperature in the vascular counter-current heat exchangers. This response would, in turn, reduce the $O_2$-driving gradient across the gills from the water to the blood.

While studies of isolated tuna Hb have provided important insights into its functional characteristics, no previous studies have investigated the effects of closed-system temperature changes on bluefin tuna blood $O_2$ affinity. We feel this to be an important omission, because the influence of closed-system temperature change on the $O_2$ affinity Hb solutions is generally not reflective of the responses of whole tuna blood treated similarly (Lowe et al. 2000). Moreover, in the *Scombridae*, the closed-system temperature response of whole blood is species-specific and independent of the presence of vascular heat exchangers. Blood $O_2$ affinity (expressed as $P_{50}$) is independent of

closed-system temperature increases in skipjack tuna (*Katsuwonus pelamis*), normal (i.e., reduced affinity) in yellowfin tuna (*Thunnus albacares*) and kawakawa (*Euthynnus affinis*), enhanced normal (i.e., large reduction in affinity) in bigeye tuna (*Thunnus obesus*), and reversed (i.e., increased affinity) in albacore (*Thunnus alalunga*) (Cech et al. 1984; Jones et al. 1986; Brill and Bushnell 1991; Lowe et al. 2000). In contrast, the $O_2$ affinity of Hb solutions prepared from bigeye and yellowfin tuna blood is independent of closed-system temperature changes, whereas the $O_2$ affinity of Hb solutions prepared from skipjack tuna blood show the expected decrease in affinity with an increase in temperature (Lowe et al. 2000).

To continue our survey of the effects of open- and closed-system temperature changes on tuna blood $O_2$ affinity, we investigated the properties of bluefin tuna blood using methods essentially identical to those in previous studies on tropical tuna species (Jones et al. 1986; Brill and Bushnell 1991; Lowe et al. 2000). This allows direct comparison of results with other tuna species as well as inferences to be drawn as to relative hypoxia tolerances (Lykkeboe and Weber 1978; Powers 1980, 1985; Yang et al. 1992; Jensen et al. 1993, 1998; Lowe et al. 2000). This latter aspect is important, as responses of various tuna species to low ambient $O_2$ conditions appears to be highly correlated with their vertical movement patterns, specific fishing gear vulnerability, and ability to exploit organisms of the deep scattering layer as prey (Ingham et al. 1977; Gooding et al. 1981; Hanamoto 1987; Cayré and Marsac 1993; Brill 1994; Brill and Lutcavage 2001; Brill and Bushnell 2001; Bigelow et al. 2002). Finally, since many of the conclusions found in the early seminal papers of Rossi-Fanelli and Antonini (1960), Carey and Gibson (1977) and Ikeda-Saito et al. (1983) were primarily based on the behavior of bluefin tuna Hb solutions, we wanted to explore their validity in whole blood from this species.

## Materials and methods

Juvenile bluefin tuna (60–62 cm fork length) were caught by hook and line in the western Atlantic (off the eastern shore of Virginia, USA), and maintained in a 40,000 l outdoor tank (≈ 26–28°C) at the Eastern Shore Laboratory of the Virginia Institute of Marine Science (Wachaprague, Virginia, USA). Fish were held in captivity for only a few days. Fish handling and catheterization procedures were as described by Brill and Bushnell (1991) and Brill et al. (1992), with the exception that fish were anesthetized with an intramuscular injection of either the steroid anesthetic Saffan (Glaxo Vet; ≈0.1–0.2 ml $kg^{-1}$) or sodium phenobarbital (≈35 ml $kg^{-1}$) (Oswald 1978) rather than initial anesthesia with benzocaine followed by spinal block with xylocaine. Blood samples were withdrawn from the anesthetized fish via a ventral aorta catheter after the fish had been given approximately 1 h to 3 h to recover from handling and catheterization procedures.

As in previous studies (Brill and Bushnell 1991; Lowe et al. 2000), we constructed $O_2$ dissociation curves under conditions mimicking those experienced by blood in vivo. Equilibration temperatures (15°C and 25°C) approximated the minimum and maximum water temperatures occupied by juvenile bluefin tuna in the western Atlantic during the summer months (Brill et al. 2002). Likewise, the two $CO_2$ levels employed cover the range of arterial and venous blood $PCO_2$ (≈ 0.4–1.3 kPa, 3–10 mmHg) measured in tuna (Jones et al. 1986; Bushnell and Brill 1992; Korsmeyer et al. 1997). We also took steps to minimize the effects of catecholamines on blood $O_2$ affinity that were most likely released during anesthesia, surgery, and blood withdrawal. Catecholamines were degraded by exposing blood samples to bright light for one hour at room temperature (≈ 22°–25°C) while being gently swirled (Gilmour et al. 1994). To ensure red blood cells were in steady state with respect to ion levels, water content, and intracellular pH prior to use in an experiment, we stored the samples overnight at 4°C in a sealed glass 50 ml Erlenmeyer flask with an air space but without agitation (Bourne and Cossins 1982; Gallardo Romero et al. 1996; Kaloyianni and Rasidaki 1996; Roig et al. 1997). In some cases plasma was harvested by centrifugation and used to reduce the hematocrit to ≈40%.

Samples of approximately 1.5 ml were placed in each of six 50 ml pear-shaped glass tononmeters

that had been flushed with humidified mixed gases for at least 1 h before the addition of blood. Samples remained in the tonometers for an additional 1 h with constant agitation before being removed for measurement.

The effects of open-system temperature change, closed-system temperature change, and $PCO_2$ on blood $O_2$ affinity were quantified by construction of a total of eight blood $O_2$ dissociation curves using blood taken from any one fish, as has been described previously (Brill and Bushnell 1991; Lowe et al. 2000). In brief, we measured both the $O_2$ content ($[O_2]$) and $PO_2$ in blood samples; the former as described by Tucker (1967) and the latter with Radiometer $PO_2$ electrodes installed in two separate MKS Mark 2 blood gas analyzers (Radiometer America, Westlake, Ohio, USA). The water jacket surrounding the $PO_2$ and capillary pH electrodes of one instrument was maintained at 15°C and the other at 25°C. The pH of all blood samples ($pH_e$), was assessed with the capillary pH electrodes of the Radiometer blood gas analyzers.

To measure the effects of open-system temperature change, we constructed two blood $O_2$ dissociation curves using samples taken from tonometers immersed in a water bath maintained at 15°C or 25°C. In these instance, $PO_2$ and $pH_e$ were measured at the same temperature to which the blood was equilibrated. To measure the effects of closed-system temperature change, we constructed two additional blood $O_2$ dissociation curves by injecting blood samples that had been equilibrated at 25°C into the $PO_2$ and pH electrodes maintained at 15°C, and blood samples that had been equilibrated at 15°C into the $PO_2$ and pH electrodes maintained at 25°C. Once injected, samples are essentially sealed, thus rapidly effecting closed-system temperature changes closely mimicking those occurring in vivo (Jones et al. 1986).

In addition, we measured whole-blood Hb concentrations ([Hb]) using the cyanmethemoglobin method (Dacie and Lewis 1984). Plasma bicarbonate concentrations ($[HCO_3–]$, in millimoles per liter) were quantified in samples taken from the blood equilibrated to a $PO_2$ of 20 kPa (150 mmHg) and 0.5% or 1.5% $CO_2$, following the procedures described in Cameron (1971). Measurements were corrected for $CO_2$ in solution using $CO_2$ solubility values for yellowfin tuna plasma from Brill et al. (1992). Plasma lactate levels were measured photometrically using an assay kit (Trinity Biotech, St. Louis, Mo., USA).

We constructed blood $O_2$ dissociation curves by fitting the $PO_2$ and $[O_2]$ data to a logistics function using a least squares regression (Sigmaplot, SPSS, Chicago, Ill., USA). We calculated maximum $[O_2]$ (i.e., 100% saturation) based on measured [Hb] and a maximum Hb $O_2$ carrying capacity corrected for temperature (1.19 ml $O_2$ $g^{-1}$ Hb at 15°C and 1.25 ml $O_2$ $g^{-1}$ Hb at 25°C; Ganong 1973). We then found the $PO_2$ at which the blood would be 50% saturated ($P_{50}$), using the regression parameters. To quantify the temperature sensitivity, we calculated the apparent heat of oxygenation (in kilocalories per mole) = $2.303 \cdot R \cdot [\Delta\log P_{50} \cdot (\Delta\ 1/T^{-1})]$, where $R$ is the universal gas constant (1.987 cal $K^{-1}$ $mol^{-1}$) and $T$ is the equilibration or measurement temperature in degrees Kelvin. We estimated $pH_e$ at $P_{50}$ ($pH_{50}$) by fitting a three-parameter logistics equation to measured blood $pO_2$ and $pH_e$ data, and then interpolating, and the Bohr effect as $\Delta\log P_{50} \cdot \Delta pH_e^{-1}$. Hill numbers were calculated by linear regression of log $PO_2$ vs log [% saturation$\cdot$ (100–% saturation)$^{-1}$], where % saturation = [(measured blood $[O_2]\cdot$maximum $[O_2]^{-1}$) $\cdot$ 100]). When data from 0% to 100% saturation are included, Hill plots are non-linear (Ikeda-Saito et al. 1983). We therefore included data only from the midrange ($\approx$ 20%–80% saturation). To calculate the Root effect, we expressed the $[O_2]$ of blood equilibrated to a $PO_2$ of 20 kPa (150 mmHg) as a percent of maximum possible $[O_2]$ based on [Hb]. (In the apparent heat of oxygenation, Bohr effect, and Hill number calculations, $P_{50}$ was expressed in millimeters of mercury to make the data comparable to previously published results.)

All results in the text, tables, and graphs are presented as means ± standard errors of the means (SEMs). Significant differences were determined from *t*-tests or the 95% confidence intervals (i.e., two times the SEM).

## Results

Blood samples were successfully obtained from five animals. Baseline parameters and responses

to changes in temperature and $PCO_2$ levels are summarized in Tables 1 and 2, and representative blood $O_2$ dissociation curves shown in Fig. 1. Blood $O_2$ binding curves were clearly sigmoid (Hill number >1.6) in all cases. Bluefin tuna blood demonstrated a Root effect only at 15°C ($P > 0.05$), in that the mean maximum $O_2$ saturation fell by approximately 10% when $PCO_2$ increased (from 0.5% to 1.5%). There was no Root effect at 25°C.

Alterations in blood $O_2$ affinity (at $P_{50}$) during open- and closed-system temperature changes (expressed as the apparent heat of oxygenation) are summarized in Fig. 2, along with data from previous studies on bigeye, yellowfin and skipjack tunas. In bluefin tuna blood there was a relatively small but statistically significant decrease in $O_2$ affinity with an increase in temperature at both 0.5% and 1.5% $CO_2$ (i.e., an apparent heat of oxygenation less than zero). In contrast, the $P_{50}$ of bluefin tuna blood showed no response to closed-system temperature changes at both levels of $CO_2$ (i.e., an apparent heat of oxygenation not different from zero) in spite of significant changes in $pH_{50}$ (Table 2); a result that is clearly dissimilar to the responses of yellowfin and bigeye tuna blood to closed-system temperature changes (Fig. 2).

The Bohr effect caused by changing $PCO_2$ alone (labeled "OPEN" in Fig. 3) was relatively large but not different from that previously recorded in bigeye, skipjack, and yellowfin tuna blood. When changes in $pH_e$ and $PCO_2$ were brought about by a 10°C closed-system temperature change, however, bluefin tuna blood showed no Bohr effect. In other words, during closed-system temperature shifts the simultaneous changes in $pH_e$ and temperature become interactive. The net result is that, during closed-system temperature changes at both levels of $CO_2$, bluefin tuna blood demonstrated no influence of temperature on $P_{50}$ (apparent heat of oxygenation not different from zero, Fig. 2). Bluefin tuna blood did, however, demonstrate a reverse temperature effect (i.e., a change in $PO_2$ directly proportional to temperature) at $O_2$ levels above $P_{50}$ (Fig. 1). These different responses to closed-system temperature changes above and below $P_{50}$ are also illustrated in Fig. 4, which plots the changes in $PO_2$ resulting from closed-system temperature changes. Note that there was no change in blood $PO_2$ (ordinate) at any equilibration $PO_2$ (abscissa) below $P_{50}$, whereas there was a reverse temperature effect at equilibration $PO_2$ above $P_{50}$ (i.e., changes opposite to those seen in yellowfin and bigeye tuna blood).

**Table 1** Blood parameters, $O_2$ affinity (expressed as $P_{50}$), $pH_{50}$($pH_e$ at $P_{50}$), bicarbonate concentration ($[HCO_3^-]$), and plasma lactate levels of bluefin tuna blood equilibrated to 15°C and 25°C and 0.5% or 1.5% $CO_2$

| Parameter | | 15°C | | 25°C | |
|---|---|---|---|---|---|
| | | 0.5% $CO_2$ | 1.5% $CO_2$ | 0.5% $CO_2$ | 1.5% $CO_2$ |
| Hematocrit (%) | 44.0 ± 1.4 | | | | |
| [Hb] (g $dl^{-1}$) | 14.5 ± 0.4 | | | | |
| Plasma lactate (mg $dl^{-1}$) (mmol $l^{-1}$) | 129 ± 35 (116 ± 31) | | | | |
| $[HCO_3^-]$ (mmol $l^{-1}$) | | 11.4 ± 1.6 | 12.7 ± 1.8 | 8.4 ± 0.6 | 11.4 ± 1.1 |
| $pH_{50}$ | | 7.93 ± 0.03 | 7.68 ± 0.02 | 7.84 ± 0.03 | 7.64 ± 0.02 |
| Maximum [$O_2$] saturation (%) | | 101 ± 3 | 90 ± 3 | 99 ± 3 | 95 ± 3 |
| $P_{50}$ (kPa) | | 3.10 ± 0.08 | 4.83 ± 0.25 | 2.57 ± 0.19 | 3.95 ± 0.28 |
| $P_{50}$ (mm Hg) | | 22.6 ± 0.6 | 36.2 ± 1.9 | 19.3 ± 1.4 | 29.6 ± 2.1 |
| Hill number | | 2.17 ± 0.06 | 1.59 ± 0.07 | 2.70 ± 0.18 | 2.21 ± 0.28 |
| | | 15°C → 25°C | | 25°C → 15°C | |
| $pH_{50}$ | | 7.72 ± 0.02 | 7.48 ± 0.01 | 8.05 ± 0.03 | 7.82 ± 0.02 |
| $P_{50}$ (kPa) | | 3.10 ± 0.11 | 4.99 ± 0.28 | 2.47 ± 0.20 | 3.73 ± 0.28 |
| $P_{50}$ (mm Hg) | | 23.3 ± 0.8 | 37.4 ± 2.1 | 18.5 ± 1.5 | 28.0 ± 2.1 |
| Hill number | | 2.35 ± 0.08 | 1.83 ± 0.12 | 2.48 ± 0.20 | 2.03 ± 0.19 |

The $P_{50}$ and $pH_{50}$ of bluefin tuna blood following closed-system temperature changes (15°C → 25°C and 25° C → 15°C) are also listed. All data are means ± SEMs

**Table 2** Mean (± SEM) change in $pH_{50}$ (i.e., the pH at $P_{50}$) of bluefin tuna blood subjected to a 10°C open- and closed-system temperature changes

| Parameter | $\Delta pH_{50}°C^{-1}$ | |
|---|---|---|
| | 0.5% $CO_2$ | 1.5% $CO_2$ |
| Open-system | – 0.0096 ± 0.0017 | – 0.0044 ± 0.0028 |
| Closed-system | – 0.0212 ± 0.0018 | – 0.0184 ± 0.0019 |

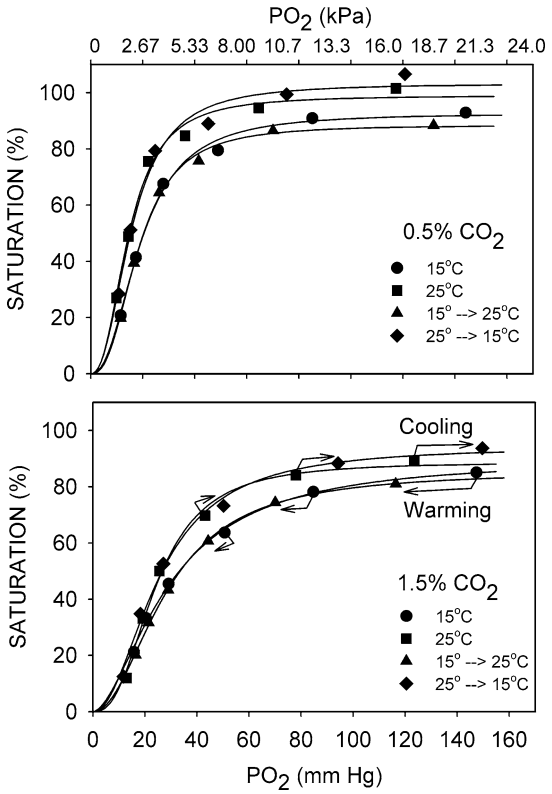


**Fig. 1** Representative $O_2$ dissociation curves from bluefin tuna blood equilibrated with $CO_2$ levels mimicking those of arterial and venous blood (0.5% and 1.5% $CO_2$, respectively) at 15°C and 25°C and subjected to closed-system (15°C → 25°C and 25°C → 15°C) temperature changes. The arrows demonstrate the reverse temperature effect (i.e., a decrease in $PO_2$ during closed-system temperature changes from 15°C → 25°C, and an increase in $PO_2$ during closed-system temperature changes from 25°C → 15°C) occurring at $PO_2$ above $P_{50}$

## Discussion

As explained in previous studies (Brill and Bushnell 1991, Lowe et al. 2000), we consider our techniques to be the best currently available for obtaining tuna red blood cells not stimulated

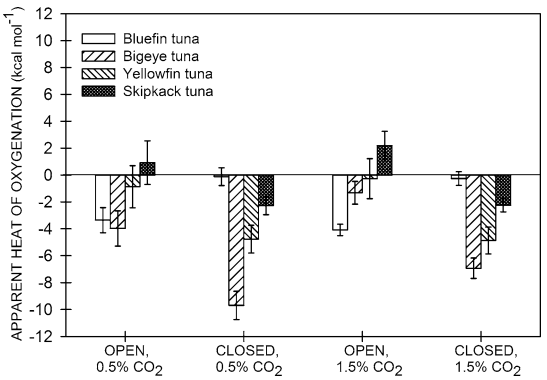


**Fig. 2** The temperature sensitivity (at $P_{50}$) of bluefin, bigeye, yellowfin, and skipjack tuna blood expressed as apparent heat of oxygenation. Data for yellowfin and skipjack tuna are from Brill and Bushnell (1991), and data for bigeye tuna are from Lowe et al. 2000). Experimental temperatures for bluefin and bigeye tuna were 15°C and 25°C, and those for yellowfin and skipjack tuna were 20°C and 30°C

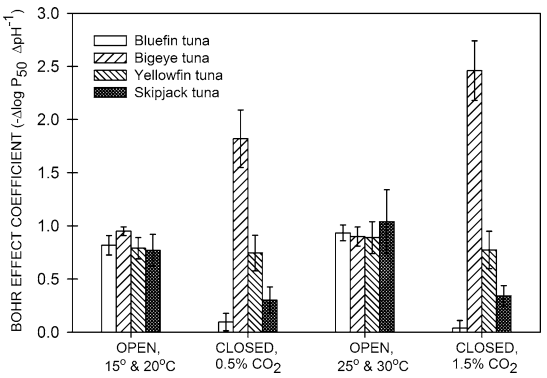


**Fig. 3** Bohr coefficients calculated for bluefin, bigeye, yellowfin, and skipjack tuna blood. Extracellular pH ($pH_e$) changes were created either by equilibration of blood to gas mixtures containing 0.5% and 1.5% $CO_2$ or by subjecting the blood to closed-system temperature changes (indicated by the terms OPEN and CLOSED, respectively). Data for yellowfin and skipjack tuna are from Brill and Bushnell (1991), and data for bigeye tuna from Lowe et al. (2000). Experimental temperatures for bigeye tuna were 15°C and 25°C, and those for yellowfin and skipjack tuna were 20°C and 30°C

by catecholamines and that respond normally in vitro. We chose to use whole blood, rather than use the alternative approach of washing and suspending the red blood cells in Ringer's solution or buffered saline solution, because the composition of these media has been shown to significantly influence fish blood $O_2$ affinity (Dalessio et al.

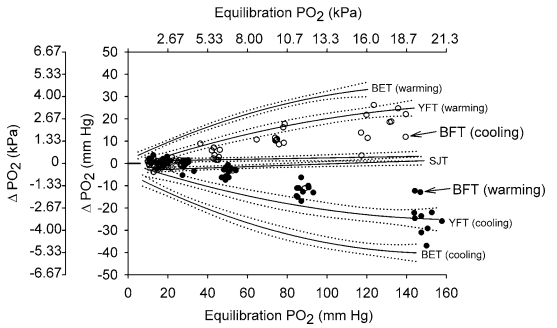


**Fig. 4** Effect of 10°C closed-system temperature change on $PO_2$ of blood from bluefin (*open and closed circles*), bigeye, yellowfin, and skipjack tuna. Data for the latter two species are from Brill and Bushnell (1991), and data for bigeye tuna from Lowe et al. (2000). Only regression lines (continuous lines) and 95% confidence intervals (dotted lines) are shown for data from bigeye tuna (*BET*), yellowfin tuna (*YFT*) and skipjack tuna (*SJT*) blood. Experimental temperatures for bluefin tuna (*BFT*) and bigeye tuna were 15°C and 25°C, and those for yellowfin and skipjack tuna were 20°C and 30°C. (Abscissa: $PO_2$ to which blood was equilibrated prior to closed-system temperature change)

1991). Blood lactate levels were elevated in comparison with those in the literature for other tuna species (<20 $mM^{-1}$; Wells et al. 1986), indicating that the fish had not recovered completely prior to blood withdrawal. As shown in Table 1, however, plasma [$HCO_3$–] was only slightly below normal values for tuna (≈13–18 $mmol^{-1}$), and $pH_{50}$ values were an average of only approximately 0.15 pH units below those observed previously in blood from skipjack, yellowfin and bigeye tuna treated similarly (Brill and Bushnell 1991, Lowe et al. 2000).

**Table 3** Summary of the presence or absence of swim bladders and blood showing a Root effect in tuna species studied to date

| Species | Swim bladder[a] | Root effect |
|---|---|---|
| Bluefin tuna | Present | Present at 15°C; absent at 25°C[b] |
| Yellowfin tuna | Present | Present[c] |
| Skipjack tuna | Absent | Present[c] |
| Bigeye tuna | Present | Present[d] |
| Albacore | Present | Negligible (<5%)[e] |

[a] Data on presence/absence of swim bladders are from Godsil and Byers (1944)

[b] Present study

[c] Brill and Bushnell (1991)

[d] Lowe et al. 2000

[e] Cech et al. (1984)

Bluefin tuna blood had an $O_2$ affinity ($P_{50}$ = 2.6–3.1 kPa or 19–23 mmHg at 0.5% $CO_2$), similar to those of skipjack tuna, yellowfin tuna, and kawakawa blood ($P_{50}$ = 2.8–3.1 kPa or 21–23 mmHg at 0.5% $CO_2$) (Jones et al. 1986; Brill and Bushnell 1991) but significantly above that of bigeye tuna blood ($P_{50}$ = 1.6–2.0 kPa or 12–16 mmHg at 0.5% $CO_2$) (Lowe et al. 2000). We therefore hypothesize that bluefin tuna are less tolerant of hypoxia than bigeye tuna are (Bushnell et al. 1990; Lowe et al 2000), and, likewise, that reductions in ambient $O_2$ can limit the vertical movements of bluefin tuna, as they do in yellowfin and skipjack tunas in the Pacific (Barkley et al. 1978; Sharp 1978; Sund et al. 1981; Hanamoto 1987; Holland et al. 1992; Cayré and Marsac 1993; Brill 1994; Mohri et al. 1996; Block et al. 1997, Graham and Dickson 2004).

Bluefin tuna blood's relatively small Root effect at 15°C (≈10% decrease in maximum blood $O_2$ content with an increase in $CO_2$ from 0.5% to 1.5%) was within the range of values reported for bigeye, yellowfin, and skipjack tuna blood (≈5% to 17% decrease under similar conditions) (Brill and Bushnell 1991; Lowe et al. 2000). Like albacore blood (Cech et al. 1984), bluefin tuna blood showed no Root effect at 25°C. As summarized in Table 3, blood with a significant Root effect is not correlated with presence of a swim bladder in tuna. Therefore, as in other teleosts (Brittain 1987), a significant Root effect does not appear necessary for excreting $O_2$ into the swim bladders of the *Scombridae*. The reasons for the differences in Root effect among tuna species remain unexplained. A significant Root effect may, however, serve to enhance $O_2$ delivery rates from the blood to the mitochondria and thus ensure adequate rates of $O_2$ delivery in muscle with relatively low capillary density (Mathieu-Costello et al. 1992; Brauner and Randall 1996).

## Temperature sensitivity of blood $O_2$ affinity and regional endothermy

The apparent heat of oxygenation does not have exactly the same meaning when calculated from whole blood as it does when based on results from

Hb solutions. As explained in Powers et al. (1979), Wood (1980), and Truchot (1987), when whole blood is subjected to temperature changes there are several additional effects (e.g., the exchange of proton equivalents between the red cells and plasma) that change the intracellular environment and influence Hb conformation and $O_2$ affinity, which do not occur when Hb solutions are subjected to temperature shifts. Nonetheless, the apparent heat of oxygenation is still a convenient measure for comparing the temperature sensitivity of the blood from various species.

During open-system temperature change at both $CO_2$ levels, the $P_{50}$ of bluefin tuna blood declined slightly with an increase in temperature (i.e., apparent heat of oxygenation $\approx$ –3 to –4 kcal $l^{-1}$; Fig. 2), although the effect of temperature was less than that seen in the blood of other teleosts (generally > –3 kcal $mol^{-1}$) (Grigg 1969; Powers et al. 1979). Similar relative insensitivity to open-system temperature has been reported for the blood from albacore, bigeye, skipjack and yellowfin tunas (Cech et al. 1984; Brill and Bushnell 1991, Lowe et al. 2000). Because of the bluefin tuna's extensive vertical and horizontal movements and concomitant changes in ambient temperature, Rossi-Fanelli and Antonini (1960) originally proposed that this reduced temperature effect "... would enable the animal to live in waters of very different temperatures without modification of the functional properties of its respiratory pigment". We think that this argument explains the commonality of near temperature independent blood $O_2$ binding within the *Scombridae*, as the functional properties of blood passing through the gills would remain unaltered by changes in water temperature.

During closed-system temperature change, however, bluefin tuna blood (similar to albacore blood, Cech et al. 1984) showed a strong reverse temperature effect at $PO_2$ above $P_{50}$ but generally insignificant changes at lower $O_2$ levels (Figs. 1 and 4). Although we have no direct evidence, we agree with previous authors (Carey and Gibson 1983; Cech et al. 1984, Hochachka and Somero 1984) that this response may be necessary to prevent massive off-loading of arterial $O_2$ stores before the blood arrives at the swimming muscle capillaries. This is particularly important, because bluefin tuna reach the largest size and are the most thermoconserving (sustained muscle temperatures may be >20°C above ambient temperature in adults) of all tuna species (Block et al. 2001a; Gunn and Block 2001). A similar reverse temperature effect has been reported in the isohemoglobins from the porbeagle shark (*Lamna nasus*), at least suggesting the convergent evolution of this necessary trait in thermoconserving fish that have steep temperature gradients between the gills and swimming muscles (Carey et al. 1985; Larsen et al. 2003). This reversed closed-system temperature effect at high levels of blood $O_2$ saturation could, however, potentially compromise rates of $O_2$ off-loading and delivery to the mitochondria. Because the reverse closed-system temperature effect does not occur at $PO_2$ below $P_{50}$ in bluefin tuna blood (Figs. 1 and 4), $O_2$, loading at the gills should not be adversely effected.

## Interactive effects of $pH_e$ and $PCO_2$ during closed-system temperature changes

It is now apparent that in at least three tuna species (bluefin, skipjack, and bigeye) there are clear, although widely divergent, interactive effects of simultaneous changes in $pH_e$ and $PCO_2$ occurring during closed-system temperature changes. The net result is that these interactions minimize the alterations in $P_{50}$ during closed-system temperature changes in bluefin and skipjack tuna blood, but exacerbate the changes in $P_{50}$ of bigeye tuna blood (Fig. 2) (Brill and Bushnell 1991; Lowe et al. 2000). This latter effect gives bigeye tuna the unique advantage of having a high $O_2$ affinity blood at the gills (generally characteristic of species tolerant of low ambient $O_2$) but normal (lower) $O_2$ affinity blood characteristic of the higher energy demand fishes (Wood 1980; Yang et al. 1992; Jensen et al. 1993) when it is warmed during passage through vascular counter-current heat exchangers (Lowe et al. 2000).

In yellowfin tuna blood there are no interactive effects of simultaneous changes in temperature, $pH_e$, and $PCO_2$ accompanying closed-system temperature changes (Fig. 2). The consequences on $P_{50}$ of changing $pH_e$ are the same, whether the change is brought about by changing the $PCO_2$

alone, or by the inevitable changes of $PCO_2$ and $pH_e$ that accompany closed-system temperature shifts. Yellowfin tuna blood, therefore, shows an equal Bohr effect under these two circumstances (Fig. 3). In contrast, bluefin and skipjack tuna blood show interactive effects and, therefore, similarly reduced closed-system Bohr effects during closed system temperature changes, whereas bigeye tuna blood shows the opposite response—a much enhanced Bohr effect (Fig. 3). The situation is further complicated in bluefin tuna blood in that the effects of closed-system temperature changes are different at equilibration $PO_2$s above and below $P_{50}$ (Fig. 4).

## Effects of temperature on $O_2$ affinity of isolated Hb solutions and whole blood

In whole blood, intact membrane exchange processes can modify one or more ligands (e.g., ATP, GTP, intracellular pH) influencing Hb $O_2$ affinity (Lykkeboe and Weber1978; Powers 1980; Nikinmaa 1990). The effect of temperature on the $O_2$ affinity of Hb solutions does not, therefore, necessarily reflect the influence of temperature on the $O_2$ affinity of whole blood (Powers et al. 1979). Indeed, the effects of closed-system temperature change on the $O_2$ affinity of bigeye and skipjack tuna Hb solutions do not match those seen in whole blood (Lowe et al. 2000). In bluefin tuna, however, our results with whole blood subjected to closed-system temperature changes generally agree with the studies by Carey and Gibson (1983) on bluefin tuna Hb solutions. Specifically, in both cases, there were reverse temperature effects (i.e., an increase in $O_2$ affinity with an increase in temperature) at high $pO_2$. In bluefin tuna, therefore, there does not appear to be a need to postulate the existence of mechanisms for changing concentrations of intracellular ligands, because, in this case, the functional properties of Hb itself appear sufficient to explain the response of whole-blood to closed-system temperature changes.

In skipjack and bigeye tuna, however, some alteration in intracellular ligands appear to underlie the shifts in whole-blood $O_2$ affinity accompanying closed-system temperature changes, as whole blood and isolated Hb solutions behave differently. It is generally reported that temperature effects on intracellular ligands usually involve time scales at least tens of hours and up to several months (Lykkeboe and Weber 1978; Jensen et al. 1998). Based on tuna blood volume, cardiac output data, and circulation time (Brill et al. 1998; Brill and Bushnell 2001), the temperature changes tuna blood experiences during its passage through vascular counter-current heat exchangers most likely occur over a few to tens of seconds. It is unknown if these time periods are too short for any active adjustments in red blood cell intracellular ligands to occur in vivo.

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