Cardiorespiratory responses of skipjack tuna (*Katsuwonus pelamis*), yellowfin tuna (*Thunnus albacares*), and bigeye tuna (*Thunnus obesus*) to acute reductions of ambient oxygen

PETER. G. BUSHNELL[1]

John A. Burns School of Medicine, Department of Physiology, University of Hawaii, 1976 East–West Road, Honolulu, HI 96822, U.S.A.

AND

RICHARD W. BRILL AND ROBERT E. BOURKE[2]

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

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Cardiorespiratory responses to acute reductions of ambient oxygen were measured in skipjack tuna (*Katsuwonus pelamis*), yellowfin tuna (*Thunnus albacares*), and bigeye tuna (*Thunnus obesus*). Prevented from swimming by a spinal injection of lidocaine, fish were placed in seawater flowing at a velocity equivalent to their normal swimming speed. Ventilation volume ($\dot{V}_g$), heart rate, cardiac output, mouth gape, and inspired water and exhalant water oxygen partial pressures (Pi_{O_2} and Pe_{O_2}, respectively) were simultaneously measured during periods of full oxygen saturation (normoxia) and brief (ca. 3–4 min) periods of reduced oxygen (hypoxia). During hypoxia, Pi_{O_2} ranged from 140 to 50 mmHg. $\dot{V}_g$ during normoxia was significantly different in skipjack, yellowfin, and bigeye tunas (6.7, 3.9, and 1.5 $L \cdot min^{-1} \cdot kg^{-1}$, respectively) and paralleled differences in oxygen consumption (740, 455, and 322 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$). All three species were sensitive to Pi_{O_2}, and mild hypoxia ($Pi_{O_2} \approx$ 115 mmHg) elicited significant cardiorespiratory adjustments, including increased mouth gape and $\dot{V}_g$ and reduced heart rate. Cardiac output was maintained until Pi_{O_2} reached 95 mmHg, but at lower oxygen levels it too began to decrease. Therefore, the three tuna species studied appear as sensitive to hypoxia as other marine teleosts and show cardiorespiratory adjustments at Pi_{O_2} values well above those eliciting swimming speed changes.

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Les réactions cardiorespiratoires à des réductions importantes de la concentration d'oxygène ont été mesurées chez la Thonine à ventre rayé, *Katsuwonus pelamis*, l'Albacore à nageoires jaunes, *Thunnus albacares*, et le Thon ventru, *Thunnus obesus*. Immobilisés par une injection de lidocaïne dans la colonne, les poissons ont été placés dans de l'eau de mer circulant à une vitesse équivalente à leur vitesse normale de nage. Le volume respiratoire ($\dot{V}_g$), le rythme cardiaque, le débit cardiaque, l'écartement des mâchoires et les pressions partielles de l'oxygène dans l'eau aspirée et l'eau exhalée (respectivement Pi_{O_2} et Pe_{O_2}) ont été mesurés simultanément durant des périodes de saturation totale en oxygène (normoxie) et durant de brèves périodes (ca. 3–4 min) de réduction de l'oxygène (hypoxie). Durant l'hypoxie, Pi_{O_2} se situait entre 140 et 50 mmHg. Le volume $\dot{V}_g$ dans des conditions normoxiques était significativement différent chez les trois poissons, (6, 7, 3,9 et 1,5 $L \cdot min^{-1} \cdot kg^{-1}$ chez la Thonine, l'Albacore et le Thon, respectivement) et correspondait à des différences dans la consommation d'oxygène (valeurs respectives de 740, 455 et 322 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$). Les trois espèces se sont avérées sensibles à la pression d'oxygène dans l'eau aspirée et une hypoxie légère ($Pi_{O_2} \approx$ 115 mmHg) déclenchait des ajustements cardiorespiratoires importants, notamment une augmentation de l'ouverture de la bouche et du $\dot{V}_g$ et une réduction du rythme cardiaque. Le débit cardiaque restait le même jusqu'à ce que la pression Pi_{O_2} atteigne 95 mmHg, mais, à des concentrations plus faibles d'oxygène, le débit aussi se mettait à diminuer. Les trois espèces étudiées semblent donc toutes trois sensibles à l'hypoxie, comme d'autres téléostéens marins, et subissent des ajustements cardiorespiratoires à des pressions Pi_{O_2} supérieures à celles qui provoquent des changements dans la vitesse de la nage.

[Traduit par la revue]

Introduction

Although some authors believe that pelagic marine fishes do not encounter low ambient oxygen (hypoxia) (Butler and Metcalfe 1983; Shelton et al. 1986), this is simply not true. Oxygen levels in the oceans are not homogeneous but are dependent upon depth, temperature, productivity, and salinity (Riley and Chester 1971), and significant reductions of ambient oxygen do occur at depths normally occupied by tunas (Barkley 1968; Sund et al. 1981). Although ambient oxygen conditions have been used to explain and predict the apparent movements, abundances, and vulnerability to specific types of fishing gear of various tuna species (Green 1967; Ingham et al. 1977; Barkley et al. 1978; Sharp 1978; Hanamoto 1987), there are few direct experimental observations of the responses of tunas to hypoxia. Tolerances of various tunas species are mostly inferred from correlations of fishery statistics and oceanographic data.

Tunas have evolved the anatomical, physiological, and biochemical adaptations necessary to reach exceptionally high maximum aerobic metabolic rates (Brill 1987). Skipjack tuna can achieve an oxygen consumption rate ($\dot{V}O_2$) of approximately 2500 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ (Gooding et al. 1981). By comparison, the maximum $\dot{V}O_2$ of other teleosts is about 1000 ± 200 mg

[1]Present address: Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, B.C., Canada V6T 2A9.

[2]Present address: Hawaii Institute of Marine Biology, University of Hawaii, P.O. Box 1346, Kaneohe, HI 96744, U.S.A.

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$O_2 \cdot kg^{-1} \cdot h^{-1}$ (Brett 1972). Because tunas have exceptionally high oxygen demands, it is reasonable to assume they would be highly sensitive to hypoxia.

Tunas are also obligate ram ventilators and rely on forward movement to develop the pressure head necessary to move water past the gills (Brown and Muir 1970; Roberts 1978). To increase ventilation volume ($\dot{V}_g$), tunas must increase one or more of the following: mouth gape, opercular openings, and swimming speed. Dizon (1977) and Gooding et al. (1981) were apparently the first to directly measure the responses of skipjack tuna (*Katsuwonus pelamis*) and yellowfin tuna (*Thunnus albacares*) to hypoxia. Skipjack tuna showed an increase in swimming speed when ambient oxygen fell to 4.0 mg $O_2 \cdot L^{-1}$ (Po_2 = 95 mmHg)[3] and definite signs of stress (high swimming speed, large gape, and absence of schooling behavior) below 3.5 mg $O_2 \cdot L^{-1}$ (83 mmHg). Yellowfin tuna subjected to similar conditions failed to show any change in swimming speed at oxygen levels as low as 2.5 mg $O_2 \cdot L^{-1}$ (59 mmHg). These results suggest that yellowfin tuna are either much more tolerant to hypoxia than are skipjack tuna and other teleosts (Davis 1975) or that they respond to hypoxia in a manner that obviates the need to increase swimming speed.

The efficacy of the cardiorespiratory system of tunas is important in determining their responses to hypoxia. Unfortunately, cardiorespiratory data previously available for tunas have come from fish either anesthetized, paralyzed, or stressed from recent handling (Stevens 1972; Graham and Laurs 1982; Brill 1987; Lai et al. 1987). Furthermore, in all these experiments, $\dot{V}_g$ was set by the experimenter rather than by the fish. Because fixing $\dot{V}_g$ can have important effects on cardiovascular and respiratory functions, our objectives were to develop a system that would allow the simultaneous measurement of several cardiorespiratory variables during normoxia and hypoxia in tunas controlling their own $\dot{V}_g$. This would then allow us to quantify the responses of tunas to acute hypoxia and determine whether physiological abilities could account for reported differences in behavioral responses to hypoxia and differences in the oceanographic distributions of bigeye tuna (*Thunnus obesus*), yellowfin tuna, and skipjack tuna (Sharp 1978; Hanamoto 1987).

Materials and methods

Skipjack, yellowfin, and bigeye tunas were purchased from local commercial fishermen and maintained in continuous-flow outdoor seawater tanks (temperature 25 ± 2°C; salinity 33‰) at the Kewalo Research Facility (Southwest Fisheries Center Honolulu Laboratory, National Marine Fisheries Service, National Oceanic and Atmospheric Administration). Animal procurement, handling, and maintenance procedures at this facility are described in Nakamura (1972), Queenth and Brill (1983), and Chang et al. (1983). Food was presented daily, but fish were not fed for approximately 20 h prior to use in an experiment. In 3 years, only three bigeye tuna became available for this study because they are only rarely caught by the boats returning fish to the laboratory. The size ranges of the fish used were 0.665–1.000, 0.579–1.278, and 1.900–2.175 kg for skipjack, yellowfin, and bigeye tunas, respectively.

Anesthesia, surgical, and data collection procedures

Initial anesthesia was induced by guiding fish into a plastic bag containing 1.0 g · L^{-1} of tricaine methanesulfonate (MS 222)[4] buffered with 1.0 g · L^{-1} $NaHCO_3$ (the complete technique is described in Jones et al. 1986)). The animal was then placed on a surgical table and force-ventilated with recirculated oxygenated seawater (cooled to 22°C and containing 0.1 g · L^{-1} MS 222) during the 20–40 min required to mount a Doppler blood-flow probe, dye injection and sampling catheters, and electrocardiogram (ECG) wires.

At the conclusion of surgery, but before the fish recovered from anesthesia, a 6 cm long, 20-gauge hypodermic needle was pushed through the dorsal body surface and down into the spinal column. The initial point of needle insertion was immediately lateral to the second spine of the first dorsal fin. Approximately 0.2 mL of a local anesthetic (2% lidocaine hydrochloride) was then injected directly into the spinal cord. This procedure paralyzed all the spinal motor nerves but left all cranial nerves intact. The syringe needle was left in place for subsequent injections.

The fish was then placed under a concave plastic holding device that fit over its dorsal surface and was held in place by two Velcro straps encircling its body. The holder was secured by a series of stainless steel bars bolted to the surface of the surgical table. The fish was positioned so that its snout was directly in front of the roughly rectangular (ca. 2.3 × 3.3 cm) seawater inflow pipe (Fig. 1) and could therefore set its own $\dot{V}_g$.

Seawater exited the pipe at approximately 77 cm · s^{-1} (35 L · min^{-1}), which is similar to the normal swimming speeds (60–80 cm · s^{-1}) of the tunas used in this study (Magnuson 1978). Fish were therefore provided with ample water flow, at a velocity that permitted a normal $\dot{V}_g$ to be achieved with a mouth gape similar to that observed in swimming fish. Fish were allowed 1–2 h to recover before the first hypoxic trial was begun.

As shown in Fig. 1, air-saturated seawater was mixed with deoxygenated seawater (Po_2 = 20 mmHg) to achieve an initial Po_2 of 50 mmHg (2.1 mg $O_2 \cdot L^{-1}$). Pure oxygen was bubbled through a gas-exchange column to bring the inflow seawater Po_2 (Pi_{O_2}) to full saturation (152–157 mmHg or 6.4–6.6 mg $O_2 \cdot L^{-1}$). A gas flowmeter controlled the amount of oxygen flowing to the column and enabled us to set the Pi_{O_2} anywhere between 140 and 50 mmHg (5.9–2.1 mg $O_2 \cdot L^{-1}$), with no change in water flow rate. Pi_{O_2} was continuously monitored with a Radiometer Po_2 electrode mounted in a temperature-controlled flow-through cuvette and calibrated with standard zero solution and air-saturated seawater. The electrode was connected to a Radiometer PHM 71 blood gas analyzer. Cuvette temperature was maintained the same as that of the inflowing seawater. All experiments were conducted at 25 ± 1°C.

$\dot{V}_g$ was measured with a dye dilution technique developed by Jones et al. (1990). Exhalant water (Pe_{O_2}, i.e., the water stream exiting the opercular openings) was sampled with two PE-50 catheters sutured through the posterior medial edge of each opercular opening about 0.5 cm ventral to the pectoral fin insertion. The ends of the catheters projected approximately 3 mm into the gill cavity and were under the opercular covers. Dye concentrations in exhalant water were determined spectrophotometrically. $\dot{V}_g$ was calculated by averaging the transmittances of the water samples from left- and right-side catheters (see Appendix for explanation).

Cardiac output was measured with a Parks model 806A directional Doppler blood flowmeter. A flat transcutaneous flow probe was fixed with tissue adhesive to the area inside the gill cavity overlying the ventral aorta. The probe was positioned 0.3–0.7 cm anterior to the bulbus arteriosus – ventral aorta junction and did not interfere with gill arch or opercular movements. Mean cardiac output was obtained by passing the pulsatile cardiac output signal through a low-pass filter. Attempts to calibrate the flow probe postmortem by infusing blood past the probe at a known flow rate were generally unsuccessful. Therefore, cardiac output measurements were recorded in arbitrary units on the basis of a known zero flow. (Zero flow was recorded between heartbeats during hypoxic bradycardia.) Heart rate was recorded with a

[3]Oxygen levels in water may be expressed either as partial pressure (mmHg) or as concentration (mg $O_2 \cdot L^{-1}$). Partial pressure and concentration are linearly related through the factor of oxygen solubility. Oxygen solubility is, in turn, affected by temperature and salinity. Whenever possible, oxygen levels will be given in both units.

[4]Use of trade names does not imply endorsement by the National Marine Fisheries Service, National Oceanic and Atmospheric Administration.

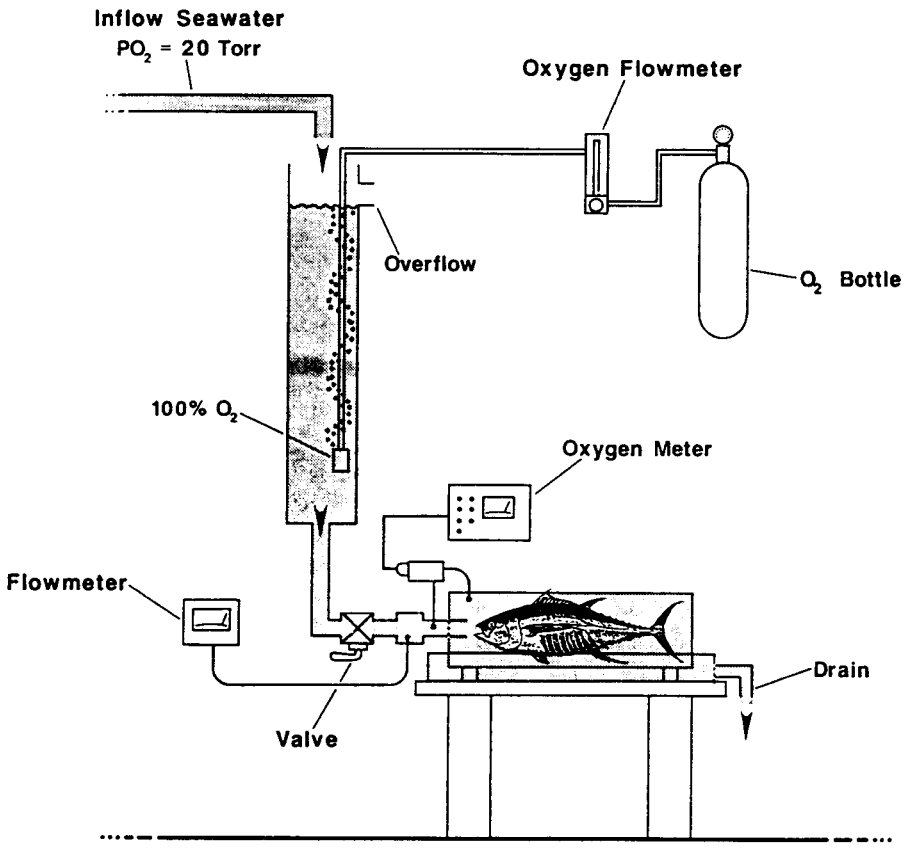

FIG. 1. Experimental apparatus for exposing tunas to hypoxia. The concave plastic holder, fitted to the fish's back, and the series of stainless steel bars holding the plastic holder and fish are not shown (1 Torr = 133.322 Pa).

cardiotachometer triggered by the ECG signal. ECG electrodes were mounted subcutaneously near the heart. Stroke volume was calculated by dividing cardiac output by heart rate.

Mouth gape, taken as the vertical distance from the tip of the snout to the lower jaw, was measured directly with a ruler fixed to the side of the seawater inflow pipe. Dynamic changes in gape were recorded with an impedance converter and electrodes glued to the lateral surfaces of the lower and upper jaws. Outputs from the cardiotachometer, Doppler flowmeter, impedance converter, and ECG amplifier were recorded on a six-channel pen recorded.

Pe_{O_2} was measured with the same Radiometer blood gas analyzer and oxygen electrode used to monitor Pi_{O_2}. Utilization (U) was calculated from Pi_{O_2} and Pe_{O_2} as

$$[1] \quad U = [(Pi_{O_2} - Pe_{O_2})/Pi_{O_2}] \cdot 100$$

Oxygen consumption ($\dot{V}o_2$, in mg $O_2 \cdot h^{-1}$) was calculated with the Fick method:

$$[2] \quad \dot{V}o_2 = \dot{V}_g (Pi_{O_2} - Pe_{O_2}) \cdot \alpha$$

where $\dot{V}_g$ is the ventilation volume ($L \cdot h^{-1}$) and α is the solubility of oxygen (mg $O_2 \cdot L^{-1} \cdot$ mmHg) at the experimental temperature and salinity.

Experimental procedure

Tuna were exposed to brief periods (ca. 3–4 min) of hypoxia (140–50 mmHg or 5.9–2.1 mg $O_2 \cdot L^{-1}$) presented in a random sequence of 10-mmHg steps. Gape, heart rate, cardiac output and Pi_{O_2} were recorded continuously. Dye was infused and samples of exhalant water (control measurements) were taken during the normoxic period immediately preceding onset of hypoxia. Dye infusion was then stopped, and Pi_{O_2} reduced to a preselected level. Two minutes after Pi_{O_2} had stabilized, dye infusion was begun again and samples of exhalant water were taken (hypoxia measurement). Pi_{O_2} was then brought back to full saturation. A 15-min intertrial period was allowed, during which time Pe_{O_2} and dye concentrations were measured.

Statistical methods

Interspecific comparisons of control measurements (excluding cardiac output and stroke volume) were made using analysis of variance (ANOVA) and multiple-range tests. All values are expressed as mean ± standard error.

Because not every fish completed all 10 trials, dividing the data into 10-mmHg ranges resulted in an inadequate number of samples in some ranges. Data were therefore combined into 20-mmHg ranges (144–125, 124–105, 104–85, 84–65, 64–45 mmHg). To determine whether any of the variables (i.e., $\dot{V}_g$, gape, heart rate, or relative cardiac output) measured in hypoxia differed significantly ($P < 0.05$) from their counterparts in normoxia, control measurements in a particular range were compared with their respective hypoxia values with a paired Student's t-test ($P < 0.05$).

To graph the effects of hypoxia, changes in gape, $\dot{V}_g$, heart rate, cardiac output, and stroke volume were calculated as the fractional change from the control measurements that immediately preceded hypoxia. Data were grouped into intervals based on Pi_{O_2}.

TABLE 1. Cardiorespiratory variables in skipjack, yellowfin, and bigeye tunas and rainbow trout

	Skipjack *tuna*	Yellowfin *tuna*	Bigeye *tuna*	Rainbow trout[a]
Mass (kg)	0.841±0.4 (10)	1.07±0.09 (10)	2.03±0.07 (3)	0.9–1.5
Pi_{O_2} (mmHg)	153.0±0.5 (10)	152.5±0.5 (10)	154.5±0.2 (3)	153
Gape (mm)	8.2±0.9[b] (10)	5.9±1.0[c] (9)	9.7±1.7 (3)	—
$\dot{V}_g$ ($L \cdot min^{-1}$)	5.5±0.9 (10)	4.1±0.7 (8)	3.1±0.2 (3)	—
$\dot{V}_g$ ($L \cdot min^{-1} \cdot kg^{-1}$)	6.7±1.0[b] (10)	3.9±0.4[c] (8)	1.5±0.2[d] (3)	0.21±0.06
Pe_{O_2} (mmHg)	101.9±6.6 (9)	93.3±7.9 (7)	74.8±2.1 (3)	101
U (%)	33.5±4.4 (9)	39.0±4.9 (7)	51.6±1.4 (3)	33.0±0.5
$\dot{V}_{O_2}$ (mg $O_2 \cdot kg^{-1} \cdot h^{-1}$)	740±103 (9)	455±66 (7)	323±33[d] (3)	49.9±0.9
Heart rate (beats/min)	117±12 (10)	99±6 (10)	99±18 (3)	70±3

NOTE: Values are given as mean ± SD, with number of fish in parentheses.
[a]Trout in fresh water at 10°C; data are from Kiceniuk and Jones (1977).
[b]Statistically different ($P < 0.05$) from yellowfin tuna.
[c]Statistically different ($P < 0.05$) from bigeye tuna.
[d]Statistically different ($P < 0.05$) from skipjack tuna.

Results

Normoxia

Mean normoxia values for all three tuna species are given in Table 1. Data for rainbow trout, *Salmo gairdneri* (Kiceniuk and Jones 1977), have been added for comparison. Total $\dot{V}_g$ is not significantly different in the three species. However, when standardized by weight, skipjack tuna had a significantly higher $\dot{V}_g$ than yellowfin tuna, which in turn had a significantly higher $\dot{V}_g$ than bigeye tuna. $\dot{V}_{O_2}$ of skipjack tuna was not significantly different from that of yellowfin tuna, but both were higher than that of bigeye tuna. Pe_{O_2}, U, and heart rates were not significantly different in the three species.

Hypoxia

Figure 2 presents a chart record of the dynamic cardiorespiratory responses of a yellowfin tuna when Pi_{O_2} was reduced to 90 mmHg (3.8 mg $O_2 \cdot L^{-1}$). Various physiological adjustments are apparent within seconds after the onset of hypoxia and the increase in Pi_{O_2} at the conclusion of hypoxia. (Note that there was an approximately 10-s lag in the response of the electrode measuring seawater Po_2; the tracings in Fig. 2 have not been corrected for this lag.) The responses to hypoxia included an increase in gape (and presumably $\dot{V}_g$) and a reduction in heart rate with little decrease in cardiac output until Pi_{O_2} fell below 100 mmHg (4.2 mg $O_2 \cdot L^{-1}$).

Yellowfin and skipjack tunas appeared to respond to the rate of change as well as to the absolute change in Pi_{O_2}. When the flow of oxygen to the gas-exchange column was reduced, Pi_{O_2} decreased exponentially. Therefore, when Pi_{O_2} was reduced from 155 to 50 mmHg (6.5–2.1 mg $O_2 \cdot L^{-1}$), for example, the initial rate of change of Pi_{O_2} was more rapid than when the reduction was from 155 to 140 mmHg. As a result, we found that fish would not respond to a change in Pi_{O_2} from 155 to 140 mmHg, but would respond within the first few seconds of the start of a change to 50 mmHg, after Pi_{O_2} had decreased to only 140 mmHg (5.9 mg $O_2 \cdot L^{-1}$). However, data recorded while Pi_{O_2} was changing were not used to determine sensitivity to hypoxia because of the difficulty in establishing the exact response time of the seawater Po_2 electrode.

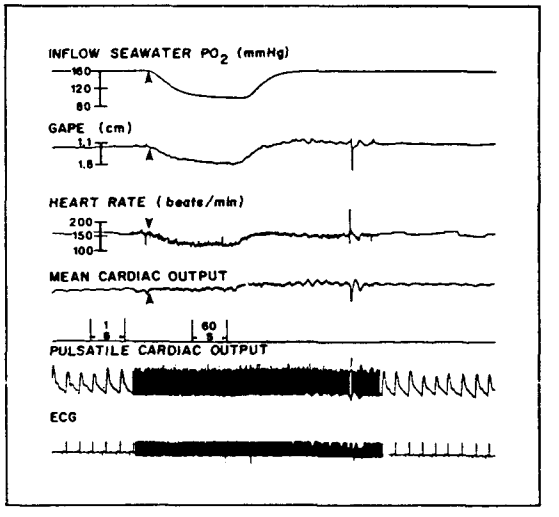

FIG. 2. A chart record of the cardiorespiratory response of a yellowfin tuna to hypoxia. Note that the changes in gape and heart rate parallel those of inflow seawater Po_2 (P_{IO_2}). Cardiac output was maintained by an increase in stroke volume until the Pi_{O_2} fell below approximately 100 mmHg (4.2 mg $O_2 \cdot L^{-1}$).

Figure 3 shows that, in skipjack tuna, no changes in the measured variables occurred until Pi_{O_2} fell to 124–105 mmHg (5.2–4.4 mg $O_2 \cdot L^{-1}$), at which point gape and $\dot{V}_g$ had

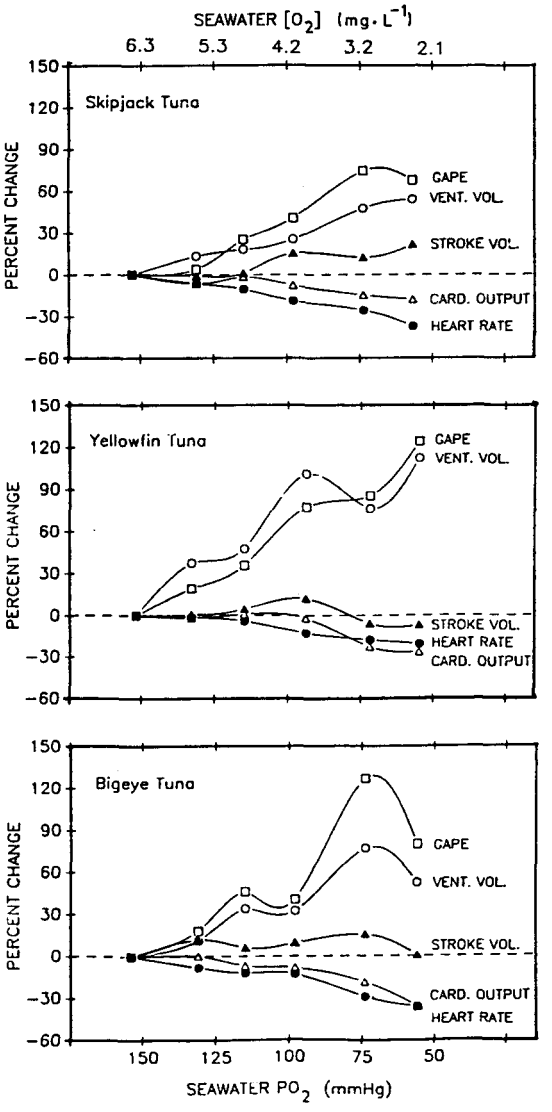

FIG. 3. Cardiorespiratory response to acute hypoxia in skipjack, yellowfin, and bigeye tunas. Data are mean percent change from the values measured during the control periods immediately preceding the hypoxic episodes. The inflow seawater P_{O_2} values are the averages of each range. Standard error bars have been omitted for clarity.

increased significantly (Table 2, Fig. 3). Heart rate and cardiac output decreased significantly while stroke volume increased significantly at the next level of hypoxia (104–85 mmHg, 4.4–3.6 mg $O_2 \cdot L^{-1}$). With the exception of relative stroke volume at 84–65 mmHg (3.5–2.7 mg $O_2 \cdot L^{-1}$), all measured variables in all three tuna species remained significantly different from control values as hypoxia became more severe.

In yellowfin tuna, gape and $\dot{V}_g$ increased and heart rate decreased at the same level of hypoxia as in skipjack tuna (Table 2, Fig. 3). Yellowfin tuna were able to maintain cardiac output at control levels until Pi_{O_2} fell to 84–65 mmHg (3.5–2.7 mg $O_2 \cdot L^{-1}$). Stroke volume was not significantly different from control values.

Bigeye tuna showed significant increases in gape when Pi_{O_2} fell to 124–105 mmHg (5.2–4.4 mg $O_2 \cdot L^{-1}$) (Fig. 3). No other changes were recorded until Pi_{O_2} fell to 104–85 mmHg (4.4–3.6 mg $O_2 \cdot L^{-1}$), by which point cardiac output was significantly reduced. There were no additional reductions in heart rate until Pi_{O_2} reached 84–65 mmHg (5.2–4.4 mg $O_2 \cdot L^{-1}$). $\dot{V}_g$ and stroke volume did not change significantly at any level of hypoxia. However, the P value was 0.06 for $\dot{V}_g$ at 124–105 mmHg (5.2–4.4 mg $O_2 \cdot L^{-1}$) and below. The large increase in gape and $\dot{V}_g$ at 74 mmHg (3.1 mg $O_2 \cdot L^{-1}$) (Fig. 3) was heavily influenced by one animal whose gape and $\dot{V}_g$ increased 230 and 134%, respectively.

When Pi_{O_2} was reduced below 70 mmHg (3.0 mg $O_2 \cdot L^{-1}$), Pe_{O_2} occasionally equalled Pi_{O_2}. Probably because of the large gape, opercular flare, or increased $\dot{V}_g$, the exhalant-water catheter could have been sampling water that passed through the gill cavity but did not come into contact with the secondary lamellae (and therefore did not exchange oxygen with the blood). While this was seen only during the most severe hypoxia, we have chosen not to report any Pe_{O_2} values and associated calculations (U and $\dot{V}O_2$) measured during hypoxia.

Discussion

Experimental techniques

Ideally, physiological studies of the cardiorespiratory system of obligate ram-ventilating fishes should be conducted on fish swimming in either a tank or a water tunnel. Water tunnels, suitable for use with large active fishes such as tunas, are difficult to design and extremely expensive to build. Although some water tunnel studies have been performed successfully on albacore (*Thunnus alalunga*) (Graham and Laurs 1982; Graham et al. 1989), such studies using skipjack or yellowfin tuna have thus far met with little success (Brill and Dizon 1979). Regardless of whether tuna are swimming in a tunnel or a tank, our experience is that they are unable to tow multiple cannulae or leads for more than a few hours. This makes it virtually impossible to measure several cardiorespiratory variables simultaneously. The spinal block preparation was developed specifically to circumvent these problems and provide a method for studying multiply instrumented, unanesthetized fish for prolonged periods.

The use of a spinal block to inhibit swimming motions appears to be a suitable technique for use with tunas. The local anesthetic selectively inhibits the spinal nerves controlling swimming muscle activity while leaving cranial nerves intact Fish were able to control their own $\dot{V}_g$ by adjusting mouth gape and opercular openings in response to changes in Pi_{O_2}. Regular eye movements and pectoral fin extension (in response to lateral tilting) also were noted. Each 0.2-mL injection of lidocaine hydrochloride was effective in blocking swimming movements for approximately 1–3 h, and fish appeared to remain in good condition for up to 10–12 h. The only side effect was a transitory (2–3 min) increase in heart rate following injection.

An effectively similar spinal transection technique has been used in a number of physiological studies on brook trout, *Salvelinus fontinalis* (McKim and Goeden 1982), and rainbow trout (McKim and Heath 1983; McKim et al. 1987). These investigators found no significant differences in heart rate, respiratory rate, ventilation volume, oxygen utilization, or oxygen consumption when fish spinally transected at the eighth

TABLE 2. Summary of statistically significant (at $P < 0.05$) changes in physiological variables at different levels of hypoxia

Oxygen level: mmHg	Oxygen level: mg $O_2 \cdot L^{-1}$	Gape	Ventilation vol. ($\dot{V}_g$)	Heart rate	Cardiac output	Stroke volume
144–125	6.1–5.3	ns	ns	ns	ns	ns
124–105	5.2–4.4	SYB	SY	ns	ns	ns
104–85	4.3–3.6	SYB	SY	SY	SB	S
84–65	3.5–2.7	SYB	SY	SYB	SY	ns
64–45	2.6–1.9	SYB	SY	SYB	SYB	S

NOTE: S, significant in skipjack tuna; SY, significant in skipjack and yellowfin tunas; SYB, significant in skipjack, yellowfin, and bigeye tunas; ns, not significant.

vertebra were compared with untransected animals. Moreover, McKim and Goeden (1982) also found that the respiratory responses of transected fish to hypoxia closely follow those of restrained but unoperated salmonids.

Because tunas will not survive the normal ≥24-h postoperative recovery period used with more docile and well-studied cyprinid or salmonid species, we decided to use measurement techniques that were as minimally invasive and disruptive to normal ventilation as possible. We chose not to use a rubber membrane sewn around the mouth to separate Pi_{O_2} and Pe_{O_2}, as is normally done with salmonids (Davis and Cameron 1971). The anatomy of tunas makes this procedure difficult because the ventral opercular openings extend anteriorly almost to the tip of the lower jaw. Also, for obligate ram-ventilating fishes a substantial pressure head would have to be provided to force water over the gills. We believe these procedures could significantly affect $\dot{V}_g$ in normoxia and the changes in $\dot{V}_g$ occurring during hypoxia. Measuring Pe_{O_2} by catheterization does have its limitations (Davis and Watters 1970), but it has been successfully used to study the responses of rainbow trout to hypoxia (Holeton and Randall 1967). Moreover, the adequacy of our methodology is supported by the fact that our metabolic rate measurements, which are dependent on accurately measuring $\dot{V}_g$ and U, agree closely with those from swimming tunas studied by Boggs (1984) and Jones et al. (1989).

Our use of a flat, transcutaneous Doppler flow probe also represents a compromise. It required no invasive surgery, but we could not calibrate the flow probe postmortem, and only data on relative changes (i.e., control versus hypoxia) could be obtained. "Cuff-type" transducers, which fix vessel diameter and provide a fixed relationship between blood velocity and volume flow rate, would have been better. Unfortunately, the area surrounding the bulbus arteriosus – ventral aorta of tunas is highly vascularized (Williams 1976). This resulted in extensive bleeding when attempts were made to mount Doppler flow cuffs. Furthermore, we believe that incisions to expose the bulbus arteriosus – ventral aorta could damage the muscles and nerves used for opening the lower jaw and, therefore, interfere with control of ventilation. This is an especially acute problem, since the tunas could be given only 1–2 h to recover from surgery.

Normoxia

The $\dot{V}_g$ of skipjack tuna in normoxia (6.7 $L \cdot min^{-1} \cdot kg^{-1}$) was higher than the 5 $L \cdot min^{-1} \cdot kg^{-1}$ predicted by Langille et al. (1983), based on data collected by Gooding et al. (1981). The U value (33%) at this high flow rate was similar to that reported for rainbow trout (Kiceniuk and Jones 1977), which have a much lower $\dot{V}_g$ (Table 1). Stevens (1972) measured U at 71% in anesthetized and artificially ventilated skipjack tuna, probably because $\dot{V}_g$ was set at an extremely low level (2.8 $L \cdot min^{-1} \cdot kg^{-1}$).

Mean $\dot{V}O_2$ values of skipjack and yellowfin tunas in our study were higher then those of fish immobilized with the neuromuscular blocker Flaxedil (gallamine triethiodide) (Brill 1987). Apparently, immobilization by spinal block results in more metabolic activity than immobilization by neuromuscular block. The metabolic rates of our spinally blocked skipjack and yellowfin tunas are closer to the routine metabolic rates of the same species measured by Boggs (1984). Mean $\dot{V}O_2$ values of skipjack tuna in our study were also similar to those of lightly anesthetized (immobile) skipjack tuna (Stevens 1972). No metabolic rate data are currently available for bigeye tuna.

Mean heart rates of our skipjack and yellowfin tunas were substantially lower than the 203 and 137 beats/min, respectively, measured in neuromuscularly blocked fish (Brill 1987). Stevens (1972) found a mean heart rate of 70 beats/min (range 46–142 beats/min) in anesthetized skipjack tuna, but this is likely due to the fact that the fish were significantly underventilated. Kanwisher et al. (1974) found telemetered heart rates of swimming skipjack tuna to be 80–240 beats/min. No data are currently available on the heart rate of bigeye tuna.

Regardless of the differences among the three tuna species used in this study (the $\dot{V}_g$ values of skipjack tuna were about 72 and 350% higher than those of yellowfin and bigeye tunas, respectively), the $\dot{V}_g$ values of tunas are much higher than the 200–900 $mL \cdot min^{-1} \cdot kg^{-1}$ of other teleosts and elasmobranchs (Johansen 1982). This is probably a reflection of the higher standard metabolic rates of tunas (Brill 1979, 1987; Gooding et al. 1981; Graham et al. 1989).

Hypoxia

Skipjack, yellowfin, and bigeye tunas were all sensitive to hypoxia. The onset of cardiorespiratory adjustments was rapid and lasted for as long as hypoxia ensued. In a few instances when hypoxia was prolonged for 5–8 min, bradycardia and increased gape were maintained until normoxia was resumed, suggesting that responses were not transient. The immediate cardiorespiratory adjustments to hypoxia are suggestive of an oxygen sensor in the gill (Bamford 1974), rather than in the central nervous system, which would have been indicated by more delayed responses (i.e., 1–2 min following the onset of hypoxia) (Saunders and Sutterlin 1971; Hughes 1978). In vitro nerve recordings from the first gill arch of yellowfin tuna showed bursting patterns sensitive to the Po_2 of both the external bathing solution and the perfusion fluid (Milsom and

Brill 1986), though the responses to changes in the P_{O_2} of the perfusion fluid were much more rapid. These results also suggest the presence of an internal oxygen receptor in the gill.

The majority of teleosts studied to date increase $\dot{V}_g$ during hypoxia. Maximum increases in $\dot{V}_g$ range from 200 to 600% (Hughes and Saunders 1970; Shelton et al. 1986). The maximum increases in $\dot{V}_g$ were only 50 and 100% in skipjack and yellowfin tunas, respectively, representing an increase to about 7–10 $L \cdot min^{-1} \cdot kg^{-1}$. While the relative changes in $\dot{V}_g$ may be modest, because of the enormous $\dot{V}_g$ during normoxia, the absolute increases in $\dot{V}_g$ of tunas far exceed those of other fishes.

The increase in $\dot{V}_g$ in response to hypoxia, although similar in sensitivity, varied in magnitude in the three species. Yellowfin tuna showed a much larger percent increase in gape and $\dot{V}_g$ than did skipjack tuna. This may be a reflection of the yellowfin tuna's lower $\dot{V}_g$ in normoxia, which allows a larger fractional increase. In view of the bigeye tuna's relatively low $\dot{V}_g$ in normoxia, one might expect to see large changes in gape and $\dot{V}_g$ in response to hypoxia. However, the increases in $\dot{V}_g$ and gape were relatively small, being more similar to those of skipjack tuna than to those of yellowfin tuna. Data on depth distributions (Hanamoto 1987; Holland et al. 1990) suggest that bigeye tuna may be relatively more hypoxia tolerant, which may explain our results. They are a far deeper-living species than skipjack or yellowfin tuna, and often occur at depths where P_{O_2} is 100 mmHg (4.9 mg $O_2 \cdot L^{-1}$) or less. Further studies on this species are clearly indicated.

The different $\dot{V}_g$ values obtained during normoxia may also explain the different behavioral responses of skipjack and yellowfin tunas to hypoxia. The former abruptly increase swimming speed at an ambient P_{O_2} below 90 mmHg (3.8 mg $O_2 \cdot L^{-1}$), whereas the latter maintain a constant swimming speed even at an ambient P_{O_2} below 50 mmHg (2.1 mg $O_2 \cdot L^{-1}$) (Dizon 1977). Because skipjack tuna are apparently limited in their ability to increase $\dot{V}_g$ by increasing gape (because of their relatively larger $\dot{V}_g$ during normoxia), they may have to increase swimming speed to generate the required flow. Yellowfin tuna, by virtue of their larger scope for changes in gape, may be able to compensate for hypoxia to a greater degree without changing swimming speed.

In most teleosts, a reduction in ambient P_{O_2} causes a reduction in heart rate (bradycardia), but no change in cardiac output because of concomitant increases in stroke volume (Randall 1982). While hypoxic bradycardia was recorded in all three tuna species, increases in stroke volume in skipjack tuna were ineffective in maintaining relative cardiac output. The inability of skipjack tuna to maintain cardiac output may have been a result of the experimental setup, however. Spinally blocked fish may lack the benefit of increased venous return resulting from tail flexion, although the contribution of tail movement to venous return is a matter of debate (Satchell 1971; Jones and Randall 1978; Farrell 1984). A statistically significant increase in stroke volume was never recorded in yellowfin or bigeye tuna.

Davis (1975) reviewed the literature, grouped teleosts by environment, and calculated the "incipient oxygen response threshold" (which he defined as "the oxygen level where some organism just starts to respond in some way to reduced oxygen availability"). The mean incipient oxygen response thresholds were 85.6 ± 5.4 (SE) mmHg (5.3 ± 0.4 mg $O_2 \cdot L^{-1}$) and 111 ± 31 mmHg (6.7 ± 2.1 mg $O_2 \cdot L^{-1}$) for freshwater and marine species, respectively. All three tuna species showed significant increases in gape at $P_{i_{O_2}}$ values between 124 and 105 mmHg (5.2–4.4 mg $O_2 \cdot L^{-1}$), and skipjack and yellowfin tunas showed increases in $\dot{V}_g$ at this level of hypoxia. Tunas therefore appear to be as sensitive to hypoxia as other marine teleosts.

Our experiments give insight into the responses of tunas in the laboratory, but their responses in less restricted environments should be considered. Our study clearly shows that skipjack and yellowfin tunas are equally sensitive to hypoxia and that the latter respond physiologically at hypoxia levels more moderate than those needed to elicit swimming speed changes. Presumably cardiorespiratory changes in response to hypoxia are aimed at maintaining the oxygen delivery to the tissues. Increased swimming speed must therefore be considered a "last resort" because of its high energetic cost. The lack of a behavioral response in tunas is not necessarily an accurate indicator of the animals' sensitivity to or tolerance of hypoxia.

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Appendix

Ideally, when a dye dilution technique is used to measure $\dot{V}_g$, the dye should be completely mixed within the mouth. If this is achieved, any opercular sampling site will suffice. Unfortunately, complete mixing is not always achieved without a mechanical stirring device mounted within the fish's mouth. However, doing this would negate the main advantages of a dye dilution system for measuring $\dot{V}_g$ in ram-ventilating fish: it is simple, light enough to be useful in swimming fish, and minimally disruptive to normal ventilatory processes.

When there are unequal dye concentrations exiting each operculum, the data can be corrected by averaging the transmittances, although this is not an intuitively obvious solution. The only required assumption is that flow rates of water exiting both opercula are equal. To the best of our knowledge, it has never been shown for any fish species whether the flow rates of water from each operculum are equal or unequal; however, equal water flow has been assumed in other studies on ventilation volume in teleosts (e.g., Lomholt and Johansen 1979).

Unequal dye concentrations in the exhalant water can be modeled as a system with one inflow but with separate outflows and separate dye injection points. The following variables are needed to describe this system:

$\dot{V}_{gT}$ is total water inflow
$\dot{V}_{gl}$ is water outflow through the left branch (operculum)
$\dot{V}_{gr}$ is water outflow through the right branch (operculum)
$\dot{V}_{dyeT}$ is total dye inflow

$\dot{V}_{dye\,l}$ is dye inflow into the left branch
$\dot{V}_{dye\,r}$ is dye inflow into the right branch
$[dye]_{in}$ is concentration of infused dye
$[dye]_{out\,l}$ is concentration of dye in the outflow of the left branch
$[dye]_{out\,r}$ is concentration of dye in the outflow of the right branch

Assuming that $\dot{V}_{g\,l} = \dot{V}_{g\,r}$, the objective is to find $\dot{V}_{g\,T}$ in terms of the measured quantities $[dye]_{out\,l}$ and $[dye]_{out\,r}$:

[A1] $$\dot{V}_{g\,l} = \dot{V}_{g\,r} = \frac{\dot{V}_{g\,T}}{2}$$

[A2] $$\dot{V}_{dye\,l} + \dot{V}_{dye\,r} = \dot{V}_{dye\,T}$$

[A3] $$\dot{V}_{g\,l} \cdot [dye]_{out\,l} = \dot{V}_{dye\,l} \cdot [dye]_{in}$$

[A4] $$\dot{V}_{g\,r} \cdot [dye]_{out\,r} = \dot{V}_{dye\,r} \cdot [dye]_{in}$$

Solving eqs. A3 and A4 for $\dot{V}_{dye\,l}$ and $\dot{V}_{dye\,r}$:

[A5] $$\frac{\dot{V}_{g\,l} \cdot [dye]_{out\,l}}{[dye]_{in}} = \dot{V}_{dye\,l}$$

[A6] $$\frac{\dot{V}_{g\,r} \cdot [dye]_{out\,r}}{[dye]_{in}} = \dot{V}_{dye\,r}$$

and then substituting eqs. A5 and A6 into eq. A2 yields

[A7] $$\frac{\dot{V}_{g\,l} \cdot [dye]_{out\,l}}{[dye]_{in}} + \frac{\dot{V}_{g\,r} \cdot [dye]_{out\,r}}{[dye]_{in}} = \dot{V}_{dye\,T}$$

Now substituting eq. A1 into eq. A7 yields

[A8] $$\frac{(\dot{V}_{g\,T}/2) \cdot [dye]_{out\,l}}{[dye]_{in}} + \frac{(\dot{V}_{g\,T}/2) \cdot [dye]_{out\,r}}{[dye]_{in}} = \dot{V}_{dye\,T}$$

and rearranging eq. A8 to get $\dot{V}_{g\,T}$ on one side of the equation, and simplifying, yields

[A9] $$\dot{V}_{g} = \dot{V}_{dye\,T} \cdot [dye]_{in} \left[\frac{[dye]_{out\,l} + [dye]_{out\,r}}{2}\right]^{-1}$$

Therefore, eq. A9 shows that the average of the transmittances of the left and right exhaled water samples should be used to estimate $\dot{V}_{g\,T}$.

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