Journal of Fish Biology (2005) **67,** 950–968
doi:10.1111/j.1095-8649.2005.00795.x, available online at http://www.blackwell-synergy.com

The metabolic and biochemical responses of tropical whitespotted bamboo shark *Chiloscyllium plagiosum* to alterations in environmental temperature

A. TULLIS* AND M. BAILLIE

Biology Department, The University of Puget Sound, Tacoma, WA 98416, U.S.A.

(Received 27 August 2004, Accepted 4 April 2005)

The capacity of tropical whitespotted bamboo sharks *Chiloscyllium plagiosum* to metabolically compensate, at both the whole-animal and biochemical levels, to prolonged exposure to temperatures higher (30° C) and lower (20 and 15° C) than their native temperature (24·5° C) was examined. As expected, whitespotted bamboo shark oxygen consumption increased upon exposure to 30° C and decreased at 20 and 15° C. Initial changes in oxygen consumption were maintained even after months at the experimental temperature, indicating that whitespotted bamboo sharks did not compensate metabolically to the experimental temperatures. Maximal activities and thermal sensitivity of citrate synthase and lactate dehydrogenase from whitespotted bamboo shark white locomotor muscle were similar between control animals maintained at 24·5° C and those maintained at 15° C, indicating that cold-exposed animals did not compensate at the biochemical level. Similarly, lactate dehydrogenase activity did not change following prolonged exposure to 30° C. White muscle from whitespotted bamboo sharks maintained at 30° C had significantly lower citrate synthase activity than did control animals. This result was surprising given the lack of metabolic compensation at the whole-animal level. Overall, whole-animal oxygen consumption measurements supported the hypothesis that animals from thermally stable environments lacked the capacity to metabolically compensate to altered temperatures. Enzymatic results, however, suggested that the metabolic potential of muscle could change following temperature acclimation even in the absence of metabolic compensation at the whole-animal level.

Key words: citrate synthase; elasmobranch; lactate dehydrogenase; metabolic compensation; oxygen consumption; temperature.

INTRODUCTION

Temperature plays a major role in controlling the physiological function of fishes, the majority of which are strict ectotherms. Moreover, the high heat capacity of water and the scarcity of thermal micro-habitats in aquatic environments mean that the body temperature of fishes generally equals water temperature (Pough *et al.*, 2002). Acute effects of temperature can be profound; in general, a 10° C change in temperature leads to two to three-fold changes in metabolic rate

*Author to whom correspondence should be addressed. Tel.: +1 253 879 2857; fax: +1 253 879 3352; email: atullis@ups.edu

(Schmidt-Nielsen, 1997; Clarke & Johnston, 1999). Studies have revealed that diverse teleosts from thermally variable, mostly temperate, environments have evolved the ability to offset the acute response to temperature through a process termed metabolic compensation (Hazel & Prosser, 1974; Johnston & Dunn, 1987; Guderley, 1990; Johnston, 1993). At the whole-animal level, metabolic compensation is reflected by a partial or complete return of metabolic rate to its original value, even though an animal remains at a higher or lower temperature. In this way, metabolic compensation counteracts the acute effects of temperature on metabolic rate and helps animals maintain a constant level of activity despite changes in environmental temperature (Sisson & Sidell, 1987; Rome *et al*., 1985).

Metabolic compensation at the whole-animal level is ultimately determined by changes at the biochemical and molecular levels (Hazel & Prosser, 1974; Goldspink & Penney, 1982; Guderley, 1990; Johnston, 1993; Johnson & Bennett, 1995; Hochachka & Somero, 2002). In general, the activities of metabolic enzymes change directly with temperature, such that enzyme activity increases with an increase in temperature. Alterations in enzyme activity following prolonged exposure to different temperatures can counteract the direct effect of temperature on enzymatic reaction rates. Specifically, in animals that show metabolic compensation, activities of key metabolic enzymes increase relative to initial values as acclimation temperature decreases (Sidell, 1980; Jones & Sidell, 1982; Kleckner & Sidell, 1985; Johnston & Wokoma, 1986; Mwangangi & Mutungi, 1994). Changes in enzyme activity in response to prolonged exposure to different temperatures (biochemical compensation) can result from alterations in enzyme concentration or expression of different isoforms (Hochachka & Somero, 2002). By whatever mechanism, changes in enzyme activity induced by prolonged temperature changes contribute to the metabolic compensation observed at the whole-animal level, and ultimately, the maintenance of locomotor capacity. Changes in enzyme activity, especially those related to the aerobic capacity of mitochondria, have also been linked to shifts in thermal tolerance limits that often accompany temperature acclimation (Pörtner, 2002).

Although comparative physiologists have learned a great deal about how teleosts respond to temperature change, there have been very few studies addressing how temperature affects the physiology of elasmobranchs and no study has examined the relationship between whole-animal metabolism and enzymatic activity in elasmobranchs. To date, the temperature dependence of metabolic rate has been examined in five subtropical elasmobranchs; three within the order Rajiformes (skates and rays; Du Preez *et al*., 1988; Hopkins & Cech, 1994) and two within the order Carcharhiniformes (ground sharks; Butler & Taylor, 1975; Carlson & Parsons, 1999; Lowe, 2001). The thermal challenge presented in these studies varied from acute exposure to different temperatures (Hopkins & Cech, 1994), to a 12 h acclimation period (DuPreez *et al*., 1988), to seasonal exposure (Butler & Taylor, 1975; Carlson & Parsons, 1999; Lowe, 2001).

The aim of the present study was to examine the metabolic responses of a tropical shark to changes in environmental temperatures. To this end, this study specifically tested for metabolic and biochemical compensation in juvenile whitespotted bamboo sharks *Chiloscyllium plagiosum* (Bennett) following prolonged exposure to temperatures higher and lower than their native temperature. Whitespotted bamboo sharks are small, nocturnal, bottom-dwelling sharks

(Compagno, 2001), which facilitates measurements of standard metabolic rate (R_S; the metabolic rate of a resting, post-absorptive ectotherm at a given temperature) without the complicating factor of locomotor activity. To assess biochemical compensation, activities of citrate synthase (CS) and lactate dehydrogenase (LDH) from whitespotted bamboo shark white locomotor muscle were measured. Citrate synthase is a commonly measured index of aerobic metabolism and would be expected to change in parallel with any changes in R_S. Lactate dehydrogenase is an indicator of the flux through anaerobic metabolism. Although LDH activity should not necessarily change in concert with metabolic rate, white muscle makes up the vast majority of the swimming musculature in sharks [*e.g. c.* 75% in *Scyliorhinus canicula* (L.) (Bone, 1988); *c.* 85% in *C. plagiosum* (A. Tullis, unpubl. data)]. Therefore, assessing white swimming muscle LDH activity will provide fundamental information about the metabolic capacity of this predominate muscle type. In addition, changes in LDH activity may also be important for maintaining glycolytic flux and locomotor capacity at different temperatures. Whitespotted bamboo sharks inhabit reefs around the Indo-West Pacific and probably experience very little temperature change on a daily or annual basis. Because of this, it was expected that they would show limited tolerance to changes in temperature and lack the capacity for metabolic compensation to different temperatures at the whole-animal or biochemical levels.

MATERIALS AND METHODS

ANIMAL CARE

Whitespotted bamboo sharks (Order Orectolobiformes) were obtained as eggs from Six Flags World of Adventure, Cleveland, OH, U.S.A., and from Sea World in San Antonio, TX, U.S.A. Eggs were raised at the University of Puget Sound in 76 l tanks at 24·5° C until hatching (water temperature was monitored daily and maintained within *c.* 24–25° C). Hatchlings were maintained in the laboratory in 76 l tanks equipped with under-gravel and external filters and air stones. Three or four animals were held in each tank. Salinity and temperature were measured once per day and corrected if necessary to maintain desired values (32 salinity). Levels of ammonia, nitrite, nitrate and pH were tested once per week and water changes (0·25 to 0·50 tank volume) were performed every 2 to 3 weeks or more frequently if necessary. Water used in the tanks was obtained from the Point Defiance Zoo and Aquarium (Tacoma, WA, U.S.A.). Hatchlings were fed squid *Loligo* spp., shrimp *Penaeus* spp. or scallops *Aequipenctin* spp. 5 days per week. Feedings were to near satiation once or twice per week with smaller portions on the remaining days. Because this study addressed the overall response to temperature change, food was not differentially restricted for any of the treatment groups. At the start of data collection, the animals had a mean ± S.D. mass of 19·7 ± 4·1 g (range 14·0–27·5 g).

TEMPERATURE MANIPULATION

After measuring the oxygen consumption of all whitespotted bamboo sharks at a mean ± S.D. 24·2 ± 0·5° C (control temperature), animals were divided into four groups. One group was placed at 20° C for *c.* 4 months, and then at 15° C for an additional 5 months (the stepped-cold group; $n = 5$). A second group was directly subjected to 15° C for 3 months (the cold group; $n = 4$). A third group was placed at 30° C for 4 months (the warm group; $n = 5$). Finally, a fourth group was maintained at the normal habitat

temperature of 24·5° C for 7 months (control group; $n = 4$). To reduce stress to the animals, temperatures were changed gradually (1–2° C per 3–4 days) and animals were monitored to ensure that they fed normally for at least two successive feedings before temperature was increased or decreased further. Because of the nature of this procedure, target experimental temperatures were reached in 2–3 weeks.

OXYGEN CONSUMPTION MEASUREMENTS

The R_S of whitespotted bamboo sharks was determined by measuring the standard resting oxygen consumption (V_{O2}, mg O_2 kg^{-1} h^{-1}). Oxygen consumption was measured using closed-system respirometry one to two times per week for the first 2 months at the experimental temperature and less frequently thereafter. Respirometers were airtight 1·3 l cylindrical plastic chambers (15 cm diameter and 8·5 cm high) equipped with a plastic-mesh partition (mesh-size 1 cm) that separated a small magnetic stir bar from the animal. To begin each trial, a respirometer was filled with water from the whitespotted bamboo sharks home tank and the animal was gently placed into the chamber. The lid was placed loosely on the chamber and the animal was allowed to adjust for a minimum of 1 h prior to data collection. During this rest period, chamber water was lightly aerated and stirred very slowly to ensure that the trials began with the water fully air saturated. Measurements of R_S were made on animals after they had settled onto the mesh partition of the respiration chamber and remained motionless for several minutes. Observations in which respiration rates of an animal in the metabolic chamber were compared to those in its home tank were also used to assess when the animal was in a sufficiently non-altered state to estimate R_S. When whitespotted bamboo sharks reached such a state (usually within 1 h), the chamber lid was secured and an oxygen electrode (DO-051, Cameron Instrument Company, Port Aransas, TX, U.S.A.) was inserted into the chamber to a depth of *c.* 1–2 cm. The decline in oxygen partial pressure within the chamber over time was measured with an oxygen meter (OM200, Cameron Instrument Company). Output from the oxygen meter was continuously recorded with a MacLab/2e (ADInstruments, Colorado Springs, CO, U.S.A.) and displayed on a PowerMac 7100/80 computer (Apple Computer Inc., Cupertino, CA, U.S.A.). Prior to each set of trials for a given day, the oxygen electrode was calibrated using nitrogen-saturated and air-saturated water. Whitespotted bamboo sharks were fasted for 48 h prior to measuring oxygen consumption.

To maintain water temperature constant during each experiment, respiration chambers were placed into individual water baths, the temperature of which was continuously monitored and adjusted with ice or warm water as necessary to remain at the target experimental temperature (15, 20, 24·5 or 30° C). Measurements of water temperature immediately before and after each trial revealed that chamber temperature generally varied by <1° C during a single experiment. Control trials that were identical to the experiments described above but without a whitespotted bamboo shark revealed that background oxygen consumption of the system was insignificant. Data were used only from trials in which the animal remained quiescent during the data collection period. Animals were weighed to the nearest 0·1 g after each trial. Oxygen consumption values for each individual were calculated following both short- and long-term exposure to an experimental temperature; oxygen consumption following short-term exposure was determined by averaging two to four measurements early (2–3 weeks) in the acclimation process, while V_{O2} following long-term exposure was determined by averaging two to four measurements late (10–14 weeks) in the acclimation process.

ENZYME ACTIVITY MEASUREMENTS

After the completion of the oxygen consumption experiments, animals were sacrificed by decapitation and tails were cut into steaks and frozen in isopentane cooled in liquid nitrogen. All tissue was stored at -70° C until analysed.

Enzyme assays were performed exclusively on white swimming muscle because this muscle type represents the majority of the swimming muscle in whitespotted bamboo

sharks (A. Tullis, unpubl. data). Assays were performed according to established protocols for fish muscle designed to elicit maximal enzyme activities (Sidell *et al*., 1987), with buffers modified for elasmobranch tissue (Watson & Dickson, 2001). Enzyme activities were measured in four control animals maintained at 24·5° C, five animals maintained at 30° C and five animals from the stepped-cold group maintained at 15° C. Enzyme activities for cold-exposed animals were only measured in animals from the stepped-cold group because these animals appeared healthier than those from the cold group, some of which had lost mass during the course of the study.

To begin each enzyme assay, frozen tail samples were thawed on an ice-cold stage and all skin, cartilage and red muscle were removed. The remaining white muscle was collected and weighed (*c*. 0·1–0·2 g wet mass). Five to 10% mass:volume homogenates of white muscle were prepared by mincing the tissue, adding the appropriate volume of extraction medium and homogenizing with a ground-glass homogenizer. The extraction medium consisted of 80 mM Imidizole, 2 mM EDTA, 400 mM urea, 200 mM TMAO, 150 mM KCl, 3 mM $MgCl_2$, 10 mM NaCl, pH 7·0 at 20° C. After homogenization, samples were sonicated at *c*. 30% maximal power for two 15 s bursts, separated by a 15 s cooling interval (60 Sonic Dismembrator, Fisher Scientific, Pittsburgh, PA, U.S.A.). All assays were conducted on crude homogenates without centrifugation. Enzyme activities were monitored with a Hitachi U2000 UV/visible spectrophotometer (Hitachi High-Technologies Inc., San Jose, CA, U.S.A.) equipped with a water-jacketed cuvette holder to maintain a constant temperature. Enzymes were assayed at 15, 20, 25 and 30° C to determine if acclimation temperature had altered the sensitivity of the enzymes to changes in assay temperature. All assays were run in duplicate. Enzyme activities are expressed in units of activity (micromoles of substrate converted to product per min) per gram wet-mass of tissue (U g-tissue^{-1}).

The lactate dehydrogenase activity was monitored by measuring the oxidation of NADH at 340 nm during the reduction of pyruvate to lactate. The reaction mixture consisted of 400 mM urea, 200 mM TMAO, 150 mM KCl, 3 mM $MgCl_2$, 10 mM NaCl, 0·15 mM NADH, 1 mM potassium cyanide, 1 mM pyruvate and 50 mM Imidizole, pH 7·4 at 20° C. Reactions were initiated by the addition of pyruvate. Control reactions, which lacked pyruvate, showed virtually no background reducing activity.

Activity of CS was measured by monitoring the reduction of DTNB [5,5′-dithiobis(2-nitrobenzoic acid)] by free coenzyme A at 412 nm. The reaction mixture consisted of 400 mM urea, 200 mM TMAO, 150 mM KCl, 3 mM $MgCl_2$, 10 mM NaCl, 0·5 mM oxaloacetic acid, 0·25 mM DTNB, 0·4 mM acetyl-CoA and 75 mM Tris, pH 8·0 at 20° C. Prior to initiating the reactions with oxaloacetic acid, background deacylase activity was measured in all samples. Background activity was subsequently subtracted from activity following addition of oxaloacetic acid.

STATISTICAL AND DATA ANALYSES

Statistical analyses were performed using StatView 5.0.1 (SAS Institute, Inc., Cary, NC, U.S.A.) or SPSS 11.0.2 (SPSS Science, Chicago, IL, U.S.A.). To determine if acclimation temperature influenced V_{O2} a one-way ANOVA with acclimation temperature as a factor and mass as a covariate was used (ANCOVA). If these results indicated a significant effect, a Tukey's HSD *post hoc* test was used to determine which treatment groups were different. A one-way ANOVA, with time as a factor and mass as a covariate, was also used to determine if V_{O2} differed between short- and long-term exposure to an experimental temperature. To determine if assay temperature influenced enzyme activity, one-way ANOVAs, with assay temperature as a factor, were performed separately for each acclimation group. Finally, to determine if acclimation temperature influenced enzyme activity at a given assay temperature, a one-way ANOVA, with acclimation temperature as a factor and mass as a covariate, was used. If these results indicated a significant effect, then a Tukey's HSD *post hoc* test was employed to determine which treatment groups were different.

Temperature quotients (Q_{10}) were calculated to determine how sensitive V_{O2} and enzyme activities were to changes in temperature. Two types of Q_{10} values were calculated: acclimation Q_{10} values and assay Q_{10} values. Acclimation Q_{10} values represented how much V_{O2} changed following exposure to a given experimental temperature. In this analysis, V_{O2} was always measured at the acclimation temperature. Acclimation Q_{10}s were calculated using the equation: $Q_{10} = (R_2R_1^{-1})^{[10(T_2 - T_1)^{-1}]}$, where R_1 and R_2 are initial and final rates of oxygen consumption, respectively, and T_1 and T_2 are initial and final acclimation temperatures, respectively (Schmidt-Nielsen, 1997). Differences between acclimation Q_{10} values were assessed using a *t*-test.

Assay Q_{10} values were determined for the enzyme activities. In this analysis, the objective was to determine if prolonged exposure of whitespotted bamboo sharks to different temperatures subsequently influenced how sensitive their enzymes were to acute changes in assay temperature. To calculate assay Q_{10}, the linear regression of $\log_{10}$(enzyme activity at T_n) *v.* $[0{\cdot}1(T_n - T_{(n-1)})]$ was generated, where T_n is the assay temperature. By this method, Q_{10} is equal to 10^b, where b is the slope (Schmidt-Nielsen, 1997). An ANCOVA, with acclimation temperature as a factor and assay temperature as a covariate, was used to determine if assay Q_{10} values for enzyme activity changed following prolonged exposure to experimental temperatures.

A significance level of alpha = 0·05 was used in all analyses and means are presented ± S.E.

RESULTS

TEMPERATURE TOLERANCE

Experimental temperature influenced feeding behaviour and body mass of whitespotted bamboo sharks. Control animals and those maintained at 20 and 30° C, ate readily and increased in mass. On average, control whitespotted bamboo sharks increased in body mass by 0·07 ± 0·01 g day^{-1}, while 20° C animals increased by 0·03 ± 0·01 g day^{-1}, and those at 30° C by 0·11 ± 0·01 g day^{-1}. By contrast, cold and stepped-cold whitespotted bamboo sharks maintained at 15° C did not eat as readily and only two of the nine animals gained body mass (*c.* 0·01 g day^{-1}). Of the remaining cold-exposed animals, three maintained a constant body mass and four decreased in body mass. All animals that decreased in body mass were from the group that was transferred directly from 24·5 to 15° C (cold group) without prolonged exposure to 20° C in between (stepped-cold group). By the end of the oxygen consumption portion of the study, whitespotted bamboo sharks in the cold group weighed significantly less than those in all other treatment groups (18·2 ± 0·8 g; ANOVA, d.f. = 3 and 14, $P < 0{\cdot}05$). Final body mass of stepped-cold (26·9 ± 2·9 g) and warm whitespotted bamboo sharks (35·1 ± 2·5 g) were not significantly different from control animals (28·2 ± 1·0 g; Tukey's HSD, $P > 0{\cdot}05$), but were significantly different from each other (Tukey's HSD, $P < 0{\cdot}05$).

OXYGEN CONSUMPTION

The initial oxygen consumption of whitespotted bamboo sharks when measured at the control temperature of 24·5° C was not significantly different among all treatment groups (ANOVA, d.f. = 3 and 13, $P = 0{\cdot}33$). Therefore, all V_{O2} values at 24·5° C were combined to yield an average control V_{O2} of 91·2 ± 5·3 mg O_2 kg^{-1} h^{-1} ($n = 18$). This average was used as a baseline V_{O2}

for which all comparisons were made following exposure to experimental temperatures.

Whitespotted bamboo shark oxygen consumption decreased when water temperature was lowered from 24·5° C to 20 and 15° C and increased when water temperature was raised to 30° C (Fig. 1). When water temperature was lowered from 24·5 to 20° C in the stepped-cold group, V_{O2} decreased to 79·3 ± 6·1 mg O_2 kg^{-1} h^{-1} ($n = 5$), a 13% decrease from the initial control value of 91·2 ± 5·3 mg O_2 kg^{-1} h^{-1}. Although this decrease was not significant (ANOVA, d.f. = 1 and 20, $P = 0{\cdot}31$), it was maintained even after 10–12 weeks at 20° C (Fig. 1). When water temperature was subsequently reduced from 20° C to 15° C, V_{O2} decreased to 34·7 ± 2·3 mg O_2 kg^{-1} h^{-1} ($n = 5$), 62% below the control value. The whitespotted bamboo sharks that were placed directly at 15° C from 24·5° C showed the same metabolic response to decrease in temperature as did the stepped-cold group (ANOVA, d.f. = 1 and 6, $P = 0{\cdot}21$); therefore, data for 15° C-exposed animals from both the cold group and stepped-cold groups were

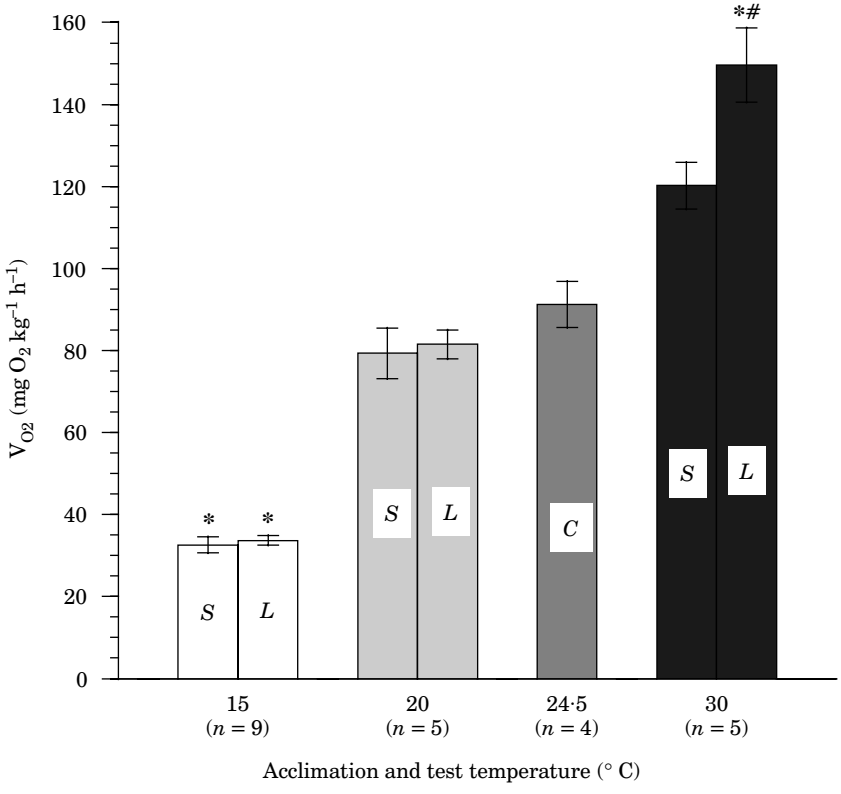

FIG. 1. Effects of acclimation temperature on the resting oxygen consumption (V_{O2}) of juvenile *Chiloscyllium plagiosum*. Mean ± S.E. oxygen consumption rates are shown for short-term (*S*; 2–3 weeks) and long-term (*L*; 10–14 weeks) exposure to the experimental temperatures. *, values that were significantly different from the control value (*C*) (ANOVA, $P < 0{\cdot}05$). #, long-term V_{O2} values that were significantly different from the short-term value at a given acclimation temperature (ANOVA, $P < 0{\cdot}05$). Sample sizes are shown in parentheses below each temperature.

combined. Importantly, the V_{O2} values from all 15° C-exposed whitespotted bamboo sharks measured early and late into the acclimation period were not significantly different (ANOVA, d.f. = 1 and 15, $P = 0{\cdot}65$; Fig. 1) but were significantly lower than the control value (ANOVA, d.f. = 1 and 24, $P < 0{\cdot}001$).

After being maintained at 30° C for 2–3 weeks, whitespotted bamboo shark V_{O2} was 32% higher than the control value ($120{\cdot}2 \pm 5{\cdot}7$ mg O_2 kg^{-1} h^{-1}, $n = 5$; Fig. 1). After 10–12 weeks the V_{O2} of these animals increased further to $149{\cdot}6 \pm 9{\cdot}0$ mg O_2 kg^{-1} h^{-1} ($n = 5$; Fig. 1). This final 30° C V_{O2} value was significantly different than the initial V_{O2} measured at 30° C (ANOVA, d.f. = 1 and 7, $P < 0{\cdot}05$), and represented a significant 64% increase over the control V_{O2} (ANOVA, d.f. = 1 and 20, $P < 0{\cdot}001$).

As expected from the V_{O2} measurements, there was no significant difference between the initial and final acclimation Q_{10} values for whitespotted bamboo sharks exposed to either 20 or 15° C (*t*-test, $P = 0{\cdot}53$ and 0·42, respectively; Table I). These results indicate that the initial metabolic response to temperature change was not altered following prolonged exposure to these experimental temperatures. The initial acclimation Q_{10} for whitespotted bamboo sharks placed at 30° C, however, was significantly lower than the final acclimation Q_{10} (*t*-test, $P < 0{\cdot}05$; Table I), indicating that prolonged exposure to high temperature accentuated the effect of temperature on the whitespotted bamboo shark R_S. The acclimation Q_{10} over the entire temperature range of 15–30° C was 2·70 (Table I).

ENZYME ACTIVITY

White muscle CS and LDH activities from control whitespotted bamboo sharks increased significantly with increasing assay temperature [ANOVA,

TABLE I. Acclimation Q_{10} values (mean ± S.E.) for whitespotted bamboo shark oxygen consumption following exposure to short- and long-term experimental temperatures

	Duration of exposure to experimental temperature	
Temperature range, ° C (*n*)	Short-term (2–3 weeks)	Long-term (11–14 weeks)
15–24·5[†] (9)	3·06 ± 0·22	2·89 ± 0·11
20–24·5[¥] (5)	1·51 ± 0·32	1·32 ± 0·14
24·5–30 (5)	1·66 ± 0·14	2·49 ± 0·26*
15–30[∫]	NA	2·70

[†]Q_{10} s for 15–24·5° C were calculated using V_{O2} values for cold and stepped-cold whitespotted bamboo sharks following exposure to 15° C ($n = 9$) because the mean V_{O2} values from these groups were not significantly different (ANOVA, d.f. = 1 and 16, $P = 0{\cdot}21$), and the average V_{O2} for all animals measured at 24·5° C (91·2 mg O_2 kg^{-1} h^{-1}).

[¥]Q_{10} s for 20–24·5° C were calculated using V_{O2} values from stepped-cold whitespotted bamboo sharks while they were maintained at 20° C, and the average V_{O2} for all animals measured at 24·5° C (91·2 mg O_2 kg^{-1} h^{-1}).

*Significant difference between short- and long-term acclimation Q_{10} values (paired *t*-test, $P < 0{\cdot}05$).

[∫]The Q_{10} value for 15–30° C was calculated from the final mean V_{O2} values for whitespotted bamboo sharks maintained at 15 and at 30° C shown in Fig. 1 and presented in Table III.

d.f. = 3 and 11, and 3 and 12, $P < 0{\cdot}05$ for both CS and LDH enzymes, respectively; Fig. 2(a), (b)], corresponding to assay Q_{10} of 1·62 and 1·80 for CS and LDH, respectively (Table II). Activities of CS and LDH from whitespotted bamboo sharks maintained at 15 and 30° C also increased significantly with increasing assay temperature [ANOVA, d.f. = 3 and 16, $P < 0{\cdot}05$ for all; Fig. 2(a), (b)]. Moreover, there was no significant difference among the assay Q_{10} values for a given enzyme among all experimental groups (ANCOVA, d.f. = 2 and 6, $P = 0{\cdot}91$ for CS and $P = 0{\cdot}44$ for LDH; Table II).

Prolonged exposure to 15° C led to mostly insignificant decreases in both CS and LDH activities relative to control and 30° C animals at all assay temperatures [ANOVA, d.f. = 2 and 10, Tukey's HSD, $P > 0{\cdot}05$; Fig. 2(a), (b)]. The one exception to this trend was a significant decrease in white muscle CS activity in 15° C whitespotted bamboo sharks relative to control animals when activity was assayed at 25° C [Tukey's HSD, $P < 0{\cdot}05$; Fig. 2(a)].

Prolonged exposure to 30° C did not significantly alter LDH activity relative to control and 15° C whitespotted bamboo sharks at all assay temperatures [ANOVA, d.f. = 2 and 10, Tukey's HSD, $P > 0{\cdot}05$ for all; Fig. 2(b)]. Muscle from whitespotted bamboo sharks maintained at 30° C, however, had significantly lower CS activity than did muscle from control animals at all assay temperatures (Tukey's HSD, $P < 0{\cdot}05$ for all), although there was no significant difference in CS activity between animals maintained at 30° C and those maintained at 15° C (Tukey's HSD, $P > 0{\cdot}05$ for all).

DISCUSSION

This study represents one of only a few studies that examines the influence of temperature on elasmobranch metabolic rate, and the first that combines both metabolic and enzymatic measurements. Results clearly showed that whitespotted bamboo sharks could tolerate a wide range of temperatures, the lowest of which is well below their normal habitat temperature. Whitespotted bamboo sharks showed no evidence of metabolic compensation at the whole-animal level, a result expected for a marine stenotherm (*i.e.* an animal that experiences a very small range of environmental temperatures). The lack of metabolic compensation, however, could also reflect an elasmobranch-specific response to changes in environmental temperature, regardless of native thermal habitat. Interestingly, enzymatic results suggested that some biochemical compensation occurred following prolonged exposure to warm but not cold conditions.

GENERAL V_{O2}, ENZYME ACTIVITIES AND TEMPERATURE TOLERANCE

Whitespotted bamboo sharks had an average R_S of $91{\cdot}2 \pm 5{\cdot}3$ mg O_2 kg^{-1} h^{-1} at the control temperature of 24·5° C. Data presented in Table III show that whitespotted bamboo shark V_{O2} was within the range expected for similarly sized sharks with comparable behavioral patterns [*e.g. Cephaloschyllium ventriosum* (Garman)]. Activities of CS and LDH from whitespotted bamboo shark white swimming muscle, and the general response of these enzymes to changing assay

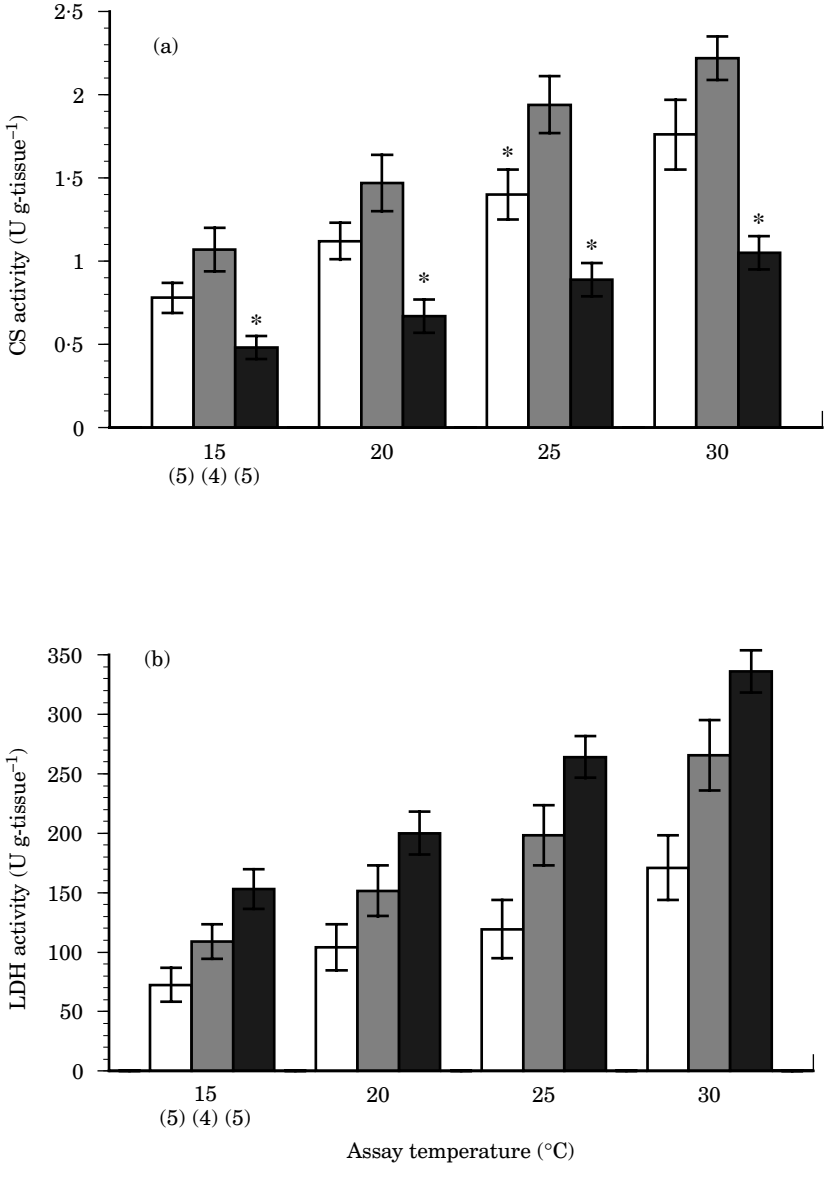

FIG. 2. The effect of assay temperature on (a) citrate synthase (CS) and (b) lactate dehydrogenase (LDH) activity (means ± S.E.) from white muscle of juvenile *Chiloscyllium plagiosum* following prolonged exposure to different temperatures: stepped-cold animals maintained at 15° C (□), control animals maintained at 24·5° C (▩), and warm animals maintained at 30° C (■). Enzyme activity increased significantly with assay temperature in all treatment groups (ANOVA, d.f. = 3 and 11, and 3 and 12, for CS and LDH, respectively, $P < 0{\cdot}05$). *, significantly different from the control (ANOVA followed by Tukey's HSD *post hoc* test, $P < 0{\cdot}05$). Sample sizes are shown in parentheses below 15° C and apply to all assay temperatures.

TABLE II. Assay Q_{10} values for enzymes [citrate synthase (CS) and lactate dehydrogenase (LDH)] from whitespotted bamboo shark white muscle following long-term exposure to different acclimation temperatures. Assay Q_{10} values were calculated for assay temperatures ranging from 15 to 30° C. Values for Q_{10} were not significantly different for a given enzyme among the three treatments (ANCOVA, d.f. = 2 and 6, $P = 0{\cdot}91$ for CS and $P = 0{\cdot}44$ for LDH)

Acclimation Temperature (° C)	CS	LDH
15 ($n = 5$)	1·69	1·70
24·5 ($n = 4$)	1·62	1·80
30 ($n = 5$)	1·67	1·69

temperature, were also within the ranges reported for other elasmobranchs (Dickson *et al.*, 1993; Bernal *et al.*, 2003; Treberg *et al.*, 2003).

One of the most surprising results of the present study was that tropical whitespotted bamboo sharks could tolerate temperatures ranging from 15 to 30° C for several months. Tolerance to 15° C was particularly unexpected given that this temperature is 10° C below their typical habitat temperature. Although numerous studies have shown that eurythermal fishes can tolerate a wide range of temperatures (Hazel & Prosser, 1974; Johnston & Dunn, 1987; Johnston, 1993), long-term tolerance to such a low temperature has not been documented in a tropical stenotherm. It is important to note, however, that whitespotted bamboo sharks showed this extreme cold tolerance only if they had first been maintained at 20° C for several months; animals that were subjected to 15° C without exposure to this intermediate temperature fed less readily and lost body mass. It has been proposed that thermal tolerance limits, to both low and high temperatures, are set by oxygen limitations caused by a progressive mismatch between oxygen supply and oxygen demand (Pörtner, 2002). According to this hypothesis, as temperature exceeds these limits, body fluids become increasingly hypoxic, reliance upon anaerobic metabolism increases, and survival become increasingly time-limited (Pörtner, 2002). Applying this concept to the present study suggests that whitespotted bamboo sharks maintained within 20–30° were not yet oxygen limited because they fed readily and displayed activity levels similar to control animals. At 15° C, however, whitespotted bamboo sharks showed evidence of time-limited survival (inactivity, anorexia and decrease in body mass), possibly due to progressive hypoxia. Results from the stepped-cold group, however, suggest that cold tolerance of whitespotted bamboo sharks can shift with thermal history, a phenomenon that is well known in teleosts (Hoff & Westman, 1966; Schmidt-Nielsen, 1997) but has not been demonstrated in elasmobranchs until the present study. The shift in cold tolerance may result from the expression of cold-induced proteins, a class of proteins that serves to stabilize RNA and prevent the formation of disruptive secondary structures as temperature decreases (Hochachka & Somero, 2002). Interestingly, the tolerance of whitespotted bamboo sharks to 15° C shifted even though there was no evidence metabolic compensation at the whole animal or biochemical levels.

TABLE III. Oxygen consumption rates and Q_{10} values for various elasmobranch species

Species, common name (scientific name)	Type of V_{O2} reported	Mass (kg)	Temperature* (° C)	V_{O2} (mg O_2 kg^{-1} h^{-1})	Q_{10}†	Source
Sharks						
Whitespotted bamboo shark *Chiloscyllium plagiosum* (Bennett)	Standard metabolic rate	0·02–0·04	15 20 24·5 30 (Prolonged exposure)	33·7 81·5 91·2 149·6	2·70	Present study
Bonnethead shark *Sphyrna tiburo* (L.)	Routine metabolic rate during slow swimming	1·10	20 25 30 (Seasonal exposure)	141·3 218·6 329·7	2·34	Carlson & Parsons (1999)
Scalloped hammerhead *Sphyrna lewini* (Griffith & Smith)	Estimated standard metabolic rate	0·51–0·93	21 29 (Seasonal exposure)	161 203	1·34	Lowe (2001)
Blacknose shark *Carcharhinus acronotus* (Poey)	Routine metabolic rate during slow swimming	0·45–3·5	28	382	–	Carlson *et al.* (1999)
Lemon shark *Negaprion brevirostris* (Poey)	Standard metabolic rate	0·8–1·3	22	136·8	–	Bushnell *et al.* (1989)
Lesser spotted dogfish‡ *Scyliorhinus canicula* (L.)	Standard metabolic rate	0·02	15	65	–	Sims (1996)
Swellshark *Cephaloschyllium ventriosum* (Garman)	Standard metabolic rate	0·14–0·23	16	44·3	–	Ferry-Graham & Gibb (2001)

TABLE III. Continued

Species, common name (scientific name)	Type of V_{O2} reported	Mass (kg)	Temperature* (° C)	V_{O2} (mg O_2 kg^{-1} h^{-1})	Q_{10}†	Source
Skates and rays						
Sandshark§	Standard metabolic rate	0·5	15	61·0	2·27	Du Preez *et al.* (1988)
Rhinobatos annulatus			20	90·8		
Muller & Henle			25	123·4		
			(12 h exposure)			
Bullray§	Standard metabolic rate	0·5	10	44·4	1·87	Du Preez *et al.* (1988)
Myliobatis aquila (L.)			15	70·5		
			20	99·2		
			25	127·7		
			(12 h exposure)			
Batray¶	Standard metabolic rate	5·0	8	50	3·0	Hopkins & Cech
Myliobatis californica Gill			14	75		(1994)
			20	280		
			26	380		
			(Acute exposure)			

*Where values are reported for more than one temperature, the type of temperature exposure is provided in parentheses; where only one temperature is presented, it represents the acclimation or acclimatization temperature.

†Q_{10} values for the species not used in the present study were obtained from the original papers. All Q_{10}s represent the value between the lowest and highest temperatures given for a particular species.

‡Oxygen consumption for *S. canicula* was calculated from the scaling relationship presented in Sims (1996).

§Oxygen consumption values for *R. annulatus* and *M. aquila* were calculated from scaling equations in Du Preez *et al.* (1988).

¶Oxygen consumption for *M. californica* was estimated from Hopkins & Cech (1994).

V_{O2} AND METABOLIC COMPENSATION

In spite of their ability to tolerate the imposed temperature challenges, whitespotted bamboo sharks did not metabolically compensate to the experimental temperatures. This conclusion is based on the observation that whitespotted bamboo shark V_{O2} decreased following cold exposure and increased following warm exposure and did not return to original control values even after 3–4 months (Fig. 1). Metabolic compensation would also lead to a decrease in acclimation Q_{10} values for V_{O2} over time, a result that was not observed in the present study (Table I).

Interestingly, the V_{O2} of whitespotted bamboo sharks placed at 30° C increased between short- and long-term exposure (Fig. 1). A response to temperature change such as this, where the initial effects of temperature on metabolic rate are amplified rather than reduced over time, has been termed inverse or paradoxical compensation (Hazel & Prosser, 1974). Cases of inverse compensation have generally been observed following prolonged exposure to low rather than high temperatures and have also been associated with high acclimation Q_{10} values for metabolic rate ($Q_{10} = 4$–5; Hazel & Prosser, 1974; Brown & Fitzpatrick, 1981; Walsh *et al.*, 1983; Hochscheid *et al.*, 2004). The apparent inverse compensation observed in whitespotted bamboo sharks, therefore, is unusual since it was in response to long-term exposure to high temperatures rather than low temperatures, and the Q_{10} value for metabolic rate was not elevated above other values in the present study (Table I). The possibility that this result is an elasmobranch-specific response to warm exposure remains to be determined.

When fishes and other ectotherms are acutely exposed to different temperatures, metabolic rates tend to change according to a Q_{10} of 2–3 (Schmidt-Nielsen, 1997; Clarke & Johnston, 1999). Animals that metabolically compensate to different temperatures tend to show acclimation Q_{10} values of less than two as metabolic rates at the new temperatures revert to the rates observed at the original temperatures. Acclimation Q_{10} values for whitespotted bamboo shark V_{O2} following long-term exposure to experimental temperatures were generally within the range expected for short-term exposure to different temperatures (Table I), reinforcing the notion that these animals did not metabolically compensate. Interestingly, acclimation Q_{10} values for whitespotted bamboo shark V_{O2} are similar to those found for other elasmobranchs, some of which had been acutely exposed to experimental temperatures while others had been exposed on a seasonal basis (Table III). Taken together, there does not appear to be any clear pattern in the magnitude of Q_{10} for V_{O2} among elasmobranchs; those that have been acutely exposed and those that have undergone long-term acclimation or acclimatization to different temperatures can have similar Q_{10} values. This finding suggests that more research is needed to determine if elasmobranchs, in general, possess the capacity to metabolically compensate to different temperatures, and, if so, under what circumstances this capacity is realized.

The lack of metabolic compensation at the whole-animal level observed in whitespotted bamboo sharks is similar to what has been found for tropical stenothermal amphibians and lizards (Feder, 1978, 1982, 1987; Tsuji, 1988). In these studies, the lack of metabolic compensation was attributed to the lack of thermal variability in the tropics (Feder, 1978, 1982). Although little is known

about the exact range of temperatures experienced by whitespotted bamboo sharks, they are reef-dwelling, tropical sharks from the Indo-West Pacific (Compagno, 2001). Given this habitat description, it is likely that whitespotted bamboo sharks do not experience temperature fluctuations of more than a few degrees on a daily or annual basis (NOAA, 2001). Thus, results from the present study support the hypothesis that animals inhabiting stable thermal environments cannot metabolically compensate to different temperatures. It must again be acknowledged, however, that the absence of metabolic compensation in whitespotted bamboo sharks could be due to a general lack of compensatory ability in elasmobranchs, regardless of native thermal habitat.

ENZYMATIC ACTIVITY AND METABOLIC COMPENSATION

Fishes and other ectotherms that show positive metabolic compensation in response to changing environmental temperatures possess a suite of changes at the biochemical and cellular levels that are ultimately responsible for the compensation of R_S (Hazel & Prosser, 1974; Goldspink & Penney, 1982; Guderley, 1990; Johnston, 1993; Johnson & Bennett, 1995; Hochachka & Somero, 2002). Therefore, since whitespotted bamboo sharks showed no metabolic compensation at the whole-animal level, it was expected that there would be no compensation at the biochemical level of the measured enzymatic characteristics (*i.e.* assay Q_{10} values and maximal activities).

Results from whitespotted bamboo sharks maintained at 15° C fit these predictions. First, prolonged exposure to 15° C did not influence the thermal sensitivity of either CS or LDH, as evidenced by comparable assay Q_{10} values between 15° C and control whitespotted bamboo sharks (Table II). This result implies that prolonged exposure to 15° C did not lead to the expression of different enzyme isoforms, a phenomenon that occurs in some fishes that exhibit metabolic compensation (Hochachka & Somero, 2002). Second, prolonged exposure to 15° C led to only insignificant changes in LDH and CS activity relative to control animals [Fig. 2(a), (b)]. Again, this result is congruent with the oxygen consumption results that showed no evidence for metabolic compensation of R_S even after 11–14 weeks at 15° C.

Enzymatic results from 30° C whitespotted bamboo sharks matched some of the predictions described above. For example, prolonged exposure to 30° C did not influence the assay Q_{10} values for either CS or LDH (Table II), again implying no change in enzyme isoforms. In addition, white muscle LDH activity from 30° C whitespotted bamboo sharks was not significantly different from control values [Fig. 2(b)]. White muscle CS activity from 30° C whitespotted bamboo sharks, however, was significantly lower than CS activity from control animals at all assay temperatures [Fig. 2(a)]. Interestingly, a decrease in CS activity following prolonged exposure to high temperature is what is expected for an animal that metabolically compensates to high temperatures (Hazel & Prosser, 1974; Johnston & Dunn, 1987; Johnston, 1993; Mwangangi & Mutungi, 1994). The decrease in CS activity that accompanies metabolic compensation to warm conditions is thought to alleviate the temperature-induced increases in the rates of biochemical processes and cellular energy demand. The reduction in

enzyme activity ultimately manifests itself as a return of R_S to initial control values even though the animal remains at a higher temperature.

An animal's R_S is determined by the cost of generating ATP that is required for maintaining cellular homeostasis. Under the resting conditions defined by R_S, this ATP is generated aerobically. Thus, the mismatch between CS results and V_{O2} measurements in warm-exposed whitespotted bamboo sharks is intriguing given that CS is involved in aerobic metabolism and is expected to reflect R_S. Indeed, a study which examined CS activity and whole-animal V_{O2} reveal that these two components change in parallel in a fish that metabolically compensates to temperature changes (Mwangangi & Mutungi, 1994). Changes in aerobic enzyme activity that accompany metabolic compensation are often linked to changes in mitochondrial volume (Johnston & Dunn, 1987). Reduced mitochondrial volume following warm acclimation has been proposed not only as a mechanism to compensate for temperature-induced increases in cellular energy demands, but also as a way to reduce baseline costs caused by proton leakage across the inner mitochondrial membrane (Pörtner, 2002), a process which has been shown to increase with increasing temperature (Hardewig *et al.*, 1999). If reduced CS activity in warm exposed whitespotted bamboo sharks does reflect reduced mitochondrial volume, the reason this was not reflected in a lower R_S needs to be explained. One possibility is that reduced baseline maintenance costs caused by a decrease in mitochondrial volume were exceeded by elevated energetic costs associated with temperature-induced increases in ATP utilization; when combined, lower CS activity could be observed with no reduction in R_S. Alternatively, or in addition, because CS is only one factor involved in determining flux through aerobic pathways, and white locomotor muscle is only one tissue type contributing to R_S, it is possible that other cellular level changes (*e.g.* substrate availability, cofactor concentrations or alterations in the activities of other aerobic enzymes) or tissue level changes (*e.g.* alterations in the aerobic capacity of other tissues) are occurring in warm-exposed whitespotted bamboo sharks that offset decreased CS activity in white locomotor muscle. It will be exciting to see if other elasmobranchs exhibit the same biochemical and metabolic patterns following prolonged exposure to high temperatures.

Results from the present study reveal that whitespotted bamboo sharks could tolerate a wide range of environmental temperatures and that thermal history can influence the level of cold tolerance. Oxygen consumption measurements revealed no metabolic compensation of R_S, although enzymatic measurements suggest that compensation may occur in some biochemical pathways following prolonged exposure to warm conditions. These results highlight the importance of using an integrative approach when studying temperature acclimation since results observed at one level of biological organization may not always reflect what is occurring at the level of the whole organism. It is only by studying additional species of elasmobranchs that physiologists will be able to develop a comprehensive picture of how this group of fishes responds to temperature challenges.

The authors would like to thank the aquarium at Six Flags Worlds of Adventure, Ohio and Sea World in San Antonio for providing shark eggs, and to J. Rupp, curator of the Point Defiance Aquarium, Tacoma, Washington, for his help and advice on animal care. Special thanks to B.R. Moon and R.L. Foster, and two anonymous reviewers for

providing helpful comments and suggestions on earlier versions of this manuscript. Thanks also to L.Z. Corum and A. Nelson for help with data collection and animal care. The experimental protocol used in this study was approved by the Institutional Animal Care Committee of the University of Puget Sound (approval number: 0304-01). This study was funded by a grant from the Murdock Charitable Trust (Murdock College Research Program for the Life Sciences #2000171) and the University of Puget Sound.

References

Bernal, D., Smith, D., Lopez, G., Weitz, D., Grimminger, T., Dickson, K. & Graham, J. B. (2003). Comparative studies of high performance swimming in sharks II. Metabolic biochemistry of locomotor and myocardial muscle in endothermic and ectothermic sharks. *Journal of Experimental Biology* **206,** 2845–2857.

Bone, Q. (1988). Muscles and locomotion. In *Physiology of Elasmobranch Fishes* (Shuttleworth, T. J., ed.), pp. 99–141. New York: Springer-Verlag.

Brown, A. V. & Fitzpatrick, L. C. (1981). Metabolic acclimation to temperature in the Ozark salamander *Plethodon dorsalis angusticlavius*. *Comparative Biochemistry and Physiology A* **69,** 499–503.

Bushnell, P. G., Lutz, P. L. & Gruber, S. H. (1989). The metabolic rate of an active, tropical elasmobranch, the lemon shark (*Negaprion brevirostris*). *Experimental Biology* **48,** 279–283.

Butler, P. J. & Taylor, E. W. (1975). The effect of progressive hypoxia on respiration in the dogfish (*Scyliorhinus canicula*) at different seasonal temperatures. *Journal of Experimental Biology* **63,** 117–130.

Carlson, J. K. & Parsons, G. R. (1999). Seasonal differences in routine oxygen consumption rates of the bonnethead shark. *Journal of Fish Biology* **55,** 876–879. doi: jfbi.1999.1031

Carlson, J. K., Palmer, C. L. & Parsons, G. R. (1999). Oxygen consumption and swimming efficiency of the blacknose shark, *Carcharhinus acronotus*. *Copeia* **1999,** 34–39.

Clarke, A. & Johnston, N. M. (1999). Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology* **68,** 893–905.

Compagno, L. J. V. (2001). *Sharks of the World*, Vol. 2. Rome: FAO.

Dickson, K. A., Gregorio, M. O., Gruber, S. J., Loefler, K. L., Tran, M. & Terrell, C. (1993). Biochemical indices of aerobic and anaerobic capacity in muscle tissues of California elasmobranch fishes differing in typical activity level. *Marine Biology* **117,** 185–193.

Du Preez, H. H., McLachlan, A. & Marais, J. F. K. (1988). Oxygen consumption of two nearshore marine elasmobranches, *Rhinobatos annulatus* (Muller & Henle, 1841) and *Myliobatus aquila* (Linnaeus, 1758). *Comparative Biochemistry and Physiology A* **89,** 283–294.

Feder, M. E. (1978). Environmental variability and thermal acclimation in neotropical and temperate zone salamanders. *Physiological Zoology* **51,** 7–16.

Feder, M. E. (1982). Environmental variability and thermal acclimation of metabolism in tropical anurans. *Journal of Thermal Biology* **7,** 23–28.

Feder, M. E. (1987). Effect of thermal acclimation on locomotor energetics and locomotor performance in a tropical salamander, *Bolitoglossa subpalmata*. *Physiological Zoology* **60,** 18–26.

Ferry-Graham, L. A. & Gibb, A. C. (2001). Comparison of fasting and postfeeding metabolic rates in a sedentary shark, *Cephaloscyllium ventriosum*. *Copeia* **2001,** 1108–1113.

Goldspink, G. & Penney, R. K. (1982). Changes in fish muscle under various environmental temperature conditions. *Molecular Physiology* **2,** 3–14.

Guderley, H. (1990). Functional significance of metabolic responses to thermal acclimation in fish muscle. *American Journal of Physiology* **259,** R245–R252.

Hardewig, I., Peck, L. S. & Pörtner, H. O. (1999). Thermal sensitivity of mitochondrial function in the Antarctic Notothenioid *Lepidonotothen nudifrons*. *Journal of Comparative Physiology B* **169,** 597–604.
Hazel, J. R. & Prosser, C. L. (1974). Molecular mechanisms of temperature compensation in poikilotherms. *Physiological Reviews* **54,** 620–677.
Hochachka, P. W. & Somero, G. N. (2002). *Biochemical Adaptation*. New York: Oxford University Press.
Hochscheid, S., Benivegna, F. & Speakman, J. R. (2004). Long-term acclimation leads to high Q_{10} effects on oxygen consumption of loggerhead sea turtles *Caretta caretta*. *Physiological and Biochemical Zoology* **77,** 209–222.
Hoff, J. G. & Westman, J. R. (1966). The temperature tolerances of three species of marine fishes. *Journal of Marine Research* **24,** 131–140.
Hopkins, T. E. & Cech, J. J., Jr. (1994). Effect of temperature on oxygen consumption of the bat ray, *Myliobatis californica* (Chondrichthyes, Myliobatididae). *Copeia* **1994,** 529–532.
Johnson, T. P. & Bennett, A. F. (1995). The thermal acclimation of burst escape performance in fish: an integrated study of molecular and cellular physiology and organismal performance. *Journal of Experimental Biology* **198,** 2165–2175.
Johnston, I. A. (1993). Phenotypic plasticity of fish muscle to temperature change. In *Fish Ecophysiology* (Rankin, J. C. & Jensen, F. B., eds), pp. 322–340. New York: Chapman & Hall.
Johnston, I. A. & Dunn, J. (1987). Temperature acclimation and metabolism in ectotherms with particular reference to teleost fish. In *Temperature and Animal Cells* (Bowler, K. & Fuller, B. J., eds), pp. 67–93. Cambridge: The Company of Biologists Limited.
Johnston, I. A. & Wokoma, A. (1986). Effects of temperature and thermal acclimation on contractile properties and metabolism of skeletal muscle in the flounder (*Platichthyes flesus* L.). *Journal of Experimental Biology* **120,** 119–130.
Jones, P. L. & Sidell, B. D. (1982). Metabolic responses of striped bass (*Morone saxatilis*) to temperature acclimation. II. Alterations in metabolic carbon sources and distributions of fiber types in locomotory muscle. *Journal of Experimental Zoology* **219,** 163–171.
Kleckner, N. W. & Sidell, B. D. (1985). Comparison of maximal activities of enzymes from muscle tissues of thermally acclimated and naturally acclimatized chain pickerel (*Esox niger*). *Physiological Zoology* **58,** 18–28.
Lowe, C. G. (2001). Metabolic rates of juvenile scalloped hammerhead sharks (*Sphyrna lewini*). *Marine Biology* **139,** 447–453.
Mwangangi, D. M. & Mutungi, G. (1994). The effects of temperature acclimation on the oxygen consumption and enzyme activity of red and white muscle fibers isolated from the tropical freshwater fish *Oreochromis niloticus*. *Journal of Fish Biology* **44,** 1033–1043.
Pörtner, H. O. (2002). Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. *Comparative Biochemistry and Physiology A* **132,** 739–761.
Pough, H. F., Janis, C. M. & Heiser, J. B. (2002). *Vertebrate Life*, 6th edn. New Jersey: Prentice Hall.
Rome, L. C., Loughna, P. T. & Goldspink, G. (1985). Temperature acclimation: improved sustained swimming performance in carp at low temperatures. *Science* **228,** 194–196.
Schmidt-Nielsen, K. (1997). *Animal Physiology: Adaptations and Environment*. New York: Cambridge University Press.
Sidell, B. D. (1980). Response of goldfish (*Carassius auratus* L.) muscle to acclimation temperature: alterations in biochemistry and proportions of different fiber types. *Physiological Zoology* **53,** 98–107.
Sidell, B. D., Driedzic, W. R., Stowe, D. B. & Johnston, I. A. (1987). Biochemical correlations of power development and metabolic fuel preferenda in fish hearts. *Physiological Zoology* **60,** 221–232.

Sims, D. W. (1996). The effect of body size on the standard metabolic rate of the lesser spotted dogfish. *Journal of Fish Biology* **48,** 542–544.
Sisson, J. E. & Sidell, B. D. (1987). Effect of thermal acclimation on muscle fiber recruitment of swimming striped bass (*Morone saxatilis*). *Physiological Zoology* **60,** 310–320.
Treberg, J. R., Martin, R. A. & Driedzic, W. R. (2003). Muscle enzyme activities in a deep-sea squaloid shark, *Centroscyllium fabricii*, compared with its shallow-living relative, *Squalus acanthius*. *Journal of Experimental Zoology A* **300,** 133–139.
Tsuji, J. S. (1988). Thermal acclimation of metabolism in sceloporus lizards from different latitudes. *Physiological Zoology* **61,** 241–253.
Walsh, P. J., Foster, G. D. & Moon, T. W. (1983). The effects of temperature on metabolism of the American eel *Anguilla rostrata* (LeSueur): torpor in the winter. *Physiological Zoology* **56,** 532–540.
Watson, R. R. & Dickson, K. A. (2001). Enzyme activities support the use of liver lipid-derived ketone bodies as aerobic fuels in muscle tissue of active sharks. *Physiological and Biochemical Zoology* **74,** 273–282.

Electronic Reference

NOAA (2001). *World Ocean Atlas 2001 Figures*. Available at http://www.nodc.noaa.gov/OC5/WOA01F/tsearch.html. Accessed July 21, 2004.