Effect of rapid temperature change on resting routine metabolic rates of two benthic elasmobranchs

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Abstract In this study, flow-through respirometry was used to test the effect of acute temperature change on resting routine metabolic rates of two benthic elasmobranchs, Atlantic stingrays, *Dasyatis sabina* ($n = 7$) and whitespotted bamboo sharks, *Chiloscyllium plagiosum* ($n = 7$) kept under fluctuating temperature regime of 24–27 and 23–25°C, respectively. Atlantic stingrays and whitespotted bamboo sharks showed a temperature sensitivity (Q_{10}) of 2.10 (21–31°C) and 2.08 (20–28°C), respectively. Not surprisingly, oxygen consumption (MO_2) increased in both species as temperature was raised. Acute increases in oxygen uptake may be useful during activities such as foraging, and some elasmobranchs may alter physiological processes by taking advantage of thermal variability in the environment. However, further investigation of different physiological processes is needed to better understand how temperature variation may affect behavioural choices of fishes.

Introduction

Temperature has such a profound effect on physiology and geographical distribution of aquatic poikilotherms, that Brett (1971) defined temperature as the *abiotic master factor*. In fact, most fishes exhibit a direct relationship between temperature and metabolic rates and a 10°C increase in ambient temperature usually translates into a doubling of physiological rate functions (Schmidt-Nielsen 1997). Respiratory rates are assumed to represent the physiological state of a fish; however, different activities, e.g. swimming, resting, and digestion, could be affected differently by temperature (Boisclair and Tang 1993). Metabolic rate may not be a reliable indicator of specific physiological processes and therefore, identifying how different activities are affected by temperature is essential in our understanding of fish movement patterns.

Several species of elasmobranchs, including Atlantic stingray, *Dasyatis sabina* (LeSueur), grey reef shark, *Carcharhinus amblyrhynchos* (Bleeker) and leopard shark, *Triakis semifasciata* Girard, move to warmer water when pregnant to presumably reduce gestation times (Economakis and Lobel 1998;

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Wallman and Bennett 2006; Hight and Lowe 2007). On the other hand, some species may show a reduction in some rate functions when moving to warmer waters (Di Santo and Bennett 2011). In fact, it is not rare for elasmobranch fishes to exploit habitat thermal variability to optimize physiological processes by moving within a range of temperatures below and above their thermal preferendum, a phenomenon known as shuttling behaviour (Casterlin and Reynolds 1979; Hopkins and Cech 1994, Sims et al. 2006). Moving across thermal gradients causes physiological processes to fluctuate, and fish may obtain some advantage by intermittently enhancing metabolic function rates. It is plausible, therefore, that fish may choose temperatures that selectively enhance specific physiological process depending on state (e.g. fed or unfed, pregnant or immature), time of the day and season. However, for shuttling behaviour to be beneficial, fish must be able to access thermal variability in their environment. Atlantic stingrays inhabit coastal waters from the Yucatan Peninsula, Mexico to New Jersey, USA. Across their latitudinal range, they experience dramatic annual as well as daily thermal fluctuations from circa 3 to over 35°C seasonally (Fangue and Bennett 2003). In coastal shallow embayments, the fish is observed to shuttle over relatively long distances where they encounter sharp thermal gradients (Teaf 1980; Fangue and Bennett 2003; Di Santo and Bennett 2011). Conversely, stenothermic elasmobranchs such as whitespotted bamboo sharks, *Chiloscyllium plagiosum* (Anonymous [Bennett]), from shallow tropical Indo-Pacific coastal and coral reef areas, experience relatively mild diel or seasonal thermal variation with minimum winter temperature of 18°C and a maximum summer temperature of 28°C (Chen et al. 2008), and observations suggest that bamboo sharks show limited foraging excursions making them unlikely to take advantage of shuttling behaviour (Compagno 2001; Tullis and Baillie 2005).

Past work on elasmobranch bioenergetics suggests that high temperature sensitivity of metabolic rates (e.g. the $Q_{10\ (14–20°C)}$ of 6.81 in bat ray measured by Hopkins and Cech 1994) may explain shuttling behaviour to enhance digestive efficiency in some species. Therefore, in the present study, the effect of rapid temperature change on metabolic rates was tested for two elasmobranchs inhabiting thermally different environments that are known to exhibit differential digestive responses to temperature (Di

Santo and Bennett 2011). Previous work has examined temperature sensitivity of metabolic rates by exposing elasmobranchs to acute change in temperature (Hopkins and Cech 1994), by acclimating fish for 8 days to the experimental temperature (Du Preez et al. 1988), or by testing seasonally acclimatized elasmobranchs (Carlson and Parsons 1999; Neer et al. 2006). Atlantic stingray and whitespotted bamboo shark metabolic rates were measured following rapid temperature change similar to those experienced by these and other elasmobranchs while shuttling between temperatures (Carey and Robinson 1981; Carey and Scharold 1990; Hopkins and Cech 1994). As it was suggested by Hopkins and Cech (1994) that elasmobranchs known to use thermoregulatory taxis as a means of enhancing digestive efficiency would show higher thermal sensitivity (Q_{10}) in metabolic rates than fish that do not utilize this strategy, the objectives of this research were to (1) determine the effect of fluctuating temperature on resting routine metabolic rates of two benthic elasmobranchs from dissimilar thermal environments and (2) determine the temperature sensitivity of these two species when exposed to rapid changes in temperature.

Materials and methods

Capture and holding conditions of experimental animals

Atlantic stingrays ($n = 7$) were collected from St. Joseph's Bay, Gulf County, Florida, USA (29°48′N; 85°20′W), whereas whitespotted bamboo sharks ($n = 7$) were donated by SeaWorld, Orlando, Florida, USA, and transported to the Marine Research Facility at the University of West Florida. At the Marine Research Facility, stingrays and sharks were transferred into biologically filtered 2000-l re-circulating tanks maintained at 35‰ salinity and a 12-h light:12-h dark photoperiod. Both species were held under a cycling thermal regime in order to prevent them from becoming acclimated to a single temperature that could influence metabolic processes (Hazel and Prosser 1974; Carlson and Parsons 1999; Neer et al. 2006). Stingrays were kept under a 24–27°C fluctuating temperature regime simulating summer temperatures registered in St. Joseph's Bay (Fangue and Bennett 2003), while whitespotted bamboo sharks

were maintained at a 23–25°C fluctuating cycle, to recreate temperature regimes similar to those experienced in nature (Tullis and Peterson 2000; Michael 2001; Tullis and Baillie 2005, Chen et al. 2008). Temperatures in holding tanks were manipulated using EBO Jager 250-W submersible heaters on timers and monitored every 30 min by Onset Stow-Away® XTI temperature loggers. During the holding period, fish were fed a mixed diet of fresh-frozen shrimp, fish or squid until sated. All fish were held for at least 2 weeks before being subjected to metabolic trials.

Respirometry

Mass-adjusted metabolic rates were determined using flow-through respirometry (Cech 1990). Fish were fasted for a minimum of 5 days before being used in trials to ensure that measurements were taken in post-absorptive state (Hopkins and Cech 1994; Di Santo and Bennett 2011). For each trial, Atlantic stingrays were placed into one of two different sized rectangular flow-through Plexiglas® respirometers depending on body size. One, measuring $48 \times 38 \times 12.5$ cm (22.8 l), was used for stingrays with pre-caudal length (PCL) measuring more than 30 cm, while a $40 \times 29 \times 13$ cm (15.08 l) respirometer was used for stingrays measuring less than 30 cm. For whitespotted bamboo sharks, a single respirometer measuring $79 \times 25 \times 16$ cm (31.6 l) was used in all trials. Respirometers were kept at a constant temperature by submerging them in a temperature-controlled water bath. Clean filtered seawater (35‰ salinity) was supplied to the respirometer from a constant-pressure head box. Stingrays and bamboo sharks were allowed to get accustomed to the respirometer chamber for 12 h (Hopkins and Cech 1994; Neer et al. 2006) before closing the respirometers. During the acclimation period, fully saturated water was allowed to flow freely through the respirometer and temperature was kept at the thermal cycle midpoint of 26°C for stingrays and 24°C for bamboo sharks. Respirometers were covered with dark plastic at all times to exclude any photoperiod effect (Miklos et al. 2003).

At the beginning of each trial, airpockets were eliminated (Carlson and Parsons 1999) and respirometer flow rates (l/h) were set based on fish body mass.

Flows were regulated, so the difference between inflow and outflow oxygen concentration (mg/l) never exceeded 17% (Cech 1990). Fish were held at the new flow conditions for 1 h, a period necessary to ensure 99% water exchange in the respirometer (Steffensen 1989). Temperature and oxygen concentrations of inflow (O_{2in}) and outflow (O_{2out}) were recorded at 30-min intervals using a Yellow Springs Instruments (YSI) oxygen meter (model 550A), and oxygen values confirmed by Winkler titration (1888). A total of four metabolic rates were measured in each trial (26, 31, 26, 21°C for stingrays; 24, 28, 24, 20°C for sharks). Metabolic rates were first obtained for the cycle mid-point temperatures to account for the effect of 12-h overnight acclimation at constant temperature. Measurements at each temperature were recorded for at least 2 h before increasing or decreasing temperature. To mimic the shuttling behaviour between environmental temperatures, temperature was raised or lowered 0.1 C/min using EBO Jager 250-W submersible heaters or an Aqua Logic Cyclone water chiller.

Mass-adjusted oxygen consumption rates (MO_2 in mg $\times$ kg$^{-0.67} \times$ h^{-1}) were calculated using the formula:

$$MO_2 = (O_{2in} - O_{2out}) \times \text{flow rate} \times \text{mass}^{-0.67}$$

The mass exponent of 0.67 was suggested for elasmobranchs by Hopkins and Cech (1994) and Meloni et al. (2002) to correct for the allometric relationships between metabolic rates and mass.

Metabolic Q_{10} determination

Temperature quotients were estimated for oxygen consumption rates to assess the effect of temperature change on overall metabolism. In both species, Q_{10} values were estimated for treatment temperatures (21–26, 26–31, 21–31°C for stingrays; 20–24, 24–28, 20–28°C for bamboo sharks). Average metabolic rate for each fish at each of the three temperatures was used to calculate Q_{10} values. The index of thermal sensitivity was determined using the following equation (Schmidt-Nielsen 1997):

$$Q_{10} = (K_2/K_1)^{(10/t_2-t_1)}$$

where K_1 is the rate at lower temperature (t_1); K_2 is the rate at higher temperature (t_2).

Statistical analyses

All experimental values are reported as means ± standard error (SE). Data were tested for normality and non-normal data were rank transformed. The effect of temperature on metabolic rates was explored using analysis of variance (ANOVA) with repeated measures followed by Tukey–Kramer Multiple Comparison Test to identify statistical differences in mean values between treatment groups. A t test was used to assess differences between double metabolic measurements at the midpoint thermal cycle; if not statistically different, then only second set of measurements were used. All statistical comparisons were based on $\alpha = 0.05$. All analyses were performed in SAS System, Version 9.

Results

Atlantic stingrays had a mean pre-caudal length of 24.9 ± 0.8 cm and a mean wet mass of 0.676 ± 0.081 kg. Whitespotted bamboo sharks had a mean pre-caudal length of 63.1 ± 0.5 cm and a mean wet mass of 0.601 ± 0.030 kg. Fish were quiescent, remaining on the respirometer bottom and exhibiting only occasional, limited movements to change position. Atlantic stingrays and bamboo sharks showed similar responses in (mass-adjusted) resting routine metabolic rates when exposed to acute changes in temperature (Fig. 1). In both species, oxygen consumption decreased with lower temperatures. Oxygen consumption rates were significantly different among temperature treatments in both Atlantic stingrays (Two-Way ANOVA: $F_{[2,12]} = 28.27$, $P < 0.0001$) and whitespotted bamboo sharks (Two-Way ANOVA: $F_{[2,12]} = 24.53$, $P < 0.0001$). Mean values at each temperature treatment were significantly different in Atlantic stingrays (Tukey–Kramer Multiple Comparison Test, $\alpha = 0.05$), while in bamboo sharks, lower temperatures means were not significantly different (20 and 24°C; Tukey–Kramer Multiple Comparison Test, $\alpha = 0.05$). In addition, both species showed similar values for total mass-adjusted oxygen consumption at similar temperatures (Fig. 1). As a result, Q_{10} values calculated across the range of experimental temperatures were 2.10 for Atlantic stingrays and 2.08 for whitespotted bamboo sharks (Fig. 1). Any potential metabolic effect of holding fish overnight at a constant
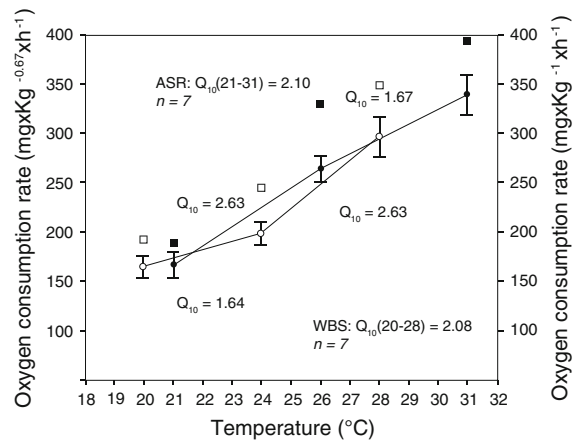
Fig. 1 Thermal sensitivity (Q_{10}) for oxygen consumption calculated across three temperature ranges for Atlantic stingray, *Dasyatis sabina* (ASR, *closed circles* and *squares*) and *whitespotted* bamboo shark, *Chiloscyllium plagiosum* (WBS, *open circles* and *squares*). *Closed* and *open circles* represent mean mass-adjusted values while *squares* are mass-dependent values. *Vertical bars* represent standard errors. All means are significantly different except the values at 20 and 24°C for WBS (Two-way ANOVA followed by Tukey–Kramer multiple comparison test, $\alpha = 0.05$)

temperature was apparently negligible, as mass-adjusted metabolic rate measurements taken at midpoint temperatures prior to, and immediately following, high temperature trials (260.7 ± 20.3 and 263.6 ± 12.9 mg $O_2 \times$ kg$^{-0.67} \times$ h^{-1}, and 192.5 ± 14.8 and 198.5 ± 7.0 mg $O_2 \times$ kg$^{-0.67} \times$ h^{-1}, for stingray and bamboo shark, respectively) were not significantly different for either species (Atlantic stingray: T-test, $P = 0.627$; whitespotted bamboo shark: T-test, $P = 0.933$).

Discussion

This work represents one of the very few studies examining the effect of temperature on elasmobranch resting routine metabolic rates. Similar to previous studies (e.g. Du Preez et al. 1988; Hopkins and Cech 1994; Carlson and Parsons 1999; Miklos et al. 2003; Neer et al. 2006), oxygen consumption rates increased with increasing temperature. Overall, Atlantic stingray and whitespotted bamboo shark show metabolic rates that fall between those of bull ray, *Myliobatis aquila* (Linnaeus; 77.0 mg O_2 kg^{-1} at 20°C) and bat ray (261.5 mg O_2 kg^{-1} at 20°C); likewise, leopard shark (Miklos et al. 2003) and cownose ray (Neer et al. 2006)

fall within this range. Neer et al. (2006) suggested that the metabolic values obtained for cownose rays differed from those of bat rays because of the effect of acclimation and acute change in temperature during the experimental trials. However, in both the present study and Hopkins and Cech (1994), double measurements at the holding point (26 or 24 and 14°C, respectively) showed that the experimental design had no significant effect on resting routine oxygen consumption rates within the temperature range. The effect of acclimation on poikilotherm physiological processes has been examined (Bullock 1955; Davies 1967; Hazel and Prosser 1974; Beitinger and Bennett 2000). Acclimation is likely to result in diminished temperature sensitivity, e.g. smaller Q_{10} values (Burggren and Roberts 1991; Hopkins and Cech 1994; Schmidt-Nielsen 1997; Neer et al. 2006); however, the fluctuating temperature acclimation regimes used in this study should have yielded oxygen consumption rates comparable to field acclimatized fish.

Some authors (Hopkins and Cech 1994; Matern et al. 2000) have suggested that the high temperature sensitivity of bat rays could be explained as thermoregulatory behaviour undertaken to enhance digestion. Therefore, it may be reasonable to assume that differences in temperature sensitivity of resting routine metabolic rates in Atlantic stingrays and whitespotted bamboo sharks reflect different foraging tactics. While Atlantic stingray and whitespotted bamboo shark show very different digestive responses to acute changes in temperature, i.e. overall digestive efficiency is affected by temperature in Atlantic stingrays while there is no significant effect on bamboo shark's digestion (Di Santo and Bennett 2011), their metabolic rates have similar sensitivity to the same temperature range. Atlantic stingrays show Q_{10} values typical for elasmobranchs moving with the tide (Teaf 1980; Miklos et al. 2003), yet studies on the effect of temperature on digestive processes in this batoid, indicated a significant increase in digestive efficiency (up to 30%) when Atlantic stingrays moved to cooler water after feeding. The stenotherm bamboo shark showed far less temperature-dependent differences in digestive rates than the eurythermal Atlantic stingray (Di Santo and Bennett 2011) but similar respiratory Q_{10} values. In conclusion, this study points out that overall resting routine metabolic responses may not be accurate indicators of significant thermoregulatory behaviour in elasmobranch fishes and that other key physiological (i.e. digestion, swimming) and ecological (i.e. prey-predators interactions) processes should also be analysed.

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