Journal of Thermal Biology 34 (2009) 220–225

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Critical thermal tolerance polygons of tropical marine fishes from Sulawesi, Indonesia

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ARTICLE INFO

Article history:
Received 15 January 2009
Accepted 13 February 2009

Keywords:
CTM
CTMax
CTMin
Sulawesi
Temperature tolerance
Thermal tolerance polygon
Tropical fish

ABSTRACT

1. Replicate thermal tolerance polygons were created using critical thermal methodology (CTM) and statistically compared.
2. Reef-associated damselfish and cardinalfish displayed the smallest total and intrinsic polygon zones and equal upper and lower acquired tolerance zones within species.
3. Two gobiids and a mullet species (resident and transient to tidepools, respectively) showed greater total and intrinsic tolerance zones than reef-associated species.
4. These CTM-polygons assess the thermal biology of fishes in habitats sensitive to global climate change and suggest that tropical Indo-Pacific fishes are likely to be affected by indirect consequences of global climate change, rather than by direct temperature mortality.

1. Introduction

The pervasive effect of temperature on fish physiology has engendered a large body of thermal tolerance literature stretching back over 120 years (Beitinger et al., 2000). The considerable interest in the thermal physiology of fishes is evidenced not only by the volume of literature produced but also by the progressive increase in experimental sophistication and complexity. The earliest studies focused on measuring single tolerance endpoints for fishes, often without regard for previous thermal acclimation history (Heath, 1884; Carter, 1887). As the importance of acclimation history emerged, however, researchers began quantifying thermal tolerance values at multiple temperatures across a species' acclimation range. By the 1940s, Fry and colleagues were using upper and lower tolerance values to create the first thermal tolerance polygons—graphical representations outlining a fishes' thermal niche. Early polygons were constructed using the Incipient Lethal Temperature technique (ILT; Fry et al., 1942), in which groups of fish were plunged into a series of high and low temperatures to determine temperatures lethal to 50% of the population (see Fry, 1967 for a complete description). Polygons derived from ILT data are rather burdensome, requiring large numbers of fish and equipment and have not been widely used since the early 1950s. Critical thermal methodology (CTM) has largely replaced the ILT technique as a means of determining fish thermal tolerance (Lutterschmidt and Hutchinson, 1997; Beitinger et al., 2000), due in part to logistical and animal-care concerns. Critical thermal tests require relatively few fish, less equipment and provide a rapid, non-lethal assessment of thermal tolerance (Fry, 1967; Beitinger et al., 2000). In 1997, Bennett and Beitinger used CTM thermal tolerance values to build the first CTM-polygon. Of the ~25 complete fish polygons, five are CTM-based (Bennett and Beitinger, 1997; Fangue and Bennett, 2003; Hernández and Bückle, 2002; Ford and Beitinger, 2005); however, the statistical fidelity with which these CTM-polygons reflect a fish's thermal ecology remains untested.

Attributes of thermal tolerance polygons provide important insights into fish ecology and distribution and have been used to identify temperature-related survival tactics (Bennett and Beitinger, 1997), predict the spread of exotic species (Bennett et al., 1997), quantify the thermal niche of endangered species (Walsh et al., 1998) and determine optimal culture conditions (Das et al., 2004). The usefulness of thermal tolerance polygons lies in their ability to impart markedly more information than tolerance endpoints alone. Overall polygon area (reported as $^{\circ}C^2$) provides a convenient and useful comparative index of eurythermicity between species. In addition, polygons define intrinsic thermal tolerance zones, i.e., tolerance independent of previous thermal acclimation history, as well as upper and lower acquired tolerance zones, i.e., thermal tolerance gained through

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0306-4565/$ - see front matter
doi:10.1016/j.jtherbio.2009.02.005

acclimation (Beitinger and Bennett, 2000). These zones characterize the relationship between thermal acclimation strategy and the thermal regimen of the fishes' life history or environment (Beitinger and Bennett, 2000; Fangue and Bennett, 2003).

Thermal tolerance polygons have been traditionally evaluated using direct percentile comparisons (Beitinger and Bennett, 2000; Fangue and Bennett, 2003), but stronger statistical inferences would make polygonal zone comparisons between fishes more meaningful. In this study, the efficacy of polygons to reflect the thermal habitats that fish exploit in the Wakatobi Marine National Park (WMNP: Banda Sea, Sulawesi, The Republic of Indonesia) was tested. The park encompasses a wide array of intertidal habitat subtypes, from thermally stable seagrass and patch reefs to shallow, hyperthermal mangrove tidepools (mangals). These habitats comprise a natural thermal gradient containing fishes that, while in close physical proximity, are exposed to very different thermal regimens. CTM-polygons were constructed and compared for five fishes with disparate spatial and temporal usage patterns of intertidal habitats in the WMNP. White-tailed humbug (*Dascyllus aruanus*; Linnaeus, 1758) and Nine-banded cardinalfish (*Apogon novemfasciatus*; Cuvier, 1828) inhabit patch reef and seagrass areas, respectively, while avoiding insolated tidepools (Myers, 1991). Squaretail mullet (*Liza vaigiensis*; Quoy & Gaimard, 1825) is a mangal transient that enters tidepools during nighttime low-tide events (Hiatt and Strasburg, 1960). Common goby (*Bathygobius fuscus*; Rüppell, 1830) and an undescribed sandflat goby species (*Bathygobius* sp.; James Van Tassell, personal communication) are mangal residents that never leave shallow tidepools and encounter local temperatures that can exceed 40 °C (Myers, 1991; Taylor et al., 2005). This study is the first to use statistical comparisons of thermal tolerance polygons (interspecific) and acquired tolerance zones (intraspecific) to provide assessment of fishes' thermal ecology, and the results of these polygons are presented in the context of sea surface temperatures in the Indo-Pacific. In addition, we report various thermal tactics used by fishes living within a coral reef thermal gradient as well as exceptional heat tolerance in species that routinely encounter temperatures near the upper biokinetic limit for vertebrate life.

2. Materials and methods

2.1. Collection, transport and maintenance of fishes

Experiments were conducted during a total of 20 weeks in the WMNP from June to August in 2003, 2004 and 2005. Fishes were collected from sites off Hoga Island (05°27.53S, 123°46.33E), transported to the Hoga Marine Research Centre and transferred to 190-L holding tanks filled with seawater at 26 ± 1.0 °C. Animals were collected under Operation Wallacea® collection permit #OP 647-03 and treated according to University of West Florida Animal Care and Use Committee protocol #2003-003.

2.2. Determination of thermal acclimation limits

Maximum and minimum acclimation temperatures were determined using chronic lethal methodology (CLM; Beitinger et al., 2000). Fish were randomly assigned to either CLmaxima or CLminima treatments. Treatments consisted of three replicate 22-L acclimation aquaria containing six to eight fish each. Aquaria were biologically filtered, and 20–25% of water changed daily to assure good water quality. Fishes were fed TetraMin® (Tetra Werke; Melle, Germany) flake food daily. Temperatures were increased or decreased from ambient at a rate of 1.6 ± 0.3 °C day^{-1} (mean ± SD) until a temperature lethal to 50% of the group was reached. Fishes used in CLM trials were not used in CTM trials.

2.3. Thermal acclimation and thermal tolerance determination

Critical thermal minimum (CTminimum) and Critical thermal maximum (CTmaximum) were estimated using the critical thermal methodology (Cox, 1974; Paladino et al., 1980; Beitinger et al., 2000). All species were randomly assigned to one of five temperature treatments across their acclimation range, except for Nine-banded cardinalfish (seven treatments). Treatments comprised three, replicate 22-L acclimation aquaria containing six to eight fish each. Aquaria were biologically filtered, and 20–25% of water changed daily to assure good water quality. Fishes were fed TetraMin® flake food daily, but not fed 24 h prior to trials. Water temperatures were increased or decreased from ambient by 1.5 °C day^{-1} until the desired acclimation temperatures were reached for each treatment. Fishes remained at each acclimation set-point temperature for at least 14 days prior to CTM trials.

For each CTM trial, randomly selected fish were placed, one each, into 250-ml Nalgene® beakers filled with clean seawater at appropriate acclimation temperatures. Beakers were suspended within a 60-L, insulated, recirculating water bath and provided with moderate aeration to prevent thermal stratification. A certified Fisherbrand® NIST mercury thermometer monitored temperature in each beaker. Temperatures in the CTM water bath were heated or chilled using a 1500-W immersable heater (custom made) or a 1/4 HP chiller (New Ocean, Universal Marine Industries, CA) at a rate of 0.31 ± 0.08 °C min^{-1} (mean ± SD) until final loss of equilibrium, LOE (inability to maintain dorso-ventral orientation for at least 1 min; Beitinger et al., 2000), was reached. This rate was slow enough to track body temperature, but fast enough to prevent thermal acclimation (Cox, 1974; Becker and Genoway, 1979). Following each trial, fish were weighed (wet mass ± 0.01 g), measured (standard length ± 0.5 mm) and returned to acclimation temperature to recover.

Upper and lower thermal tolerance for each replicate treatment group was calculated as the mean of the CTminima or CTmaxima trials. The grand mean of the collective replicate endpoints was taken as the CTminimum or CTmaximum for the population (Cox, 1974). Simple linear regression (SLR) analysis was used to model the relationship of thermal tolerance on acclimation temperature for each species. Analysis of covariance (ANCOVA) was performed on tolerance endpoint data within each species, and least square mean (LSM) values used to assess potential mass on tolerance effects.

2.4. Construction and interpretation of thermal tolerance polygons

Thermal tolerance polygons were constructed from the CTM and CLM limits of each species using a modified version of the methods described by Bennett and Beitinger (1997). Polygons were created by connecting CLminima and CLmaxima with CTM regressions to produce a quadrilateral figure expressed quantitatively using the areal units, °C^2. Polygons were divided into an intrinsic tolerance zone (i.e., thermal tolerance independent of previous thermal acclimation) and acquired tolerance zones (i.e., thermal tolerance gained through acclimation) by dividing polygons with horizontal lines from extrapolated CTminimum and CTmaximum values at CLM limits. A one-way analysis of variance (ANOVA) was used to compare total polygonal area, intrinsic tolerance area and acquired tolerance areas between species, and a Tukey's Studentised Range (TSR) *post-hoc* test separated values into statistically distinct subsets. Student's *t*-test was used to examine intraspecific differences between upper and lower