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Heat tolerance and its plasticity in Antarctic fishes

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ABSTRACT

The adaptive radiation of the Antarctic notothenioid ancestral benthic fish stock within the chronic freezing waters of the Southern Ocean gave rise to five highly cold adapted families. Their stenothermy, first observed from several high-latitude McMurdo Sound species, has been of increasing recent interest given the threat of rising polar water temperatures from global climate change. In this study we determined the heat tolerance in a geographically diverse group of 11 Antarctic species as their critical thermal maximum (CTMax). When acclimatized to their natural freezing water temperatures, environmental CTMaxs ranged from 11.95 to 16.17 °C, well below those of fishes endemic to warmer waters. There was a significant regional split, with higher CTMaxs in species from the more northerly and thermally variable Seasonal Pack-ice Zone. When eight of the Antarctic species were warm acclimated to 4 °C all showed a significant increase over their environmental CTMaxs, with several showing plasticity comparable in magnitude to some far more eurythermal fishes. When the accrual of heat tolerance during acclimation was followed in three high-latitude McMurdo Sound species, it was found to develop slowly in two of them, which was correlated with their low metabolic rates.

1. Introduction

Antarctica's coastal fishes are divided between largely non-overlapping geographic distributions in the High-Antarctic Zone (HAZ) and the Seasonal Pack-ice Zone (SPZ) (Kock, 1992). Both lie within the chronically cold Southern Ocean where water temperatures range between −1.9 and 3 °C (Deacon, 1984; Eastman, 1993). Despite this narrow temperature range, these region's endemic ichthyofaunas are exposed to distinct temperature conditions which may have led to the differentiation of their respective heat tolerances.

The HAZ encompasses much of the continental coast where water temperatures remain at or near their freezing point throughout the water column and for most of the year (DeVries and Steffensen, 2005). In the HAZ waters of McMurdo Sound, where seasonal variation in water temperatures has been investigated, the upper 50 m of the water column rises above its freezing point during summer peaking at −0.5 °C for a few weeks in January (Hunt et al., 2003). The more northerly SPZ includes the waters along the Western Antarctic Peninsula (WAP) and the southern Scotia Arc Islands. While the winter surface waters here can be as cold as those in the HAZ, they show a comparably larger temperature range with increases of 3 °C along the WAP during the summer months (Barnes et al., 2006). Furthermore, in some places along the WAP warm circumpolar deep water (CDW) periodically intrudes onto the continental shelf raising deep water temperatures up to 1 °C (Clarke et al., 2009). While the seasonal changes are small relative to those in temperate environments, there still appears to be a strong correlation between these small temperature differences and certain physiological/biochemical traits in the Antarctic notothenioids. For example the warmer and mostly ice free habitats in the SPZ region have been noted to correspond with a reduction in freeze avoidance relative to fishes endemic to the HAZ (Bilyk and DeVries, 2010) and a similar correlation may exist with their heat tolerance.

The heat tolerance of Antarctic fishes was first studied by Somero and DeVries (1967) who determined the upper incipient lethal temperature (UILT) in three HAZ nototheniids. This technique, based on lethal dosage methodologies, measures heat tolerance as the temperature at which median mortality is no longer time dependent. They found that the species *Pagothenia borchgrevinki*, *Trematomus bernacchii*, and *Trematomus hansoni* shared UILTs between 5 and 7 °C when acclimatized to the water temperature in McMurdo Sound (−1.9 °C). Given that the lower boundary of their tolerance is dictated by the temperature at which they freeze (−2.2 °C), this results in a remarkably narrow thermal range when compared to temperate and tropical fishes (Elliot, 1981).

Most animals do not have a static heat tolerance; rather it changes in response to their recent thermal history through acclimation. However, given the long residence of Antarctic fishes in constant freezing seawater, this plasticity had long been thought either lost or marginal (Brett, 1970). Recently though, Podrabsky and Somero (2006) noted that the two McMurdo Sound nototheniids *T. bernacchii* and *Trematomus pennellii* both shared the ability to extend heat

Abbreviations: CDW, Circumpolar Deep Water; CTMax, Critical Thermal Maximum; HAZ, High Antarctic Zone; SPZ, Seasonal Pack-ice Zone; UILT, Upper Incipient Lethal Temperature; WAP, Western Antarctic Peninsula.

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tolerance through acclimation. When transferred to 14 °C water, which is fatal to these fishes within minutes, specimens acclimated to 4 °C for four to six weeks survived for significantly longer than those held at their environmental water temperatures. While the ability to extend heat tolerance in these two species which are endemic to particularly cold stable Antarctic waters suggests that this ability is widespread throughout the Antarctic ichthyofauna, the study's use of survival time at a single temperature as a measure of heat tolerance makes it difficult to compare either tolerance or the ability to extend it between Antarctic, temperate, and tropical fishes.

The critical thermal maximum (CTMax) is a commonly used technique equating an animal's heat tolerance to the temperature at which it loses the ability to escape from constant rapid warming (Paladino et al., 1980). While this technique has been used extensively in temperate and tropical fishes it has never been used to determine heat tolerances in Antarctic fishes. Though it is a measure of acute tolerance, it has proven correlated with longer term measures of tolerance, and in some cases to a species' physiological optima (Kilgour and McCauley, 1986; Garland et al., 1991; Bennett and Beitinger, 1997). Combined with its ease of determination, these make it a useful tool for identifying species' relative sensitivity to long term increases in local water temperatures.

However, caution must be applied when comparing CTMaxs as they have been shown sensitive to experimental parameters. Warming rate, which often differs between studies, is positively correlated with CTMax even in specimens that are held and tested under otherwise identical conditions (Becker and Genoway, 1979; Terblanche et al., 2007). Despite this, when consistent heating rates are used the CTMaxs of species are repeatable with little intraspecies variability and thus can be used for comparisons between species and experimental groups.

In this investigation we used the CTMax methodology to survey heat tolerance in a geographically diverse group of 11 species of Antarctic fishes acclimatized to the cold water temperatures of their natural habitats. These include representatives of nototheniid species endemic to both the HAZ and SPZ allowing us to examine regional differences in heat tolerance as well as two zoarcid fish species. CTMaxs were also determined in eight of the species following warm acclimation to 4 °C, which when compared to their environmental CTMaxs provided a measure of the plasticity of their heat tolerance. Finally, the accrual of heat tolerance during this warm acclimation was followed over three weeks in three HAZ species.

2. Materials and methods

2.1. Collection of fishes

Specimens of the four HAZ nototheniid species were collected from the shallow waters of the ice-covered McMurdo Sound (77°S) by hook and line during the Austral spring (Oct and Nov) before warming of the surface waters. The two species of zoarcid fishes were trapped between September and December of 2007 at a depth of 500 m where the water is a constant −1.9 °C throughout the year (Littlepage, 1965). Following collection, specimens were transported in aerated insulated coolers at −1.9 °C to the Crary laboratory's aquarium facility at McMurdo Station. CTMaxs were then either immediately determined, or if this was not possible, the fish were placed into 7000 L aquaria where they were held for up to 21 days in a constant flow of local seawater that ranged in temperature from −1.5 to −0.9 °C. The McMurdo aquaria had windows and thus the fish were exposed to 24 h of daylight. At Palmer Station the aquaria also had widows and specimens thus were exposed to 10 h of subdued light. No attempt was made to control the lighting during the determination of the CTMax as from our experience it seems to have little effect on the behavior in the aquarium. Most would feed with interior lights turned on.

Specimens of the five notothenioid SPZ species were collected along the WAP near Anvers Island (64°S) during the Austral winter months of July and August of 2008. Most were collected aboard the *R.V. L.M. Gould* by bottom trawl and trapping with additional specimens of *Notothenia coriiceps* collected by hook and line in 2 m of water from the shore at Palmer Station. Most ship trawls and trap lines were set in 100 m or less where the temperature was less than −1 °C. Some *N. coriiceps* and *Notothenia squamifrons* were captured at 300 m where the water temperature was 1 °C. Specimens were transported in flow-thru sea water aquaria for two to four days until they could be transferred to Palmer Station. Since surface waters were freezing, tank temperatures were held at −1.5 °C using submersible 300 W aquarium heaters as several of the SPZ species were at risk of freezing in the ice-laden sea water supply.

The SPZ specimens were transferred to aquaria at Palmer station which received a constant flow of local seawater from Arthur Harbor ranging between −1.7 to −1.0 °C, the variation depending upon whether Arthur Harbor was ice covered and wind direction. CTMaxs were determined immediately for shore collected specimens, while those collected aboard the research vessel were allowed three to seven days to recover from collection stress.

2.2. Determination of the environmental CTMaxs of Antarctic fishes

Environmental CTMaxs were determined for the 11 Antarctic species following the methodology of Paladino et al. (1980). As these had been caught or held at temperatures below −0.9 °C this served as a measure of the heat tolerance at their habitat temperatures. Specimens were transferred into 40 or 80 L plexiglas aquaria at the same water temperature as their holding tanks and which were large enough to allow free movement. Specimens were allowed several minutes to adjust to their new surroundings and then they were warmed at 0.3 °C/min through the activation of submerged stainless steel aquarium heaters.

This rate allows core body temperature to closely track the surrounding water temperature while fast enough to avoid the onset of substantial warm acclimation (Becker and Genoway, 1979; Lutterschmidt and Hutchison, 1997b). During warming the tank was vigorously aerated to prevent thermal stratification and maintain oxygen saturation. Water temperature was monitored once a minute using a DiGi sense model 8525 thermistor thermometer (Eutech Instruments Inc.) with an attached YSI series 401 temperature probe (Measurement Specialties Inc.) suspended in the aquaria.

Warming was continued until a persistent loss of equilibrium was observed as the inability of the fish to right itself for one minute after rolling on its side. While other investigators have argued for the onset of respiratory tremors or muscle spasms as signifying the CTMax (Lutterschmidt and Hutchison, 1997a,b), only a persistent loss of equilibrium was clearly observed in all of the Antarctic species. The temperature at which the specimen's persistent loss of equilibrium was first observed was then taken as their CTMax. They were then removed from the warming tank, quickly weighed to the nearest 0.1 g, then returned to their holding aquarium at its original temperature for observation during recovery.

Most specimens were tested following a brief period in the research station's holding aquaria which could be up to 0.5 °C warmer than local surface waters. To gauge whether any significant warm acclimation occurred during their residence in the holding aquaria, CTMaxs were determined on freshly caught and aquaria held specimens of *P. borchgrevinki* and *N. coriiceps* then compared between groups by species using a two-tailed Student's *t*-test.

Log_{10} transformed CTMaxs were then compared among all specimens for significant differences between species using a one-way analysis of variances (ANOVA). This transformation was necessary to achieve the homogeneity of variance necessary for an ANOVA which was rejected by Levene's test in the untransformed CTMaxs. Significant groupings among the Antarctic species were then determined using a Student Newman–Keuls multiple range test performed on the log_{10}