Heat tolerance of the secondarily temperate Antarctic notothenioid, *Notothenia angustata*

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Abstract: Although most of the notothenioid fishes have geographic distributions restricted to the Southern Ocean, several species with inferred Antarctic origins have come to permanently inhabit the warmer waters around New Zealand and southern South America. However, it remains unknown whether the Antarctic ancestry of these secondarily temperate species continues to influence their modern heat tolerance. We investigated the heat tolerance of one such secondarily temperate nototheniid, *Notothenia angustata*, which is now endemic to the waters around the South Island of New Zealand. Their heat tolerance was determined using the critical thermal maximum (CTMax) both when acclimatized to their winter water temperatures (7.9°C), and warm acclimated (15°C) near the summer water temperatures in Otago Harbour. When compared to equivalently acclimated specimens of the basal New Zealand notothenioid *Bovichtus variegatus*, *N. angustata* have consistently lower CTMaxs, though they are significantly greater than those determined from 10°C acclimated specimens of its endemic Antarctic congener, *N. coriiceps*. While this shows greater heat tolerance in the secondarily temperate *N. angustata* than in endemic Antarctic species, it also suggests that some of its ancestral intolerance to heat persists.

Introduction

The evolution of the Antarctic notothenioid fishes in the cold waters of the Southern Ocean has resulted in a marked and shared intolerance to heat (Somero & DeVries 1967, Bilyk & DeVries 2011). Though most extant members of the suborder Notothenioidei inhabit the Southern Ocean, a few species are only found in the warmer waters around New Zealand and southern South America. Divided into two distinct groups, these include both the members of the three basal notothenioid families (Bovichtidae, Pseudaphritidae, Eleginopidae), which diverged prior to the origin of the cold adapted Antarctic notothenioid clade, and at least 16 species nested within the Antarctic notothenioids but now permanently found outside of Antarctic waters (Eastman 2005). Unlike the basal species, the ancestors of this latter group of secondarily temperate species presumably once shared the reduced heat tolerance noted among the endemic Antarctic species.

The shallow coastal waters of southern New Zealand are home to one such secondarily temperate species, the black cod (*Notothenia angustata* Hutton). Despite a residence time in cold temperate waters that may reach back 11 m.y.a., *N. angustata* continues to exhibit a number of cold adaptations characteristic of the endemic Antarctic species. Although they inhabit continually ice-free waters, this species retains some functional genes for antifreeze

glycoprotein (AFGP) not found among the basal notothenioid families (Cheng *et al.* 2003). At their environmental water temperatures, they also show intermediate levels of both cellular membrane lipid saturation (Logue *et al.* 2000) and ubiquitin conjugated proteins (Todgham *et al.* 2007) between cold temperate and endemic Antarctic species. However, whether whole organism traits, like heat tolerance, show a similar pattern remains unknown.

Heat tolerance in fishes has typically been determined using two methodologies, either the measurement of their upper incipient lethal temperature (UILT), or their critical thermal maximum (CTMax) (Kilgour & McCauley 1986). The UILT, based on lethal dosage methodologies, measures tolerance as the temperature at which median mortality first becomes independent of the length of exposure, typically determined from exposure times ranging from a day to a week (Fry 1947, Cossins & Bowler 1987). The first study of heat tolerance in the Antarctic notothenioids used this methodology and found UILTs between 5 and 7°C in three species of high latitude nototheniids acclimatized to their natural freezing water temperatures (-1.9°C), which were the lowest UILTs then recorded (Somero & DeVries 1967).

The CTMax is an increasingly used alternative, which determines tolerance as the temperature at which a specimen becomes incapacitated from warming at a constant rate (Hutchison 1961, Paladino *et al.* 1980). As a measure of acute

tolerance, CTMaxs always exceed the UILT for equivalently acclimated specimens (Kilgour & McCauley 1986). Determinations of CTMax also differ according to experimental parameters, most notably warming rate and starting temperature (Becker & Genoway 1979, Terblanche *et al.* 2007). However, when care is used to ensure identical experimental conditions, CTMaxs are highly repeatable and useful for making comparisons within and between species. Additionally, this latter approach benefits from greater economy of specimens, time, and space when compared with the UILT, while producing results that still appear correlated to more chronic measures of heat tolerance. When the CTMaxs of 11 species of Antarctic fishes were recently determined from specimens acclimatized to their natural freezing water temperatures, these ranged from 11.95–16.17°C that, like measures of UILT, were well below CTMaxs reported from cold temperate and temperate fishes (Bilyk & DeVries 2011).

Despite the polar ancestry of *N. angustata*, this species largely shares its present-day geographic and bathymetric distributions with a basal notothenioid, the New Zealand thornfish (*Bovichtus variegatus* (Richardson)). In their modern thermal range these species inhabit water temperatures continually above those experienced by the endemic Antarctic notothenioids. While the endemic Antarctic species experience relatively little thermal variation, in Otago Harbour where the specimens used were collected, surface water temperatures have varied between monthly average highs of 14.2–18.2°C and lows of 5.6–8.6°C (Bev Dickson, personal communication 2010). The goal of this project was to compare CTMaxs between similarly acclimated specimens of the secondarily temperate *N. angustata*, the endemic cold temperate *B. variegatus*, and the endemic Antarctic *N. coriiceps* (Richardson). This was done to determine whether the heat tolerance of the secondarily temperate Antarctic notothenioid had increased to the extent that it matched that of the temperate *B. variegatus* which lack their Antarctic ancestry.

Materials and methods

Collection of specimens

Specimens of *N. angustata* were purchased in June of 2010 from the Portobello Marine Laboratory located on the Otago Peninsula. These had been collected with baited crayfish traps placed on the rocky bottom in shallow waters (10 m) around the Otago Heads. After collection, they were held in a large outdoor aquarium (10 x 10 m, 2 m deep) with a constant flow of local seawater. Specimens of *B. variegatus* were collected by hand net and by hook and line off the rocky shore and from tide pools along the Otago Peninsula and in the vicinity of the mouth of Bull Creek during June and July 2010. Prior to experimentation both species were moved into 390 L shallow indoor aquaria for one to two weeks where they received a constant flow of local seawater ranging in temperature from 6.7–9.0°C and averaging 7.9°C, mirroring the conditions of their winter environment. While in captivity after specimens of *B. variegatus* were isolated in covered plastic mesh cages to prevent escape and predation. Specimens of both species were held and treated in accordance with protocol 39/09 administered through the University of Otago Animal Ethics Committee.

Specimens of the Antarctic nototheniid *N. coriiceps* were collected during July and August 2008 by traps deployed from the RV *L.M. Gould* at sites along the western Antarctic Peninsula (WAP) and by hook and line from the shore of Anvers Island (64°S) near Palmer Station. Those collected aboard the ship were transported to Palmer Station in aquaria with a constant flow of ice laden surface waters but kept above -1.5°C through the use of a 300 W aquarium heater. At Palmer Station all specimens were allowed several days to recover from collection stress before the start of warm acclimation.

At both Portobello and Palmer Station specimens were exposed to their regular light cycles through windows in the aquaria facilities. Prior to experimentation all specimens of *N. coriiceps* and *B. variegatus* were weighed to the nearest tenth of a gram, and *N. angustata* to the nearest 10 g. To track individual specimens of *N. coriiceps* and *N. angustata* Floy tags (Floy Tag & Mfg. Inc) were inserted into the muscle just behind the first ray of the second dorsal fin. Due to their small size *B. variegatus* could not be tagged and instead individuals were kept separated by tank partitions.

Determination of environmental CTMax

Following a seven to ten day period of adjustment to captivity, environmental CTMaxs were determined for specimens of both of the New Zealand notothenioids. The environmental CTMax of a specimen is a measure of the heat tolerance present while acclimatized to their natural environmental temperatures, which were only determined for winter acclimatized specimens of *N. angustata* and *B. variegatus*.

CTMaxs were determined according to the protocol of Bilyk & DeVries (2011) adapted from Paladino *et al.* (1980). To summarize, specimens were transferred to test aquaria at their holding tank temperature then after a ten minute adjustment period they were warmed at 0.3°C min^{-1} until the onset of a persistent loss of equilibrium. The temperature of this endpoint was taken as the specimen's CTMax and species' CTMaxs were calculated as the arithmetic mean across all specimens at each acclimation temperature.

Following a specimen's persistent loss of equilibrium they were immediately returned to their original water temperature and their recovery monitored over 24 hours. On account of the large size of *N. angustata*, all CTMaxs were determined in a 60 L aquarium which afforded even

the largest specimens sufficient space for free movement. Temperature homogeneity within this aquarium was maintained through vigorous aeration and the use of a recirculation pump.

Warm acclimation of N. coriiceps to 10°C

Eight specimens of *N. coriiceps* were warm acclimated at 10°C for three weeks. As attempts to directly transfer specimens from their environmental water temperatures (-1.5 to -1.0°C) to 10°C resulted in notable mortality, these were instead warmed in steps up to their final acclimation temperature. First, specimens were held at 6°C for five days, then 8°C for two days, and finally at 10°C for three weeks after which their CTMaxs were determined as previously described. Water temperature was maintained using a 300 W submersible heater and temperature controller activated solenoid valve which allowed the addition of cold local seawater only when their tank water temperature rose above the set point. During acclimation, their tank water was vigorously aerated to maintain oxygen saturation, specimens were fed white nototheniid muscle every three days to satiation with any excess removed, and the tank was cleaned of detritus daily with the sides brushed every three days to prevent fouling.

Warm acclimation of N. angustata and B. variegatus to 15°C

Following two to five days to recover from the determination of their environmental CTMaxs, specimens of *N. angustata* and *B. variegatus* were placed at 15°C. This water temperature was maintained using two 300 W aquarium heaters activated when water temperatures fell below their set point and a submersible pump added cold seawater from a reservoir tank when the water temperature rose above its set point. Over the next three weeks water temperatures ranged from 14.6–15.4°C cycling every 6 to 38 minutes. During acclimation, white fish muscle was provided twice per week with any uneaten food removed

immediately, and to reduce fouling, tanks were cleaned of detritus every three days. After three weeks CTMaxs were again determined as previously described.

Statistical analysis

CTMaxs of *N. angustata* and *B. variegatus* were compared using a two-way ANOVA testing for significant differences between species, acclimation temperature, and any interaction between the two. The CTMaxs of 10°C acclimated *N. coriiceps* were compared with the environmental CTMaxs of *N. angustata* and *B. variegatus* using a one-way ANOVA with species as the independent variable, followed by a post-hoc Tukey test. For both, the ANOVA assumption of homoscedasticity was confirmed using Levene's test and the residuals were tested for normality.

Results

During this study all warm acclimating specimens appeared healthy showing no visible change in colouration, behaviour, skin abrasions, or gross weight, though none of the *B. variegatus* were observed to feed. While direct transfer of *N. coriiceps* from their environmental water temperatures (-1.5 to -1.0°C) to 10°C resulted in some mortality after three or four days, neither stepped acclimation of *N. coriiceps* to 10°C, nor direct acclimation of the New Zealand species to 15°C resulted in any mortality or visible distress.

In addition to the warm acclimation of the two New Zealand species, an attempt was made to cold acclimate these fishes to 4°C which would have allowed for a direct comparison to the CTMaxs of eight Antarctic species previously reported by Bilyk & DeVries (2011). While three *B. variegatus* specimens readily tolerated 4°C, six *N. angustata* showed increasing pallor, refusal of food, and declining health ultimately leading to the abandonment of this part of the experiment. However, specimens of *N. angustata* have previously been cold acclimated to

Table I. Comparison of CTMaxs between endemic Antarctic (*N. coriiceps*), secondarily temperate (*N. angustata*), and continually cold temperate (*B. variegatus*) notothenioids. Values are reported as mean ± their standard deviation. CTMaxs of notothenioid species investigated during this study. *N. angustata* and *B. variegatus* tested at 7–8°C were winter acclimatized specimens while all other specimens were warm acclimated to their described temperatures. WAP = western Antarctic Peninsula.

Species	Collection site	n	Mass	Environmental temperature range	Acclimation temperature	CTMax
			g	°C	°C	°C
Notothenia coriiceps†	WAP	20	479.4 ± 194.6	-1.9–3	-1.9	16.17 ± 1.40
Notothenia coriiceps†	WAP	10	345.1 ± 139.9		4	17.39 ± 1.2
Notothenia coriiceps	WAP	8	404 ± 273.2		10	21.58 ± 0.67
Notothenia angustata	Otago Harbour	8	2150 ± 992.5	7–16	7–8	24.88 ± 0.79
Notothenia angustata	Otago Harbour	8	2150 ± 992.5		15	26.54 ± 0.57
Bovichtus variegatus	Otago Harbour	4	87.9 ± 42.8	7–16	7–8	26.80 ± 0.37
Bovichtus variegatus	Otago Harbour	4	87.9 ± 42.8		15	28.45 ± 0.28

†CTMaxs for environmental acclimatized and 4°C acclimated *N. coriiceps* are reprinted from Bilyk & DeVries (2011).
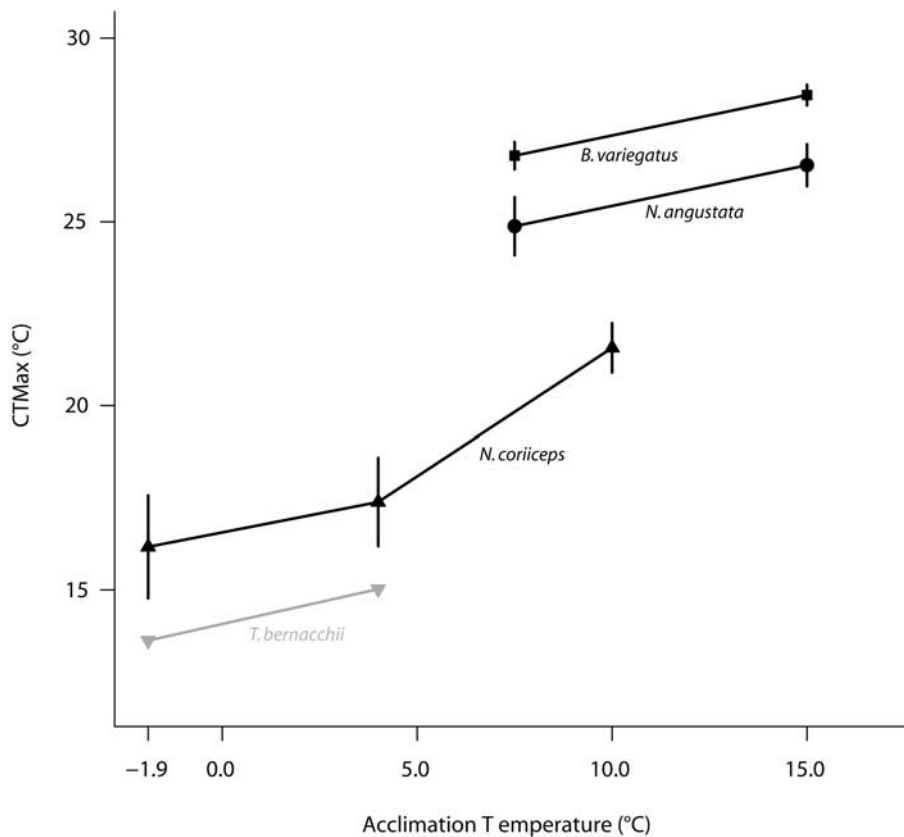
Fig. 1. Comparison of CTMaxs against acclimation temperature in four species of notothenioid fishes. All points represent the mean value with vertical bars denoting their standard deviation. Values for *Trematomus bernacchii* and *Notothenia coriiceps* at -1.9 and 4°C were taken from Bilyk & DeVries (2011).

2°C without issue over several weeks by DeVries (unpublished), and it is unclear whether the observed decline during this study was due to sub-optimal holding conditions or from stepping them down to 4°C too rapidly.

Environmental and warm acclimated CTMaxs for all three species are reported in both Table I and Fig. 1. Though some caution is warranted in comparing CTMaxs between acclimatized and acclimated specimens, the one-way ANOVA found significant variation between the environmental CTMaxs of *N. angustata*, *B. variegatus*, and 10°C acclimated *N. coriiceps* ($F_{2,17} = 90.38$, $P < 0.0001$, $r^2 = 0.91$), with the post-hoc Tukey test noting the lowest CTMaxs in *N. coriiceps*. The two-way ANOVA on *N. angustata* and *B. variegatus* found a significant effect of both species ($F_{1,20} = 53.53$, $P < 0.0001$) and acclimation temperature ($F_{1,20} = 40.15$, $P < 0.0001$) on CTMax, though no significant interaction between the two ($F_{1,20} = 0.0004$, $P = 0.9718$). The absence of an interaction between species and acclimation temperature indicates a similar increase in CTMax in both species following warm acclimation despite the lower CTMaxs of *N. angustata*.

With the exception of a single specimen out of eight *B. variegatus*, there were no fatalities following the determination of CTMaxs and all of the New Zealand specimens readily recovered within several hours of their CTMax. Unlike the New Zealand species, 10°C acclimated *N. coriiceps* were directly returned to their environmental

water temperatures after their CTMax and 50% failed to recover. This reduction in survivorship suggests that their gain in heat tolerance from warm acclimation came at a notable expense in cold tolerance raising it above the freezing point of seawater.

Discussion

A species' heat tolerance often reflects its ancestral thermal environment with tolerance apparently driven by selection from the highest temperatures experienced over their evolutionary history (Mongold *et al.* 1996, Stillman & Somero 2000). The removal of positive selective pressure for heat tolerance during the evolution of the Antarctic notothenioids in the cold stable waters of the Southern Ocean has been suggested as a cause for these species' modern low heat tolerance (Somero *et al.* 1998). While the ancestors of the secondarily temperate Antarctic notothenioids once presumably shared the low heat tolerance noted among modern endemic Antarctic notothenioids, their presence in cold temperate waters shows that they now have greater tolerance than species that have continually inhabited Antarctic waters. However, it has been unclear whether the upper thermal tolerances of these species are similar to fishes of non-Antarctic origin that share their current thermal habitat.

Comparing heat tolerances between the secondarily temperate N. angustata *and the basal notothenioid* B. variegatus

Although they inhabit the same cold temperate waters, the CTMax of *N. angustata* was significantly less than that of *B. variegatus*, both when compared between winter acclimatized specimens and between specimens warm acclimated to 15°C (Table I, Fig. 1). However, it is probable that the difference in heat tolerance between these species is understated when measured by CTMax as this fails to account for the effects of thermal lag on the much larger black cod.

Thermal lag is the time required for heat to be conducted from the periphery of an animal to the heat sensitive tissues in its body core. The greater thermal lag of larger animals means that it takes longer for heat to reach these sensitive sites and as a result they can resist warming longer simply by virtue of their size (Fry 1971). For assays such as the CTMax that rely on continuous warming, the greater thermal lag of larger species therefore acts to inflate their measured tolerance relative to smaller species. As the tolerance of heat sensitive tissues is often of greater interest than the time needed for heat to reach them it is important to account for differences in thermal lag when comparing species that vary greatly in body size.

When thermal lag was directly compared between three species of Antarctic nototheniids warmed at 0.3°C min^{-1}, core temperatures in *N. coriiceps* (791 ± 414 g) were 0.79°C cooler than *Pagothenia borchgrevinki* (Boulanger) (112 ± 39 g), and 1.11°C cooler than *Trematomus bernacchii* Boulanger (114 ± 42 g) (Bilyk & DeVries 2011). The difference in mass between the *N. angustata* and *B. variegatus* in this experiment was greater yet, with *N. angustata* specimens averaging 24 times the mass of *B. variegatus* (Table I) indicating that our determinations of CTMax are overstating the already low heat tolerance of *N. angustata* relative to *B. variegatus*.

While sharing a modern thermal environment with the endemic temperate *B. variegatus*, the lower CTMax of *N. angustata* occur along with the persistence of several biochemical and cellular cold adaptations found in endemic Antarctic species. As previously noted, at its environmental water temperatures this species shows incomplete homeoviscous adaptation of cell membranes (Logue *et al.* 2000) and intermediate lability of their cellular protein pool values which fall between cold temperate New Zealand and endemic Antarctic fishes (Todgham *et al.* 2007). It also retains circulating AFGPs found in most endemic Antarctic notothenioids, though at vestigial levels insufficient to provide protection from freezing conditions (Cheng *et al.* 2003). Unlike the endemic Antarctic species, *N. angustata* shows a modest inducible cellular heat shock response which is believed to have been lost in the endemic Antarctic species (Hofmann *et al.* 2000, Clark *et al.* 2008) but less than that of *B. variegatus* (Hofmann *et al.* 2005).

Despite the lower CTMax of *N. angustata* compared to *B. variegatus*, both species showed an equivalent capacity to increase their CTMax in response to warm acclimation (Fig. 1). Recent investigations of endemic Antarctic species have shown notable organismal and systems level capacity for warm acclimation even with their comparably low heat tolerance (Podrabsky & Somero 2006, Franklin *et al.* 2007, Robinson & Davison 2008, Bilyk & DeVries 2011). As such, it is not surprising *N. angustata* appears to show a similar capacity to increase heat tolerance through warm acclimation. However, the plasticity of heat tolerance in *N. angustata* and *B. variegatus* was only tested over a small temperature range and it remains unknown whether these species share an equivalent response across their full acclimatory ranges.

Comparing the heat tolerance of the secondarily temperate Antarctic notothenioid N. angustata *and the endemic Antarctic species* N. coriiceps

Though *N. angustata* is found in the same cold temperate waters as *B. variegatus*, it is more closely related to endemic Antarctic species including its congener *N. coriiceps*. The latter is restricted to Antarctic waters below 54°S (Gon & Heemstra 1990) which remain continually below 4°C throughout the year (Barnes *et al.* 2006). When the environmental CTMaxs of winter acclimatized (7.9°C) *N. angustata* were compared to the CTMaxs of 10°C acclimated *N. coriiceps*, those of the *N. angustata* were significantly higher despite the slightly warmer acclimation temperature of *N. coriiceps*.

Averaging 3.3°C, this difference is larger than that observed between *N. angustata* and *B. variegatus* though it is probably partly overstated due to greater thermal lag in the larger *N. angustata* (Table I). However, unlike the comparison between *N. angustata* and *B. variegatus*, that fraction of the CTMax difference due to thermal lag is less because of the smaller difference in body size between *N. angustata* and *N. coriiceps*. A CTMax intermediate to cold temperate and polar fishes is consistent with the reduced presence of some polar characteristics in *N. angustata*, and with recent work showing an increase in standard and total metabolic rates when compared to *N. coriiceps* (Campbell *et al.* 2007). The intermediate heat tolerance of *N. angustata* between *B. variegatus* and *N. coriiceps* remains despite an apparent lengthy period under selection for higher heat tolerance from the warmer water temperatures shared by the endemic cold temperate *B. variegatus*.

Although the method of dating the origin of the secondarily temperate Antarctic notothenioids remains in question, the divergence of *N. angustata* from endemic Antarctic species probably extends to at least the early Pliocene. The prevalent hypothesis for the appearance of Antarctic notothenioids in New Zealand waters is that their ranges extended northward during glacial maxima

(Petricorena & Somero 2007). During prior maxima, the Antarctic circumpolar front which forms the boundary of the Southern Ocean has shifted northward by up to five to ten degrees of latitude in open ocean areas (Nelson & Cooke 2001). This could have brought the Antarctic Front near New Zealand allowing for the dispersal of the eggs and larva of *N. angustata*'s progenitor. Given the persistence of Southern Ocean fronts between New Zealand and Antarctica and the associated temperature gradient, once *N. angustata*'s progenitor arrived in New Zealand waters it would have experienced continually warmer water temperatures than Antarctic species (Kennett 1982, Nelson & Cooke 2001, Sikes *et al.* 2002).

The length of their isolation from Antarctic waters has been estimated at 11 m.y. by Cheng *et al.* (2003) using the divergence in the mitochondrial ND2 gene between the two New Zealand nototheniids. Though this places their origin near a glacial maximum, the reliance of this analysis on universal substitution rates may not allow it to withstand future scrutiny and other investigators have instead suggested a more recent origin during the late Miocene or early Pliocene (Eastman & McCune 2000, Petricorena & Somero 2007).

Comparing the heat tolerance of 10°C acclimated N. coriiceps to other Antarctic species

Most prior studies of increased heat tolerance from warm acclimation in the endemic Antarctic nototheniods have been limited to acclimation temperatures of 4–5°C. This has been in deference to the apparent stenothermy of high-Antarctic species, where chronic exposure to temperatures as low as 5–7°C can prove fatal in specimens acclimatized to their natural freezing water temperatures (-1.9°C) (Somero & DeVries 1967). However, comparably greater heat tolerance is present among Antarctic fishes endemic to the WAP and more northerly Scotia Arc Islands such as *N. coriiceps* which may more readily acclimate to warmer temperatures (Bilyk & DeVries 2011).

While 10°C acclimated *N. coriiceps* have lower CTMax than comparably acclimated cold temperate fishes (Otto & Ohararice 1977, Becker & Genoway 1979, Fangue & Bennett 2003), including the winter acclimatized New Zealand nototheniods, these showed a notable increase in CTMax over environmental and 4°C acclimated specimens (Table I). At 10°C they similarly exceed the CTMaxs of seven other 4°C acclimated nototheniods, predominantly from high latitude waters (Bilyk & DeVries 2011). Both their survival and continued normal activity at this temperature show a broader thermal range than anticipated even among the fishes found in the relatively more thermally variable waters along the western Antarctic Peninsula.

Summer acclimatized specimens of the nototheniid *Leptonotothen nudifrons* (L.), which is restricted to more northerly Antarctic waters, warmed at 1°C per day resulted

in the mortality of most upon reaching 8–9°C (Hardewig *et al.* 1999). Similarly, Van Dijk *et al.* (1999) noted a cellular shift to anaerobic metabolism at 9–10°C in the phylogenetically distant Antarctic zoarcid, *Pachycara brachycephalum* (Pappenheim) when also warmed at 1°C per day, which suggests the limits of their heat tolerance lies near this temperature. However, while these studies warmed specimens slowly they may not have allowed sufficient time for the physiological and cellular processes that occur during acclimation to fully track rising temperatures as some high latitude Antarctic species required up to two weeks to fully manifest their increased organismal heat tolerance during warm acclimation at 4°C (Bilyk & DeVries 2011). The failure of *N. coriiceps* to directly acclimate to 10°C in this study would also suggest that while acclimating nototheniods to cold temperate water temperatures is possible it may require very slow rates of warming or staged increases.

Despite the survival of *N. coriiceps* at 10°C, it is unclear whether this species could survive the even warmer summer water temperatures experienced by the secondarily temperate species. If it could survive such extremes of heat, the capacity for activity, growth, and reproduction are under more stringent thermal constraints than survival (Fry 1971) and the temperature limitations of these remain unknown in *N. coriiceps*.

Gains in heat tolerance among fishes from warm acclimation typically come at the expense of an even greater loss in cold tolerance (Bennett *et al.* 1998). The mortality of warm acclimated *N. coriiceps* when returned to their natural freezing water temperatures indicates that such a loss in cold tolerance occurred. That acclimation to just 10°C costs them the ability to tolerate freezing Antarctic water temperatures would argue that the endemic Antarctic species have a particularly narrow thermal range despite the presence of plasticity in their heat tolerance.

Conclusion

Evolution in chronic cold has led to reduction of heat tolerance in the Antarctic nototheniods. While the Antarctic *N. coriiceps* shows a surprising ability to warm acclimate to 10°C its heat tolerance was still less than that of equivalently acclimated cold temperate and temperate fishes. Several members of the Antarctic nototheniods are now permanently distributed outside Antarctic waters even though their Antarctic ancestors presumably once shared the reduced heat tolerance now common throughout endemic Antarctic species. Our examination of heat tolerance in *N. angustata*, one such secondarily temperate nototheniod, is consistent with an increase in heat tolerance following their arrival in New Zealand waters. Heat tolerance can serve as an important physiological constraint on geographic distribution (Hochachka & Somero 2002) and the capacity of the Antarctic nototheniods

to adapt to rising water temperatures will probably be important for their future survival in the Southern Ocean in light of predicted future impacts of global climate change on the Antarctic ecosystem (Clarke *et al.* 2007).

Acknowledgements

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