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Can acclimation of thermal tolerance, in adults and across generations, act as a buffer against climate change in tropical marine ectotherms?

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Abstract

Thermal acclimation capacity was investigated in adults of three tropical marine invertebrates, the subtidal barnacle *Striatobalanus amaryllis*, the intertidal gastropod *Volegalea cochlidium* and the intertidal barnacle *Amphibalanus amphitrite*. To test the relative importance of transgenerational acclimation, the developmental acclimation capacity of *A. amphitrite* was investigated in F_1 and F_2 generations reared at a subset of the same incubation temperatures. The increase in CT_{max} (measured through loss of key behavioural metrics) of F_0 adults across the incubation temperature range 25.4°C to 33.4°C was low: 0.00°C (*V. cochlidium*), 0.05°C (*S. amaryllis*) and 0.06°C (*A. amphitrite*) per 1°C increase in incubation temperature (the acclimation response ratio; ARR). Although the effect of generation was not significant, across the incubation temperature range of 29.4°C to 33.4°C, the increase in CT_{max} in the F_1 (0.30°C) and F_2 (0.15°C) generations of *A. amphitrite* was greater than in the F_0 (0.10°C). These correspond to ARR's of 0.03°C (F_0), 0.08°C (F_1) and 0.04°C (F_2), respectively. The variability in CT_{max} between individuals in each treatment was maintained across generations, despite the high mortality of progeny. Further research is required to investigate the potential for transgenerational acclimation to provide an extra buffer for tropical marine species facing climate warming.

Keywords: Acclimation; transgenerational acclimation; CT_{max}; tropical marine invertebrate; warming tolerance; climate change

1. Introduction

Studies of current latitudinal patterns of thermal tolerance provide evidence for how physiological capacity has evolved across physical gradients (Gaston, 2009; Rehfeldt et al., 1999). These global patterns allow species vulnerability to climate warming to be assessed (e.g., Deutsch et al., 2008; Sunday et al., 2011; Sunday et al., 2014) and improve predictions of how species and populations will persist in the face of increasing global temperatures. These broad scale patterns can then be interpreted through organism-based studies that take species' ecological and evolutionary patterns into account (Polgar et al., 2015).

Whilst geographic patterns of acute thermal tolerance have been most widely studied (Gaston et al., 2009), acclimation capacity (Deutsch et al., 2008; Sunday et al., 2011; Sunday et al., 2014), the capacity of physiological systems to adjust to a new stable state in response to an altered environment (Somero, 2012) will likely be an important moderator of these patterns (Bozinovic et al., 2011; Peck et al., 2014). This increase in functionality can be measured at the biochemical level or at the level of the whole animal, in the form of an increased temperature tolerance (Fry et al., 1942). Acclimation capacity can also be assessed through comparisons of the change in critical thermal maximum per degree increase in incubation temperature: the acclimation response ratio (ARR, Claussen, 1977). A higher ARR indicates that a species has a greater capacity to shift physiological pathways in response to incubation temperature. In response to predictable warming, acclimation can provide an important buffer, improving the chances of species persisting in altered environments (Anderson et al., 2012; Barrett and Hendry, 2012; Bell, 2013; Charmantier et al., 2008; Hendry et al., 2011; Peck et al., 2014; Somero, 2012), resulting in improved physiological fitness of their progeny (Burgess and Marshall, 2011; Shama et al., 2014)

through non-genetic inheritance, allowing more time for evolution to occur (Chevin and Lande, 2010; Somero, 2010).

Species physiological capacities will likely vary between life history stages and the mechanism of acclimation may vary. This study focused on comparisons of the relative importance of phenotypic plasticity among adults and developmental plasticity during ontogeny (Salinas et al., 2013)), as measured through changes in CT_{max}. Acclimation of adults is expected to be reversible whilst developmental plasticity is more likely to be fixed during ontogeny (Donelson et al., 2012; Munday et al., 2013). The effects of acclimation can be restricted to the current generation, but when they affect the reproductive development of the adults they can also affect their offspring's phenotype. This interactive effect on both adult reproduction and offspring development has been termed transgenerational plasticity (Salinas et al. 2013). To understand organism response to gradual environmental warming it is important to determine the relative contribution of adult and subsequent transgenerational acclimation capacity to species, or population, acclimation capacity (Donelson et al., 2012).

Strong selection, due to the introduction of species into new environments (Hendry et al., 2000; Huey et al., 2000), manipulation of laboratory environments (Bennett et al., 1990), or climate change (Skelly et al., 2007), can lead to rapid evolution, even in relatively long lived species (Hendry et al., 2011; Sanford and Kelly, 2011; Shaw and Etterson, 2012; Thompson, 1998). While acclimation capacity in tropical marine ectotherms may be reduced compared to mid latitude species (Peck et al., 2014) and can therefore be considered less resilient to climate change, many tropical species have shorter generation times, thus possessing a greater potential for adaptive change than higher latitude species (Peck, 2011). The current study sets out to further investigate the resilience of tropical marine species by testing the relative importance of acclimation in adults of three species versus transgenerational shifts in thermal tolerance in one of these species. The ARR of adults of

three tropical marine ectotherm species from Singapore, the barnacles *Amphibalanus amphitrite* (Darwin 1854) and *Striatobalanus amaryllis*, (Darwin 1854) and the gastropod mollusc *Volegalea cochlidium*, (Linnaeus 1758), was tested at incubation temperatures between 25.4 and 35.4°C. These species were chosen as they occur at different tide heights: two are intertidal, high shore *A. amphitrite* and mid shore *V. cochlidium*, and the subtidal *S. amaryllis*. As they live at different shore heights, and are expected to experience different microhabitats, they are likely to have different thermal limits (Stillman and Somero, 2000). To test the relative importance of developmental acclimation capacity, transgenerational shifts in CT_{max} were tested through two generations of *Amphibalanus amphitrite*, reared under the same temperature regimes.

2. Materials and methods

Three species in this study were collected from different marine locations in Singapore to maximise the chances of differences in thermal response. The high intertidal barnacles (*Amphibalanus amphitrite*) were found on rocks or mangrove roots and branches in Kranji (1.43°87'N, 103.75°46'E). The gastropod mollusc (*Volegalea cochlidium*) was collected from Pasir Ris beach (1.38°34'N, 103.94°84'E). The subtidal barnacle (*Striatobalanus amaryllis*) was collected from the submerged undersides and anchor chains of navigation buoys in the Strait of Singapore. The barnacle *A. amphitrite* was cultured and spawned, following the methods of Rittschof (Rittschof et al., 1992; Rittschof et al., 2003).

2.1 Acclimation of adults

Around 100 adults of each species were collected from the field and held for 24 hours in water at their naturally experienced salinity: *A. amphitrite* at 20 psu (lim, 1984); *S. amaryllis*

and *V. cochlidium* at 30 psu (Gin et al., 2006). *A. amphitrite* and *S. amaryllis* were acclimated for three months, at the above salinities, at five different temperatures (25.4°C, 27.4°C, 29.4°C, 31.4°C and 33.4°C), which bracketed and extended the naturally experienced seawater temperature range (27 to 31°C; Chou and Lee, 1997) whilst, due to logistical constraints, *V. cochlidium* was only incubated at the warmest three temperatures. To assess acclimation of CT_{max}, constant incubation temperatures (±0.1°C) were maintained by conducting experiments in a constant temperature controlled room with heating provided by thermostatically controlled heaters (Aquamedic) and cooling provided by a grant GP200/R4 thermocirculator (Grant Instruments, Shepreth, UK). During acclimation, all the animals were fed daily: *A. amphitrite* and *S. amaryllis* with *Artemia* sp. nauplii and a mixed algal culture (containing *Tetraselmis suecica* (Kylin) Butcher 1959 and *Cheatoceros muelleri* Lemmemann, 1898), and *V. cochlidium* with barnacles (*Amphibalanus amphitrite*) and commercially reared bivalves (*Meretrix meretrix* (L., 1758) and *Gari togata* (Deshayes, 1855)) (after Tan and Phuah, 1999). Before feeding, seawater was changed every day obtained from a flow-through aquarium at the incubation temperature and salinity adjusted accordingly using distilled water. Mortality was recorded every day. After three months, acclimated animals were subjected to thermal tolerance tests, that started at the incubation temperature, using a Grant GP200/R4 thermocirculator to increase water temperature at a rate of 1.0 ± 0.1°C h^{-1} (following Nguyen et al., 2011). CT_{max} of the two barnacle species was recorded when an individual was unable to close their opercula plates after a standard force was applied with a pair of fine tweezers. As the barnacles were attached to hard substrata it was not possible to return only those individuals at CT_{max} to ambient temperatures to check for survival. It is therefore possible that some of the barnacles had also reached their lethal temperatures. *V. cochlidium* was tested in a similar fashion to the barnacles with CT_{max} determined as the temperature at which they could not retract their operculum into the shell. All *V. cochlidium* recovered when returned to ambient water temperature.

2.2 Generational shifts in thermal tolerance

100 individuals of *Amphibalanus amphitrite* were acclimated at all five temperatures (25.4°C, 27.4°C, 29.4°C, 31.4°C and 33.4°C: acclimation temperatures). The incubation of adults, larval release and subsequent rearing of the next generation, were all conducted under the same incubation temperature and salinity, with conditions controlled as previously described. Adults were dried overnight in air at ambient temperature to stimulate spawning (following Rittschof et al., 1992; Rittschof et al., 2003) and when they were re-submerged in sea water under strong light, nauplii were released and swam towards the light. Nauplii were collected into glass beakers containing 400 ml of aerated seawater at the appropriate salinity and temperature for up to 4 hours or until a concentration of 2-3 larvae/ml was achieved. The nauplii were fed twice a day with 5 x 10^5 cells ml^{-1} of the mixed algal culture (following Rittschof et al., 2003). They were examined under the microscope daily until they metamorphosed into cyprids within 3-5 days. When larvae reached the cyprid stage, 50 were collected into petri dishes and left undisturbed to settle at the experimental acclimation temperature of their parents in systems heated and cooled by heaters and thermocirculators as detailed previously. Settlement was stopped and water was changed after 48 hours. The number of successfully settled barnacles was recorded and the unsettled cyprids were removed and counted. After settlement was finished, petri dishes containing F_1 barnacles were transferred into separate 500 ml glass beakers, maintained at the same incubation temperature as the adults. They were fed daily with the same density of algae, at the acclimation temperature, and monitored until they reached adult stage and were ready for spawning. Water was changed every day and *Artemia* sp. was added to their diet after two weeks. In an additional trial, the size of F_1 nauplii, recently settled cyprids, and 1 month old cyprids was measured using a calibrated microscope eyepiece graticule. F_1 adults were then

spawned to produce the F_2 generation. Once the F_1 and F_2 generation reached adulthood (circa 1 month), spawning was attempted weekly, using the protocol detailed above. After another month a batch of 20 sexually mature F_1 and F_2 adults were subjected to acute heating using the previously described protocol.

2.3 Statistical analysis

Statistical analysis was conducted using Minitab 17. CT_{max} values could not be normalized so data were ranked and a Scheirer-Ray-Hare extension of the Kruskal-Wallis test (Sokal and Rohlf, 1995) was used to test for two way effects of incubation temperature and species on CT_{max}. ARR, the degrees Celsius increase in CT_{max} per Celsius degree increase in incubation temperature, was also calculated from regressions fitted separately to the individual CT_{max} data for each species, following Claussen (1977).

Differences in percentage survival and success during development were compared with Bonferroni-corrected χ^2 analyses. The effect of incubation temperature on the mean size of *A. amphitrite* F_1 nauplii and cyprids at settlement and cyprids after 1 month was tested by regressing mean size against temperature. Residuals from these analyses were normally distributed with homogenous variances across treatments. For *A. amphitrite*, transgenerational acclimation effects were also tested with a Scheirer-Ray-Hare extension of the Kruskal-Wallis comparing the mean CT_{max} values of adults (n = 20) across generations and incubation temperatures. The equality of variances of CT_{max} values of the three generations within each treatment was also tested with the Levene's test.

3. RESULTS

3.1 Acclimation of adults

For all three species across all temperatures, the only adult mortality during the 3-month incubation period was 10% mortality of *S. amaryllis* at 33.4°C, which occurred within a few weeks. Although no further mortality occurred, *S. amaryllis* at 33.4°C often had closed opercula or decreased cirral activity, suggesting that, for *S. amaryllis*, 33.4°C was close to critical thermal limits. CT_{max} of *S. amaryllis* was therefore not tested at 33.4°C. In all other incubations F_0 adults remained healthy and were able to spawn at least once.

Incubation temperature did not have a significant effect on the CT_{max} of F_0 adults ($H = 3.3$, $P > 0.05$) but there was a significant difference in the slope of the response of CT_{max} to temperature between species (species by incubation temperature interaction; $H = 8.7$, $P < 0.05$). CT_{max} for all individuals of *V. cochlidium* occurred at the same temperature, 45.4°C, an intermediate temperature to *S. amaryllis* and *A. amphitrite*. The subtidal *S. amaryllis* had a lower ARR, a 0.05°C increase in CT_{max} per 1°C increase in incubation temperature, than the intertidal *A. amphitrite* (0.06°C).

3.2 Transgenerational development and plasticity

Larval survival and development of *A. amphitrite* incubated at the control temperature (29.4°C) was similar to that recorded by Rittschof (1992; 2003) (Table 1, 29.4°C). There were differences both between generations and between different temperature treatments; effects were observed on the density of the nauplii produced by adults, on the percent survival of nauplii, on the percent survival to metamorphosis from nauplii to cyprids, and on the percent survival to settlement of cyprids (Table 1). At 25.4°C and 27.4°C 100% mortality of F_1 and F_2 adults occurred, after spawning, but before CT_{max} could be measured, but there was no other post settlement mortality of F_1 or F_2 adults. The density of hatching F_1 nauplii was lower ($\chi^2 = 1000$, $P < 0.01$) at the two highest incubation temperatures (1.25 nauplii ml^{-1}

at 31.4°C and 33.4°C; Table 1). The reproductive success of adult F_1 generation was lower than F_0 adults (density of F_2 nauplii < 0.63ml^{-1}; $\chi^2 = 66.0$, $P < 0.01$).

There was no effect of incubation temperature on the mean size of F_1 nauplii (ANOVA; $F_{1,4}$=0.45, P=0.55), cyprids at settlement ($F_{1,4}$=0.62, p=0.49) or cyprids after 1 month ($F_{1,4}$=0.16, P=0.72; Table 2).

While the mean CT_{max} of adult F_1 *A. amphitrite* was consistently higher (range of means: 0.05 to 0.25°C) than that of the F_0 generation incubated at the same temperature, there was no significant effect of generation (H = 1.8, P > 0.05) or its interaction with incubation temperature (H = 2.0, P > 0.05; Fig. 2). There was, however, a significant effect of incubation temperature on CT_{max} (H = 4.5, P < 0.05). Across the 4°C range of incubation temperatures CT_{max} increased by 0.10°C in the F_0, 0.30°C in the F_1 and 0.15°C in the F_2. This resulted in an ARR of 0.10 between 29.4°C and 31.4°C for both the F_1 and F_2 generations, which was higher than the ARR of F_0 adults (0.025). Within generations, the ARR between the higher incubation temperatures, 31.4°C and 33.4°C, was reduced, and even negative, for the F_2 generation (-0.025 to 0.05). The individual variance in CT_{max}, remained constant across generations at each temperature (Levene's test of equal variances = 1.8, P = 0.09).

4. Discussion

The results of the current study confirm the limited acclimatory capacity of CT_{max} of adults of three tropical marine invertebrates, ranging from 0 to only 0.06°C for every 1°C increase in acclimation temperature. As predicted, the intertidal *A. amphitrite*, had the highest CT_{max} whilst the subtidal *S. amaryllis* had the lowest CT_{max}. The mid-shore *V. cochlidium* was intermediate to these two. Whilst intertidal organisms live in a complex environment with a mosaic of exposed and sheltered micro-habitats (Helmuth et al., 2002), they are regularly subject to a wide range of temperatures as they are alternately immersed and emersed through

each tidal cycle. The tropical intertidal environment can be extremely variable, with temperatures ranging between 25 and 50°C (Williams and Morritt, 1995) requiring animals to have high acute temperature limits. This contrasts to the very stable 27 to 31°C temperature range experienced by Singapore subtidal species throughout the year (Chou and Lee, 1997). Intertidal *A. amphitrite* also had the greatest potential to acclimate to elevated temperatures, with the steepest slope (Fig. 1). Microhabitat temperatures were not measured and so it is not known if the high shore *A. amphitrite* experiences the warmest temperatures or is at least partially shaded by the mangrove trees they live on. Previous studies have found that marine ectotherms from the warmest microhabitats have a reduced acclimation capacity compared to those from cooler microhabitats (Stillman and Somero, 2000; Vinagre et al., 2015), suggesting that *A amphitrite* may benefit from habitat shading.

Although not significant, the greatest magnitude of the shift in mean CT_{max} was in F_1 *A. amphitrite* adults (0.08°C) which was more than twice that measured in F_0 adults (0.03; Fig. 2). Despite the fact that in the current study, the F_2 generation was spawned from the survivors of the selective breeding of the F_1 generation, the variation in thermal response between individuals was retained across generations and between temperature treatments. The maintenance of this individual variation across the F_1 and F_2 generations indicates that selection did not reduce the individual variability in CT_{max} during transgenerational acclimation. However, survival of F_2 generation *A. amphitrite* was markedly reduced and the acclimation response was also reduced, inducting there were strong negative trade-offs of acclimation at warmer temperatures. For a population with an elevated temperature tolerance to persist, there need to be sufficient survivors so that the population is self-sustaining (Bell, 2013; Munday et al., 2013). Without further research it is difficult to know how the low survival measured in the laboratory would translate into demographic effects in the wild. Experimentally induced mortality could have masked selection effects across temperature

treatments, so it is hard to predict whether populations would decline beyond the point where extirpation becomes likely.

Transgenerational plasticity results from the modification of the surviving offspring phenotype in response to environmental conditions (Munday et al., 2009; Salinas et al., 2013). While in the current study, there was no effect of temperature on the size of developing *A. amphitrite*, one of the commonest responses is for ectotherms to be a smaller size at higher temperatures (Chambers, 1997; Forster et al., 2012), which has a range of effects on the next generation (Burgess and Marshall, 2011).

Whether acclimation to higher temperatures is ultimately beneficial will depend on how other aspects of fitness co-vary with thermal tolerance (Leroi et al., 1994). Whilst elevated acclimation capacity indicates that physiological pathways have been altered to improve survival in the face of warmer environmental temperatures, long term survival will depend on a combination of factors (Clark et al., 2013). Re-allocating energy and resources to biochemical pathways that result in more elevated temperature lethal limits may have associated costs that reduce the energy available for other key ecological functions, such as growth and reproduction (Donelson et al., 2011). Improved performance at elevated temperatures may also cause a reduction in performance at lower temperatures, which may reduce overall fitness in seasonal environments or colder years (see e.g. Glanville and Seebacher, 2006).

Acclimation capacity is just one of the mechanisms by which organisms can respond to change. Maintaining adults at elevated temperature during gonad development and maturation can affect subsequent generations, regardless of future temperatures, through epigenetic effects, which can speed up the rate at which genetic evolution can occur (Klironomos et al., 2013). Without being able to follow single parent crosses it is not possible to assess the heritability of thermal acclimation from the current study. Transgenerational plasticity is, however, increasingly being recognized as a mechanism providing mitigation for

some of the negative impacts of climate change (Donelson and Munday, 2015; Zizzari and Ellers, 2014) including increasing CT_{max} across generations (Ho and Burggren, 2012).

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Figure Legends

Figure 1. CT_{max} under 1°C $hour^{-1}$ rate of warming for three tropical marine ectotherms, after incubation for three months at different temperatures: the high intertidal barnacle *Amphibalanus amphitrite* (closed circles) from 25.4°C to 33.4°C, the low intertidal gastropod *Volegalea cochlidium* (open circles) from 29.4°C to 33.4°C and the subtidal barnacle *Striatobalanus amaryllis* (triangles) from 25.4°C to 31.4°C. Each data point represents the mean CT_{max} and S.D. (n = 20) of each species at each incubation temperature. *A. amphitrite*, $CT_{max} = 0.058T + 44.3$, $R^2 = 0.96$, $p<0.01$; *S. amaryllis*, $CT_{max} = 0.045T+40.0$, $R^2=0.98$, $p<0.05$; where T = incubation temperature. CT_{max} of *V. cochlidium* had no acclimation response.

Figure 2. Relative CT_{max} under 1°C $hour^{-1}$ change for three groups of *Amphibalanus amphitrite* incubated at 29.4°C, 31.4°C and 33.4°C. Relative CT_{max} are °C above the lowest recorded CT_{max}. F_0 adult animals collected from the field (filled circles) were kept at the required temperature and spawned after one month. F_1 (open circles) and F_2 (filled diamond) adults were generations grown in the laboratory at three constant incubation temperatures. Each data point represents the mean CTmax ± S.D. (n=20) of each generation at each incubation temperature. Incubation temperatures for F1 and F2 are offset by +0.2 and +0.4°C, respectively, for clarity.

Table 1. The density of *Amphibalanus amphitrite* nauplii at hatching (F_1 and F_2) and the % of these that survived, metamorphosed and settled at the different incubation temperatures.

			Temperature, °C				
			25.4	27.4	29.4	31.4	33.4
F_0	Adults	Survival	100%	100%	100%	100%	90%
F_1	Nauplii	Number per ml‡	2.5	2.5	2.5	1.25	1.25
		Survival	90%	90%	70%	70%	75%
	Cyprid	Metamorphosis	45%	45%	56%	63%	64%
		Settlement	36%	36%	45%	50%	57%
F_2	Nauplii	Number per ml‡	0.63	0.42	0.42	0.42	0.42
		Survival	50%	50%	50%	20%	30%
	Cyprid	Metamorphosis	15%	15%	15%	15%	15%
		Settlement	5%	6%	6%	6%	6%

‡ Nauplii density obtained from spawning, collected in 400ml of aerated seawater at incubation temperature and salinity

Table 2. The size of F_1 nauplii and cyprids of *Amphibalanus amphitrite*, at settlement and after 1 month, in response to incubation at five incubation temperatures. N= 8 except nauplii at 29.4°C when N=1.

		Incubation temperature, °C				
F_1	Size mm	25.4	27.4	29.4	31.4	33.4
Nauplii	Mean	64.7	58.8	53.3	59.6	59.7
	SD	19.8	23.0	-	28.6	17.1
Cyprids at settlement	Mean	162.6	176.9	151.5	159.9	158.9
	SD	37.8	24.1	28.0	50.4	39.5
Cyprids after 1 month	Mean	5.7	6.1	5.3	5.7	5.7
	SD	0.8	1.1	0.5	1.0	0.9

Fig. 1

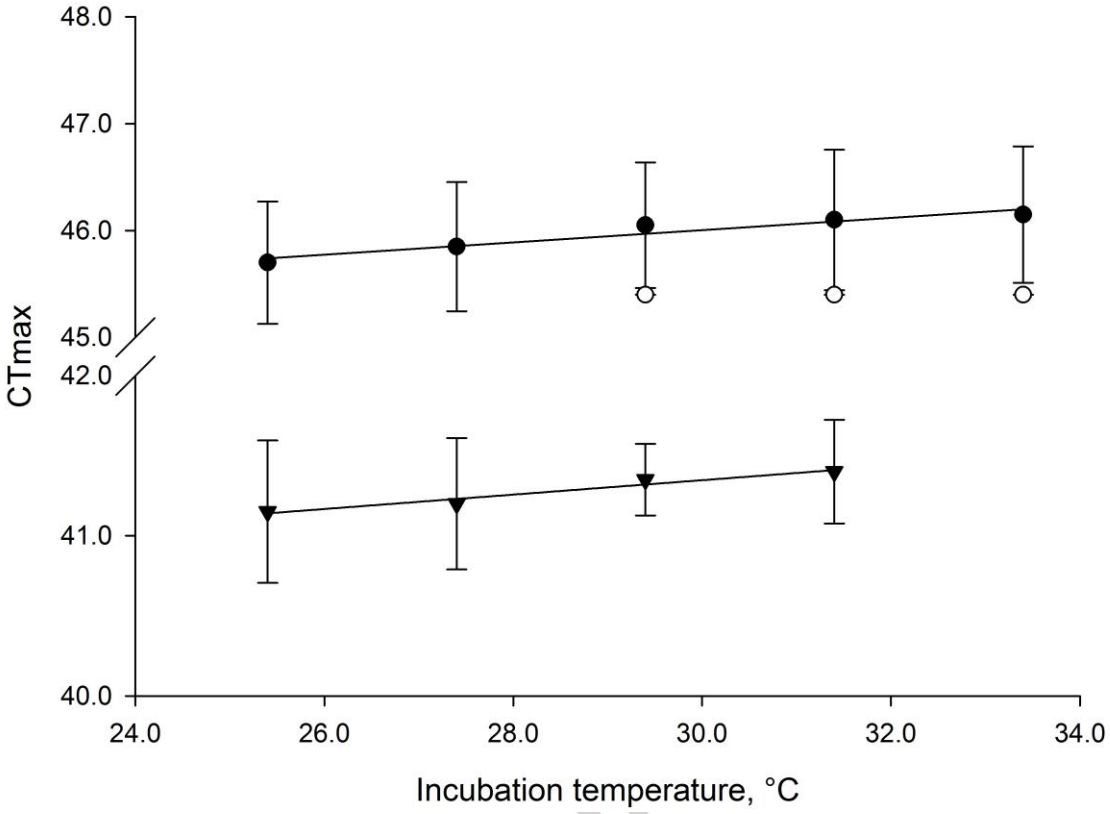
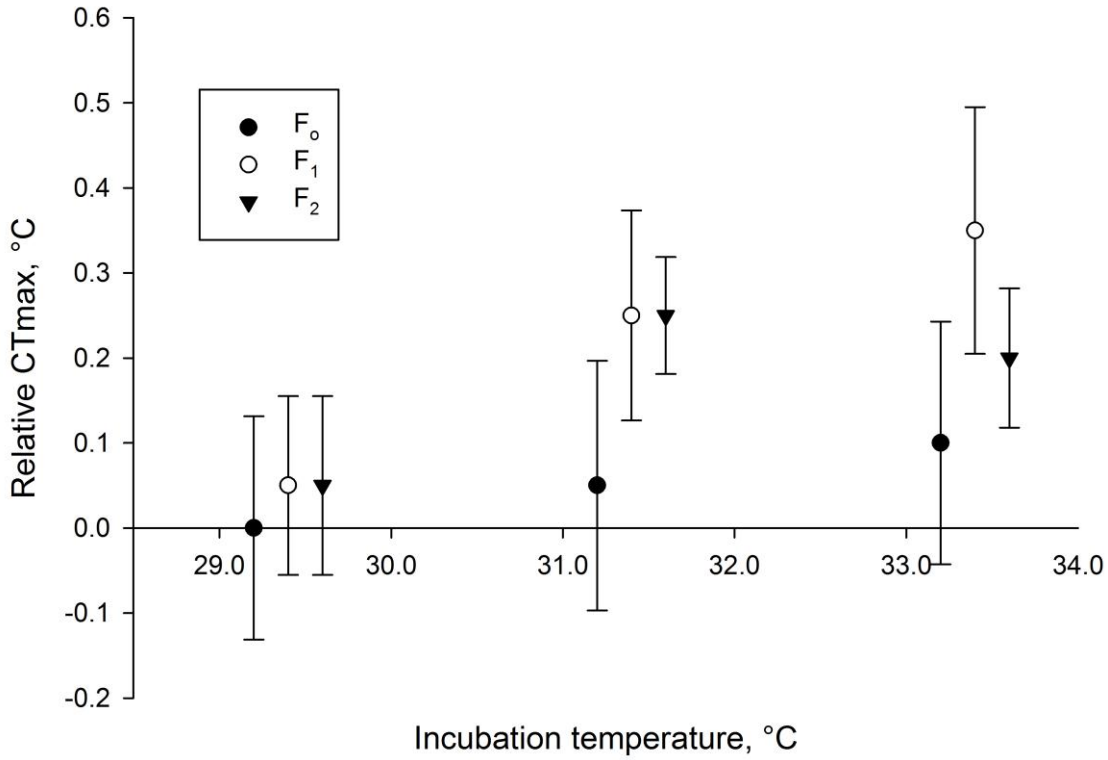


Fig 2.



Highlights

- Adult acclimation of CT_{max} was low; 0 to 0.06°C per 1°C increase in temperature
- Transgenerational acclimation was up to 5 times greater than adult acclimation
- Transgenerational acclimation may buffer climate change in tropical ectotherms