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NOTE

Ghosts of thermal past: reef fish exposed to historic high temperatures have heightened stress response to further stressors

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Abstract Individual exposure to stressors can induce changes in physiological stress responses through modulation of the hypothalamic–pituitary–interrenal (HPI) axis. Despite theoretical predictions, little is known about how individuals will respond to unpredictable short-lived stressors, such as thermal events. We examine the primary neuroendocrine response of coral reef fish populations from the Îles Eparses rarely exposed to anthropogenic stress, but that experienced different thermal histories. Skunk anemonefish, *Amphiprion akallopisos*, showed different cortisol responses to a generic stressor between islands, but not along a latitudinal gradient. Those populations previously exposed to higher maximum temperatures showed greater responses of their HPI axis. Archive data reveal thermal stressor events occur every 1.92–6 yr, suggesting that modifications to the HPI axis could be adaptive. Our results highlight the potential for adaptation of the HPI axis in coral reef fish in response to a climate-induced thermal stressor.

Keywords Climate change · Phenotypic plasticity · HPI axis · Thermal stressor · Cortisol · Acclimation

Introduction

The earth's climate is currently warming more rapidly than over the last 1,000 yr, local climate fluctuations are more extreme, and natural populations are affected (e.g., Walther et al. 2002). Organisms may cope by shifting latitudinal distributions, adapting to new conditions, or adjusting via

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plastic changes in their behavior, physiology, and/or morphology (e.g., West-Eberhard 1989; Parmesan 2006; Angilletta 2009). An organism's sensitivity will affect its ability to acclimate (physiological/morphological/biochemical nongenetic modifications enabling a population to cope with environmental stressors) to the climate change. Tropical populations have a narrow thermal tolerance range (Tewksbury et al. 2008), and equatorial fishes living close to their thermal optima (Rummer et al. 2014) have low aerobic scope at high temperatures (Gardiner et al. 2010); as such, coral reef fishes may show a low capacity for thermal acclimation. Nonetheless, some reef fish populations demonstrate developmental (Donelson et al. 2011; Grenchik et al. 2013) and trans-generational acclimation (Donelson et al. 2012) in their response to thermal stressors.

The teleost thermal stress response follows a stereotypic three-component response (Barton and Iwama 1991; Sumpter 1991; Barton 2002). There is an immediate release of catecholamines and stimulation of the hypothalamic–pituitary–interrenal (HPI) axis, culminating in glucocor-ticoid (GC) hormones, such as cortisol, released into blood circulation (Mazeaud et al. 1977). This primary response triggers secondary responses that adjust physiological mechanisms, which may cascade into changes in whole-organism performance (tertiary responses). Recent work on thermal plasticity in reef fishes has concentrated on secondary and tertiary responses such as metabolic rate, aerobic scope, and reproductive endocrinology (Gardiner et al. 2010; Donelson et al. 2011; Pankhurst and Munday 2011). While phenotypic plasticity in the primary neuroendocrine response is well documented in response to ecological stressors (Angelier and Wingfield 2013), much less is known about such plasticity in response to thermal stressors (Wingfield et al. 2011). Early life events permanently affect HPA (adrenal) axis development and GC stress responses later in life (e.g., Sheriff et al. 2010), and reversible phenotypic modifications may habituate (Romero and Wikelski 2002) or sensitize (e.g., Mullner et al. 2004) the GC response throughout life. We aim to determine whether coral reef fish show plasticity in the HPI axis in response to historic thermal events.
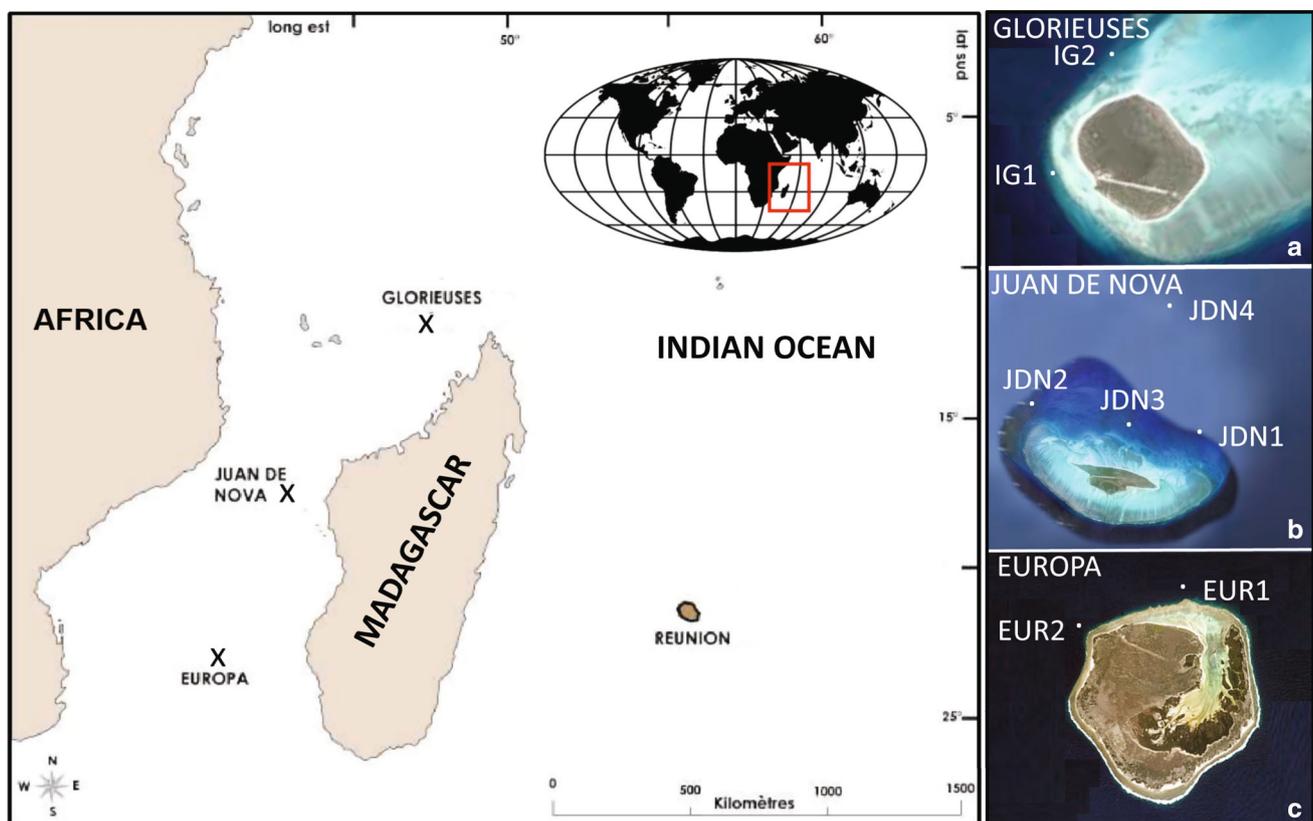
Fig. 1 Three Îles Eparses within the mozambique channel, Indian Ocean, surveyed and sampled indicated with an "x." a Glorieuses: IG1 = S11°34.880′, E47°16.862′ ($n = 6$), and IG2 = S11°33.678′, E47°17.480′ ($n = 3$); b Juan de Nova: JDN1 = S17°02.083′, E42°46.251′ ($n = 6$), JDN2 = S17°01.152′, E42°40.779′ ($n = 2$), JDN3 = S17°01.968′, E42°44.148′ ($n = 4$), and JDN4 = S16°57.163′, E42°45.534′ ($n = 6$); c Europa: EUR1 = S22°19.766′, E40°21.905′ ($n = 5$) and EUR2 = S22°20.723′, E40°19.662′ ($n = 2$). Satellite photographs taken from Google Earth
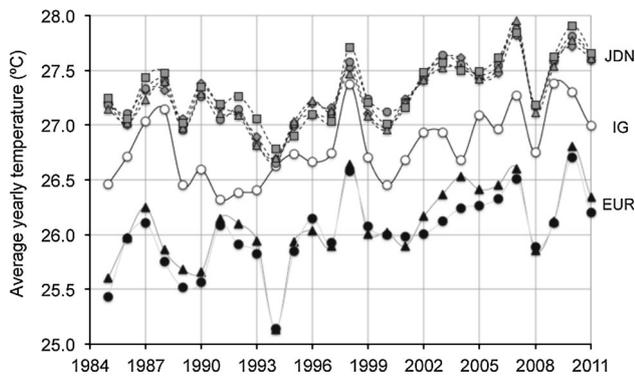
Fig. 2 Average yearly sea surface temperatures for the Îles Eparses from 1985 to 2011. The sites are indicated in: *black* for Europa (EUR1 = *triangles*, EUR2 = *circles*), *white* for Glorieuses, and *gray* for Juan de Nova (JDN1 = *diamonds*, JDN2 = *circles*, JDN3 = *triangles*, JDN4 = *squares*)

We compare the primary stress-induced response of skunk anemonefish, *Amphriprion akallopisos*, to a generic stressor (acute handling) between populations along an 11° latitudinal gradient in the Îles Eparses (Fig. 1) that experienced different temperature histories (Fig. 2). The Îles Eparses are uninhabited apart from small military bases, have minimal direct anthropogenic impacts, and are geographically isolated from neighboring human populations (Naim and Quod 1999). Due to the different thermal histories, we hypothesize that stress responses should (1) differ between islands and (2) be greater in populations exposed to greater historic thermal stressors if the HPI axis shows plasticity.

Materials and methods

We sampled 34 adult skunk anemonefish, *Amphriprion akallopisos*, at eight sites across three Îles Eparses in April 2011 (Fig. 1). We took caudal venous puncture blood samples 2 h after a generic stressor (capture and bucket confinement) to measure stress-induced cortisol levels (Pankhurst and Sharples 1992; Frisch and Anderson 2000; Grutter and Pankhurst 2000; Pankhurst 2001). We measured plasma cortisol using a Cortisol EIA Kit (Mills et al. 2010) validated for this species (Electronic Supplementary Materials, ESM, Appendix 1).

We obtained weekly, remotely sensed sea surface temperature (SST) time series from the NOAA Pathfinder v5.2 dataset (resolution 4 km²; Casey et al. 2010) for all sites between 1985 and 2011. Yearly temperature was lowest at Europa (Fig. 2) and showed larger variation over time (26.0 ± 0.07 °C; mean ± SE) compared to Juan de Nova (27.3 ± 0.05 °C) and Îles Glorieuses (26.8 ± 0.06 °C). We defined the summer season as the 3-month period inclusive of the month with the highest average

temperature and each month either side (February–April excepting EUR2, which is January–March). Total length of anemonefish males ranged from 48 to 91 mm ($n = 16$) and females from 62 to 112 mm ($n = 17$), and we conservatively estimated fish ages between 1.75 and 8 yr (ESM Table S1). To match exposure of historic temperatures with maximum age, we assessed SST metrics over the previous 8 yr (ESM Table S2). For the eight summer seasons between 2003 and 2010, we determined three SST metrics: maximum weekly temperature (maximum SST), maximum temperature anomaly (anomalies), and average number of degree heating weeks (DHWs). We calculated anomalies as the maximum positive difference between the weekly temperatures over the monthly averages, and DHWs are the sum of those positive anomalies per week. We also analyzed our archive data from 1985 to 2011 to determine the frequency of thermal events.

The release of GCs by individuals could also be altered by multiple stressors in the natural environment (Busch and Hayward 2009). Therefore, the stress-induced GC response of anemonefish was considered within the context of historic thermal stressors while controlling for present-day natural stressors, including social context and reproductive state (only sampling breeding adults without eggs), as well as food availability (live coral cover) and predator density measured in situ 60 min prior to blood sampling. We surveyed live coral cover using three replicate 20-m line intersect transects (LIT) (English et al. 1997; Conand et al. 1998; Chabanet et al. 2002). We visually censused three 50 × 5 m belt-transects (250 m²) distributed on either side of the LIT to record fish species density (juveniles and adults; Chabanet et al. 2002). We calculated anemonefish-known predators from the total number of families/genera described in ESM Table S3 (Fricke 1975; Moyer 1980).

Generalized linear mixed effects models (LMM) fit by maximum likelihood were used (after log transformation when necessary), to test for the fixed effects of historic thermal stressors (maximum SST, anomalies, and DHWs) and 2011 ecological parameters (live coral cover and predator density) on anemonefish stress-induced cortisol levels while controlling for the random effects of island and site (nested within island). To establish the best fitting model, terms were eliminated one by one from a maximal model. Simplified models were compared with more complex ones using Chi-square statistics and were retained if they were not significantly worse with a term removed.

Results and discussion

Anemonefish cortisol levels in response to a generic stressor were significantly different between islands ($F_{2,31} = 13.229$, $p = 0.005$; site (nested within island): $F_{5,28} = $

Table 1 Adult skunk anemonefish, *Amphiprion akallopisos*, stress-induced cortisol level as a function of historic thermal stressors [maximum sea surface temperature (SST), average degree heating weeks (DHWs), and maximum SST anomaly], current ecological parameters in 2011 (maximum SST, live coral cover, and predator density) as fixed factors and island, as well as the eight sites (nested within island) as random factors

Model	df n, d	F	p
A			
Historic thermal stressors			
Max SST (2003–2010)	**1, 1.06**	**177.051**	**0.041**
Max SST anomaly (2003–2010)	1, 23.9	0.921	0.347
Average DHWs (2003–2010)	1, 8.5	0.137	0.720
Ecological parameters 2011			
Live coral cover (2011)	1, 0.6	525.920	0.099
Predator density (2011)	1, 3.8	1.463	0.296
Max SST (2011)	1, 2.8	3.595	0.161
B			
Historic thermal stressors			
Max SST (2003–2010)	**1, 2.0**	**336.265**	**0.003**

(A) Full model and (B) the final reduced model after removing non-significant factors individually are presented

Values in bold are significant

(A) Full model: Akaike's information criterion (AIC) = 7.103; Island effect: variance = 0.013, standard deviation = 0.029; Site (nested within island) effect: variance = 3.8×10^{-5}, standard deviation = 0.001

(B) Final model: Akaike's information criterion (AIC) = −11.975; Island effect: variance = 0.018, standard deviation = 0.025; Site (nested within island) effect: variance = 0.0001, standard deviation = 0.0008

Fig. 3 Relationship between maximum experienced summer sea surface temperature at eight Îles Eparses sites and stress-induced cortisol level in skunk anemonefish, *Amphiprion akallopisos* (linear regression: $F_{1,32} = 21.776$, $p < 0.001$; $r^2 = 0.446$; $y = -971.74x + 33.67$). The sites are indicated in: *black* for Europa (EUR1 = *triangles*, EUR2 = *circles*), *white* for Glorieuses (IG1 = *circles*, IG1 = *diamonds*), and *gray* for Juan de Nova (JDN1 = *diamonds*, JDN2 = *circles*, JDN3 = *triangles*, JDN4 = *squares*)

2013). Glucocorticoids produced in response to historic high temperatures may have had activational effects on the already developed HPI axis (Love et al. 2012) inducing sensitization by enhancing memory formation and retrieval (McEwen and Sapolsky 1995). The GC stress response may also have been modified to go beyond the preexisting individual range via changes in GC secretion, binding globulin, receptors, and/or clearance rate (Angelier and Wingfield 2013). This study is the first to find plasticity in the primary neuroendocrine stress response following exposure to thermal stressors.

Stressors not only compromise survival, but may also create trade-offs in the allocation of resources between immediate survival (the stress response) and other life-history traits, such as reproductive performance and fitness (Sumpter 1997). As such, irreversible modifications in the stress response mostly appear detrimental to individuals, especially if the stochasticity of environmental changes is increasing as a mismatch between the GC stress response and environmental conditions will render the modifications maladaptive (but see Sheriff et al. 2010; Love et al. 2012). In this study, the frequency of thermal events across the Îles Eparses was on average every 1.92–6 yr, and as such, a heightened GC stress response, via either irreversible modifications to the HPI axis or sensitization, is a potential physiological mechanism for fish, with a lifespan greater than 6 yr, to cope with future climate change events. However, a GC stress response can be effective in coping with one type of stressor, but not another, and here we only measured responses to acute handling, whereas plasticity may affect the response to chronic/prolonged stressors differently. Nevertheless, the primary stress response, via its cascading effects on secondary and tertiary stress responses, has

0.843, $p = 0.532$; sex: $F_{1,32} = 0.140$, $p = 0.712$); lower in EUR (29.5 ± 2.9 ng ml^{-1}; mean ± SE) compared to both JDN ($p < 0.001$; 68.7 ± 5.3 ng ml^{-1}) and IG ($p = 0.001$; 61.4 ± 6.5 ng ml^{-1}). After controlling for effects of island (LMM: variance = 0.018, SD = 0.025) and site (nested within island) (LMM: variance = 0.0001, SD = 0.0008), anemonefish stress-induced cortisol levels significantly increased with increasing historic maximum summer SST ($F_{1,2} = 366.3$, $p = 0.003$; Table 1b; Fig. 3). The highest maximum summer SSTs occurred in 2005 (JDN4 = 31.14 °C), 2009 (EUR2 = 29.68 °C, JDN2 = 31.25 °C), and 2010 (JDN3 = 30.85 °C, JDN1 = 30.82 °C, IG1 and 2 = 30.61 °C, EUR1 = 30.5 °C, JDN4 = 30.85 °C). Cortisol levels were not explained by any other historic temperature metric nor by 2011 ecological data (all $p > 0.05$; Table 1). Our results suggest that the greater the historic exposure to temperature, the greater the response of the HPI axis later in life, i.e., the stronger the GC stress response to acute handling.

The HPA axis is known to show plasticity after exposure to stressful stimuli, permanently modulating the GC stress response to subsequent stressors (Angelier and Wingfield

positive effects on immediate survival; therefore, the potential of organisms to permanently modify their HPI axis may indeed provide an adaptive response to thermal stressors.

Low-latitude populations, adapted to a narrow temperature range (Rummer et al. 2014), are predicted to be unable to acclimate to global warming (Gardiner et al. 2010). Despite the 11° latitudinal range, we did not find a latitudinal gradient in either stress response or average yearly temperatures (Fig. 2). JDN is located within seasonal eddies with abnormal temperatures (Lutjeharms 2006; Swart et al. 2010), resulting in thermal similarity to IG in both SST average and range. Low-latitude populations at JDN and IG may be living closer to their thermal optima and may be locally adapted to their narrower thermal range, yet high SSTs resulted in greater stress responses than at EUR. However, whether EUR populations show greater or lesser plasticity than lower-latitude populations can only be ascertained by comparing phenotypic variation in GC responses across the same thermal gradient.

The differences in stress responses between islands could also have originated during the two-week larval dispersal phase. Fish larvae are more thermally sensitive than adults (Pankhurst and Munday 2011), and early life events shape the development of the HPI axis (Angelier and Wingfield 2013). Local oceanographic features, local currents, eddies (Lutjeharms 2006), and thus thermal profiles lend support to the hypothesis that larvae from JDN and IG populations may have experienced higher SSTs during dispersal than from EUR.

Climate change exposes populations to massive unpredictable events; thus, understanding the consequences of primary stress responses is critical for predicting secondary and tertiary responses, as well as population trajectories. Links between climatic stressors and increases in GCs are predicted theoretically (Wingfield 2008), and our paper shows modifications of the HPI axis in fish that have experienced historic thermal stressors. We suggest that such plasticity would be beneficial to fish populations experiencing subsequent thermal events. Our results reinforce the local adaptation potential of coral reef fish to global warming environments through plasticity of the GC stress response, even at low latitudes.

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