

BES Grant Report:

Susceptibility of black bream to climate warming: Considerations of body size and populations

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Section 1 - Scientific Research Report

What was the overall aim of the work supported by this grant?

Climate warming is seeing temperatures breach exceptional thresholds as the frequency and intensity of heat waves worsen. Efforts to forecast species vulnerability to climate warming often focus on upper thermal limits threatening survival, but vulnerability assessments typically overlook the role that intraspecific variation (e.g., differences in body mass and populations among individuals) may play in determining climate change vulnerability. Using an estuarine fish (black bream, *Acanthopagrus butcheri*) as a model, this research aimed to explore how intraspecific variation in body mass and among populations affects upper thermal tolerance.

Have the overall aims of the project been met?

Climate change will affect individuals and populations within a species, yet data on intraspecific variation in warming tolerance is currently lacking. Using black bream, this project shows that intraspecific variation in body mass does not impact upper thermal limits over a 500 g body mass range. However, intraspecific variation in thermal tolerance exists among some black bream populations, with southern populations being more sensitive to acute warming than northern populations of black bream.

Background and Rationale

Climate change is forcing an increase in average temperatures, coupled with an increased frequency and intensity of heat waves (Oliver et al., 2018; Stillman, 2019). These warming trends are expected to cause population declines, local extirpation, or even extinction as thermal limits of animals are surpassed (Stillman, 2019). Ectothermic animals, such as fishes, are particularly vulnerable to warming temperatures because their internal temperature often matches their surrounding environment (Ern et al., 2023). The risk posed by climate warming has prompted a flurry of research into thermal limits across taxa (e.g., Comte and Olden, 2017; Gomez Isaza et al., 2020; Gunderson and Stillman, 2015), which have been instrumental in building a strong understanding of how climate change impacts the physiology and ecology of animal life. However, these data are often applied broad stroke to infer the vulnerability of a species to climate warming (e.g., Comte and Olden, 2017; Dahlke et al., 2020) and overlook intraspecific variation in thermal limits (McKenzie et al., 2021). Understanding intraspecific variation in thermal limits will be crucial to comprehensively forecast the sensitivity of species to climate change.

Body mass is a key factor that can contribute to intraspecific variation in temperature tolerance. Understanding if and why temperature tolerance varies with body size has a rich history in thermal ecology (e.g., Illing et al., 2020; Mora and Maya, 2006; Stevenson, 1985); a question which has only garnered more attention due to rising global temperatures and the need to identify critical life stages to protect species from warming (Dahlke et al., 2020; Rodgers et al., 2019). For example, a recent analysis compiled thermal tolerance data for over 600 fishes and report a “thermal

bottleneck” in the lifecycle of fishes (Dahlke et al., 2020); that is, embryos and spawning adults consistently have narrower tolerance ranges than larvae and nonreproductive adults and are therefore more susceptible to climate warming. Hypothesised explanations for body size differences in temperature tolerance revolve around size-dependent differences in metabolic rate (Gillooly et al., 2001), oxygen transport (Pörtner, 2010), diffusion distances (Gillooly et al., 2016), enzyme function (Ern et al., 2023), and energetic stores (Chung and Schulte, 2020; Leiva et al., 2024), among others, though no consistent explanation has been reached. There is also no consistent outcome for the effects of body mass on thermal tolerance. Some research has shown that larger body size correlates with lower thermal tolerance (Clark et al., 2017; Di Santo and Lobel, 2017; McPhee et al., 2023; Messmer et al., 2017), while others have shown that larger fish have higher thermal limits (Illing et al., 2020; Moyano et al., 2017), and others have found no association between body mass and thermal tolerance (Blasco et al., 2022; Ospina and Mora, 2004; Recsetar et al., 2012). This incongruence between studies suggests a high degree of species specificity and poses a challenge for identifying sensitive life stages that may be of conservation concern.

Thermal limits could also differ among populations of a species. Intraspecific variation in thermal tolerance could arise among populations due to heritable differences between individuals of a population or due to phenotypic plasticity in response to varying environmental conditions (Anttila et al., 2013; Muñoz et al., 2015; SandovalCastillo et al., 2020). There are several examples that show variation in thermal limits among populations, with evidence being strongest among populations with little gene flow (Eliason et al., 2011; Sandoval-Castillo et al., 2020). For example, thermal tolerance between populations has been shown to differ by 1 – 4 °C in widely distributed species (Fangue et al., 2006; Pereira et al., 2017; Villeneuve et al., 2021). Similarly, thermal tolerance can differ among populations exposed to different thermal conditions spread within just a few hundred kilometres (e.g., mountain versus desert populations) (Chen et al., 2018; Eliason et al., 2011). Therefore, assigning species a single thermal tolerance value can be inappropriate. Understanding if intraspecific variation in thermal limits exists among populations is of considerable conservation and ecological significance, since some populations might be at greater risk from climate warming while others could serve as safety populations.

To examine if intraspecific variation in body mass and populations impacts upper thermal limits, we quantified intraspecific variation in thermal tolerance using a widely distributed estuarine fish, black bream (*Acanthopagrus butcheri*). Specifically, we tested the hypothesis that 1) thermal tolerance would decrease with increasing body mass; and 2) that differences in thermal tolerance would occur across populations of black bream that correlate with latitudinal temperature ranges experienced by each population. Black bream are an ideal study species to test these hypotheses because they range in size from a few grams to ~1 kg (though can reach up to 4 kg) and inhabit the same conditions throughout their lifecycle. Black bream are an estuarine fish that display strong philopatry (Chaplin et al., 1998; Sarakinis et al., 2024). There is evidence of isolation by distance between populations of black bream (Chaplin et al., 1998). Isolation between black bream populations provides a basis for the potential for population differences in thermal tolerance to arise.

Methods

Field locations

We selected four estuaries along the West Australian coastline as field sites spanning ~ 800 km of coastline (Fig. 1A). The four estuaries cover the upper (Moore River; 31° 20' S, 115° 30' E), mid (Serpentine River 32° 33' S, 115° 45' E) and lowermost (Blackwood River 34° 17' S, 115° 9' E; and Kalgan River 34° 56' S, 117° 58' E) distribution of black bream in Western Australia, but their distribution extends south to Victoria and Tasmania (Sarakinis et al., 2024). The four estuaries were chosen because they differ in their annual thermal profiles (Fig. 1B – E) and could therefore contribute to population-level difference in upper thermal tolerance. This work was conducted during the Austral summer (February – March of 2024). At each field site we collected basic environmental data, including water temperature, pH, and salinity.

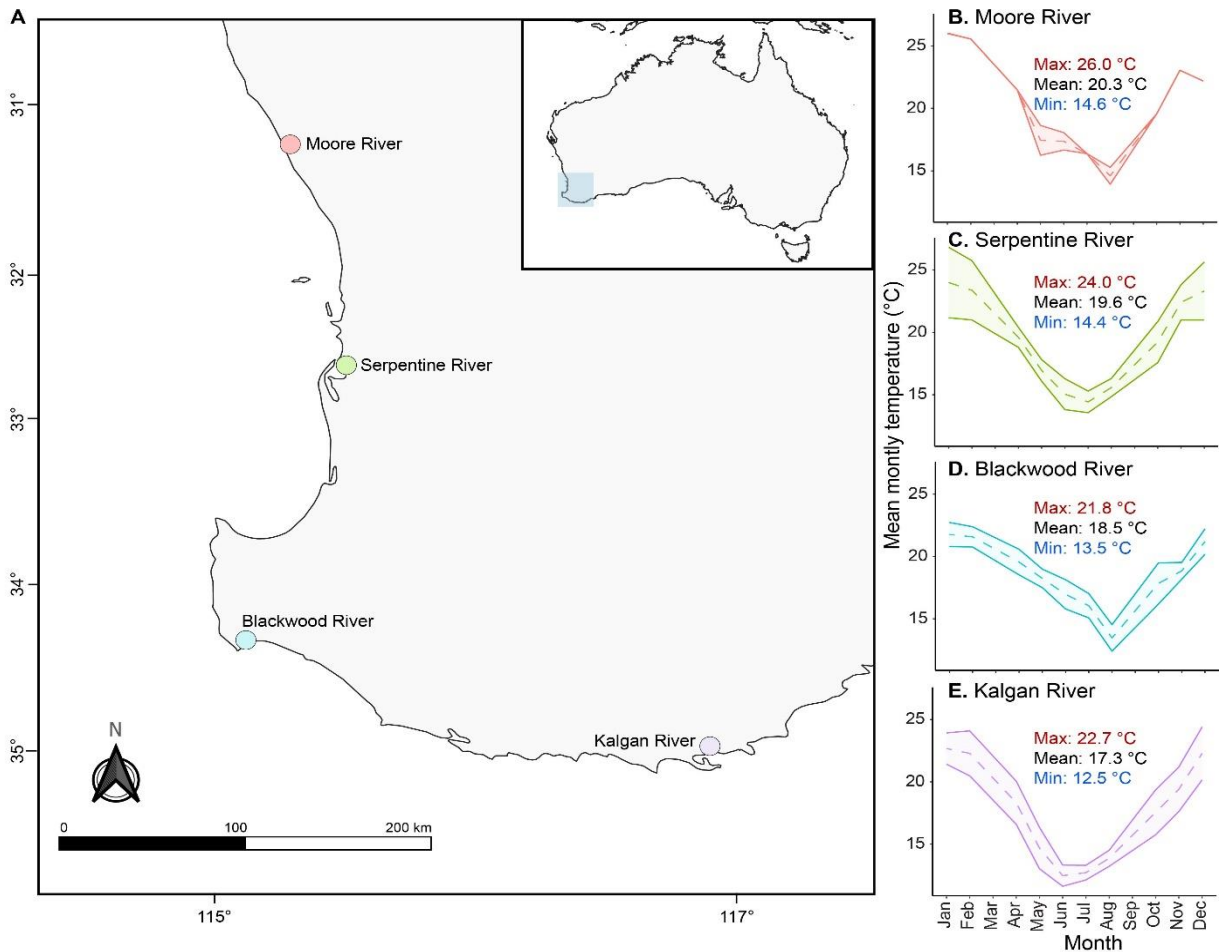


Figure 1: (A) Map of study locations distributed across a ~5 latitudinal cline along the southwest corner of Western Australia (insert). Monthly temperature profiles composed of mean monthly temperature (°C) for the (B) Moore River, (C) Serpentine River, (D) Blackwood River, and (E) Kalgan River. Data are presented as mean \pm standard deviation for temperature data collected between Jan 2016 – Dec 2023. Mean maximum (red), median (black), and mean minimum (blue) temperatures of each location. Data were accessed from the Western Australian Department of Water and Environmental Regulation.

Animal collection

Fish were collected ($n = 28 - 53$ per population) using a 21.5 m seine net. To test the influence of body mass on thermal tolerance, we aimed to collect fish of varying sizes from each population. Once collected, fish were immediately placed within an instream holding pen ($50 \times 50 \times 150$ cm, $l \times w \times d$) that allowed fish to recover from netting within the river. Fish were kept in the holding pen for at least 2 h prior to testing.

Thermal tolerance tests

Upper thermal tolerance was quantified as the critical thermal maximum (CT_{max}). CT_{max} trials were performed in the field on the streamside of the riverbank at each of the four study sites. The CT_{max} set-up consisted of a heating tank ($58 \times 38.5 \times 34$ cm) filled with 37 L of water, two 350 W heating coils (Korjo WB 51 water boiler), and a water pump (5W Magi-200, Magic-Jet Filter).

A group of randomly selected fish (4 – 12, depending on body mass) were caught from the holding pen and transferred to the CT_{max} test tank. Fish were allowed 30 min to habituate to the testing tank before thermal ramping began. After this time, the water in the testing tank was increased at a rate of 0.3 °C per minute. Loss of equilibrium (LOE), defined as the failure to maintain dorsal-ventral orientation for 10 s, was chosen as the CT_{max} endpoint. Once a fish lost equilibrium, the water temperature was recorded, and the fish was transferred to an aerated bucket filled with fresh river water for recovery. Fish were allowed to recovery from the CT_{max} test for about 1 h before being weighed, measured, and returned to the wild. Any fish that did not regain equilibrium (~ 5 %) were euthanised with an overdose of Aqui-S Pty Ltd (Lower Hutt, New Zealand).

Statistical analysis

The effect of population and body mass on CT_{max} were analysed using linear mixed effects models using the lme function of nlme package (Pinheiro et al., 2023) in R (version 4.3.1). Population and centred mass were included as fixed effects, with CT_{max} trial number as a random effect. Mass was centred by subtracting the grand mean from all values to simplify the interpretation of parameter estimates (Clark et al., 2017). We conducted Tukey post hoc comparisons using the emmeans package (Lenth, 2023).

Results and discussion

Thermal tolerance is often assumed to be consistent within a species, which risks misguiding species' management if there is variation in heat tolerance among life stages or across populations. Here, we used black bream to test the hypothesis that thermal tolerance would decrease with increasing body mass. Contrary to our first hypothesis, there was no significant relationship between body mass and CT_{max} in black bream ($F_{1, 154} = 1.19, P = 0.28$; CT_{max} ~ mass slope \pm s.e.m. = -0.001 ± 0.0001 ; Fig. 2). We collected black bream over an approximate 950-fold body size range across the four populations (mean = 52.4g, range = 0.57 – 541 g; Fig. 2A). This range covers a substantial mass range of black bream found in Western Australian estuaries (Cottingham et al., 2018).

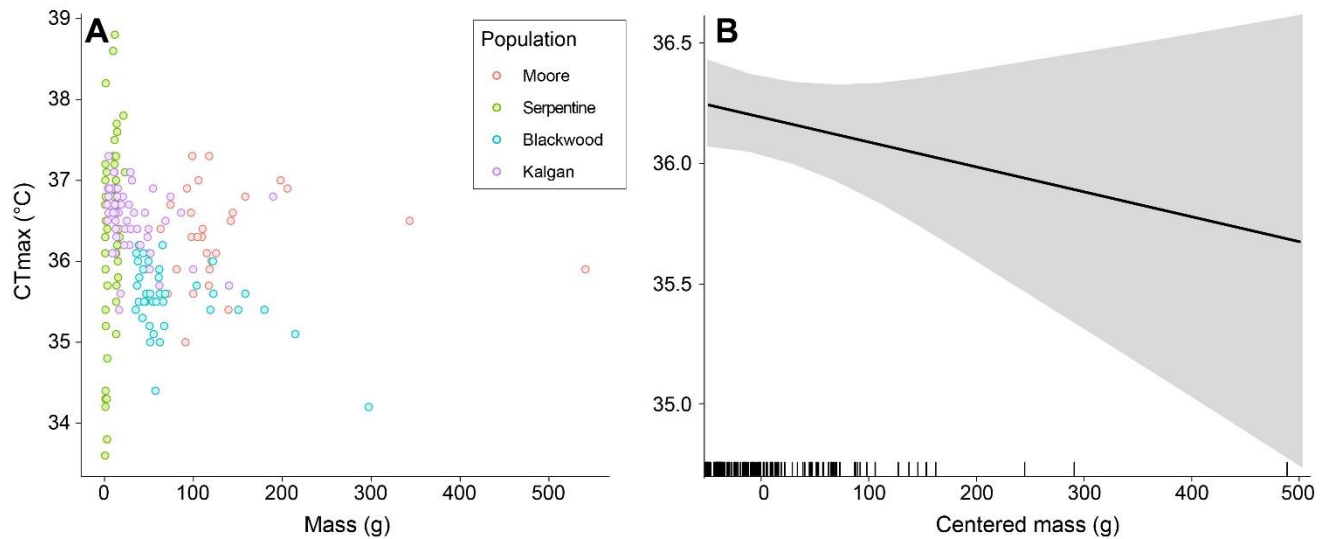


Figure 2: (A) Effect of body mass on CT_{max} of black bream, with colours representing the four study populations. (B) Linear mixed-model predictions with 95% confidence intervals is presented. Mass was centred (overall mean value [52.4 g] subtracted from each value) so that zero represents the mean mass for all populations.

Our results support those on several tropical and temperate fishes where no association between body mass and thermal tolerance has been found (Blasco et al., 2022; Ospina and Mora, 2004; Recsetar et al., 2012). Unfortunately, we were unable to measure the thermal tolerance of larval or spawning fish, which are predicted to be especially susceptible to acute warming (Dahlke et al., 2020). In fact, our data are skewed towards small juvenile black bream (mean mass = 52 g), so potentially a body size effect exists if a wider body mass range is considered. That being said, previous studies on the relationship between body size and thermal tolerance typically involved smaller size ranges than the size range in black bream examined here. For instance, Di Santo and Lobel (2017) report a negative correlation between body mass and thermal tolerance in tropical gobies (*Elacatinus oceanops* and *E. lobeli*) over 0.5 g mass range, whereas Illing et al. (2020) report a positive correlation between body mass and thermal tolerance in two tropical fish larvae (*Amphiprion melanopus* and *Lates calcarifer*) over a 6 g and 40 g body mass range, respectively. Studies that have found an association between body mass and thermal tolerance using a large size range tend to report modest differences. In leopard coral grouper (*Plectropomus leopardus*), Messmer et al. (2017) report ~ 1 °C difference in CT_{max} between small ~ 0.5 kg and large ~ 3 kg fish, while only a 0.7 °C difference in CT_{max} was found between small (~ 0.2 g) and large (~ 1.2 kg) bodied Murray cod (*Maccullochella peelii*) (McPhee et al., 2023). Together, these findings suggest that the effect of body mass on acute thermal tolerance using CT_{max} protocols is weak and species dependent.

We further tested the hypothesis that differences in thermal tolerance would occur across populations of black bream that correlate with latitudinal temperature ranges experienced by each population. CT_{max} differed among populations of black bream ($F_{3,18} = 6.07$, $P = 0.005$; Fig. 3). Black bream from Blackwood River (southern population) had the lowest CT_{max} (mean \pm s.d. = 35.57 ± 0.43 °C) and differed from the other three populations. The CT_{max} of the Moore ($36.32 \pm$

0.70 °C), Serpentine (36.36 ± 1.15 °C), and Kalgan (36.52 ± 0.41 °C) populations did not differ from one another. The documented effect was small, with only a 0.7 °C mean difference between the Blackwood (southern) and Moore (northern) populations. Fish from the Blackwood population typically experience habitat temperatures that are 2 – 3 °C lower than fish in the northern (Moore River) and mid (Serpentine River) latitude populations (Fig. 1B – E), which could explain the slightly lower CTmax value. Larger differences in mean habitat temperature may be required for stronger differences among populations to arise. However, although the effect is small, it is similar to other published studies testing intraspecific variation in thermal tolerance among more pronounced thermal gradients (Chen et al., 2013; Fanguie et al., 2006; Villeneuve et al., 2021). This result provides evidence that black bream populations should be managed separately due to their different warming tolerances.

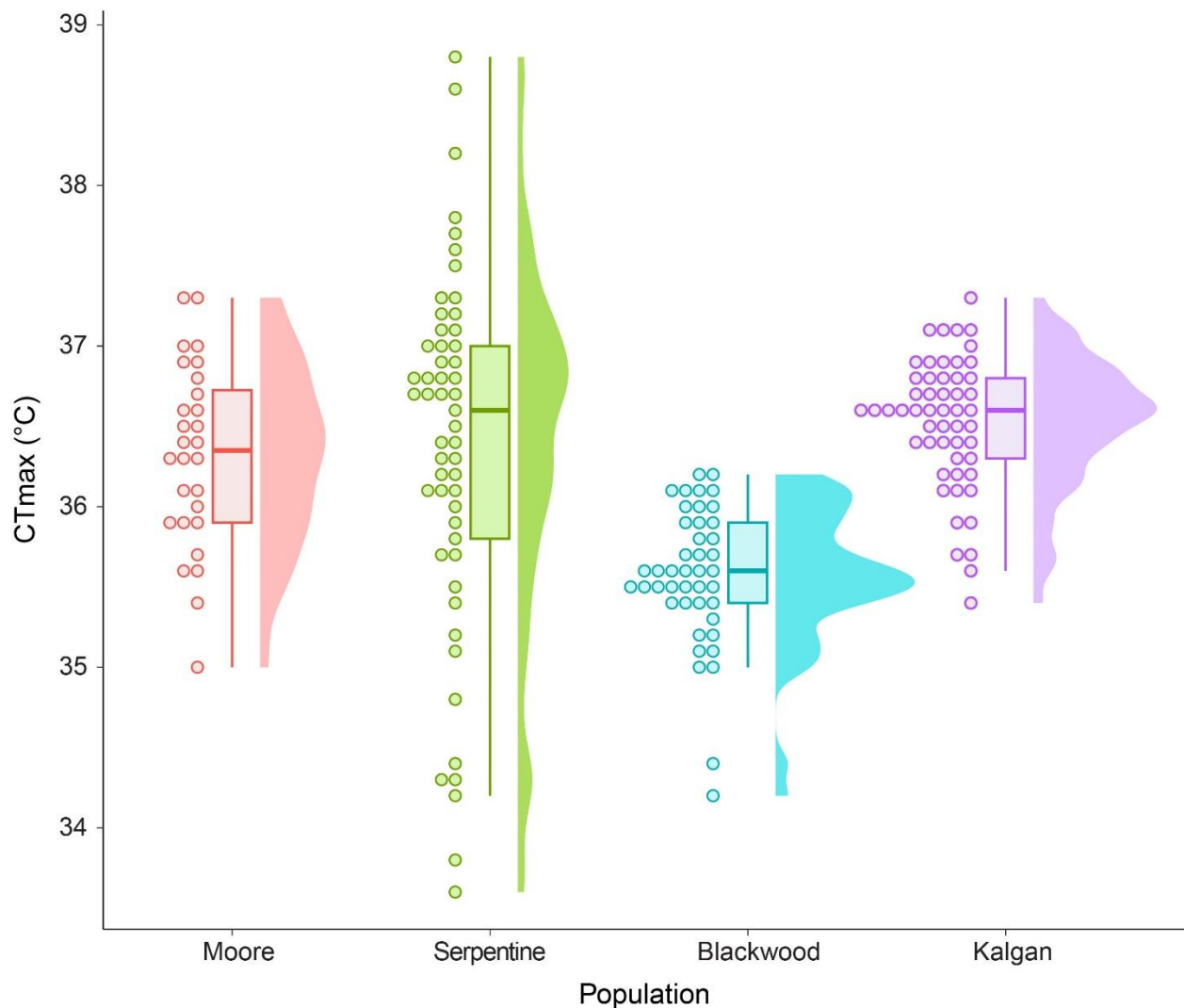


Figure 3 CTmax of black bream (*Acanthopagrus butcheri*) across populations. Data are presented as rainforest plots, showing the density distribution, boxplot (with median and interquartile ranges), and individual points showing the distribution of the raw data.

Unlike most literature on CT_{max}, we conducted CT_{max} trials in the field with wild fish to better capture estimates of thermal tolerance. This approach ensured that fish were fully acclimatised to local conditions to test if differences exist among populations (Desforges et al., 2023). However, this approach does not allow us to disentangle the relative contributions of plasticity versus local adaptation in shaping thermal limits among populations. Distinguishing between plastic and heritable variation will require future common garden experiments or pedigree studies (Chen et al., 2013; McKenzie et al., 2021). Surprisingly, fish from the southernmost population (Kalgan River population) from the current study, who also experience lower mean annual temperatures than northern populations, had the highest CT_{max}. This would suggest that thermal tolerance is unlikely to be linked to latitudinal differences in mean temperatures experienced by each population. However, the Kalgan River population were tested following three heatwaves in February 2024 in Western Australia (Poncet and Rowe, 2024), which could have resulted in heat-hardening effects that increased the population's mean CT_{max} (Morgan et al., 2018). Further work is required to establish if population differences in thermal tolerance are consistent across seasonal and annual timescales.

Conclusions

Climate change will affect individuals and populations within a species, yet data on intraspecific variation in warming tolerance is currently lacking. Using black bream, this study shows that intraspecific variation in body mass does not impact upper thermal limits over a 500 g body mass range. We show that intraspecific variation in thermal tolerance exists among some black bream populations. Further research is required to disentangle the relative contributions of plasticity versus local adaptation in shaping thermal limits among populations. Nonetheless, this work underscores the importance of observing intraspecific variation in thermal limits and the need for conservation efforts to consider not only species but also the unique characteristics and capabilities of individuals and populations.

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Section 2 - Monitoring and Evaluation

Regarding your BES award, which of the following do you feel to be true:

- Influenced you receiving funding from sources other than the BES
- Increased your knowledge of ecology
- Resulted in work that has the potential to influence policy
- Improved your access to equipment
- Provided travel assistance
- Provided field assistance

Please provide any specific feedback or comments that you would like to make on your award:

The award of a BES grant allowed me to carry out a research project independently. The award enabled me to conduct a project that I was very interested in, but had not secured the funding from other sources. Through this grant, I was able to provide baseline evidence that I will use to help gain future funding.

Section 3 - Impact Report

Project Description.

Please describe the work funded by the BES grant in terms that would be understood by a member of the public. Please do not use detailed scientific terms.

Climate change is causing temperatures to reach extreme levels, making heat waves more frequent and intense. When predicting how species will be affected by climate change, scientists often focus on the highest temperatures that can threaten their survival but tend to overlook the differences within a species that might influence their vulnerability. In this study, we examined an estuarine fish called black bream (*Acanthopagrus butcheri*) to see how variations in body size and different populations affect their ability to withstand high temperatures. We measured the highest temperatures these fish could survive (known as critical thermal maxima or CT_{max}) in wild fish. The fish we studied ranged in size from 0.57 grams to 541 grams, with an average size of 52.4 grams, to see if body size influenced thermal tolerance. We also looked at four different populations of black bream spread over a 5° range of latitude to see if there were any differences in thermal tolerance among these groups. Contrary to what we expected, body size did not affect the fish's ability to withstand high temperatures. However, we found significant differences among the populations. Fish from the southern population had a lower thermal tolerance (CT_{max} of 35.57 ± 0.43 °C) compared to those from the northern (36.32 ± 0.70 °C) and mid-latitude (36.36 ± 1.15 °C) populations, which matched the average temperatures of their habitats. These findings highlight the importance of considering differences within a species when studying their ability to cope with climate change. This can help improve the management and conservation of species, particularly those in vulnerable life stages or populations. **Personal Impact.**

What impact did receiving this grant have on you personally and the development of your career in ecology?

Receiving this grant has profoundly shaped both my personal journey and professional trajectory in the field of ecology. Personally, it has been a validation of my dedication and potential in the field, boosting my confidence and reaffirming my commitment to aid the conservation of fishes at risk of climate warming. Professionally, the grant has provided crucial financial support, enabling me to pursue an ambitious research project. Moreover, the grant has allowed me to obtain necessary equipment for and provided travel funds to remote locations in Western Australia required for this project. I think this project has allowed me to carve out my own little niche in thermal ecology of fishes in Western Australia and will help build my track record as an expert in this field. Importantly, this grant has allowed me the opportunity to obtain baseline information on the thermal ecology of fish native to Western Australia, and I will use this information as a springboard for future funding. The award of this grant has also allowed me to build relationships with government and natural

resource managers, which will help my science generate impact as I will be sharing the results of this research with local managers. Overall, receiving this grant has been greatly impacted my career and I think the benefits will persist beyond the 1-year tenure of the grant. It has not only advanced my scientific endeavours but also empowered me to make a tangible impact in the field of ecology. I am grateful for the support that the BES have awarded me through this grant and the personal, professional, and scientific impact that this work will bring

Scientific Impact.

What impact did receiving this grant have on the research community?

This grant has provided significant evidence of the intraspecific differences in thermal tolerance of fishes. The impacts of climate warming on animals have been generally applied broad stroke, and important intraspecific variables within individuals has been largely ignored. Through this grant, I showcase the importance of considering important intraspecific factors such as body mass and population when considering the vulnerability of species to climate change. I found that different populations of black bream are more sensitive to acute warming, but body mass does not affect upper thermal limits. These data will hopefully drive more researchers and conservation managers to consider intraspecific variables when assessing the susceptibility of animals to climate warming. Additionally, these data were collected in the field rather than in the laboratory where they are typically conducted. This works highlights the importance of conducting ecological research in the field so that it better represents the responses of animals in their natural environment. Overall, receiving this small research grant has not only benefited my own research endeavours but has also had a great impact on the broader research community.

Wider Impact and Outcomes.

What impact and outcomes do you think the work funded by the BES may have within fields outside of academia? Please take into account all wider implications e.g. society/policy/public.

The funded research is expected to make crucial policy and societal impacts. In particular, the research can help aid fisheries policy by understanding the risk of different populations to climate warming. The research identified that southern populations of black bream are more vulnerable to warming than northern populations. As such, populations require different levels of protection. These data will be disseminated to the Western Australian Department Primary Industries and Regional Development and the RecFish West (Recreational Fisheries Western Australia) with the aim of providing evidence of populations that warrant more stringent protection from climate warming. More broadly, I believe that these data can be provide foundational evidence to conservation and managers in Australia and abroad on the importance of considering intraspecific vulnerability to climate warming and the need for tailored conservation strategies to protect the most vulnerable life-stage, population, or local environment at risk from warming. This research will be made publicly available, as required by Western Australia fisheries permits. As such, the work can help raise public awareness of conservation of different populations of black bream. Overall, this work crucial policy and societal impacts by raising awareness of the intraspecific differences of fish and how these differences affect their susceptibility to warming.

Publications and Outputs.

Please provide us with a summary of any key outputs and publications. Have you published/are you intending to publish any papers relating to this work? (e.g. Published/submitted/in preparation)

This work has been submitted for publication to the Journal of Thermal Biology and it is currently under review (Gomez Isaza DF and Rodgers EM. in review. Upper thermal limits are 'hard-wired' across body mass but not populations of an estuarine fish). This work was also presented as a poster presentation at the Society for Experimental Biology Conference in July 2024 in Prague (Title: Are all fish the same? Importance of considering how intraspecific factors shape thermal limits).

Your Shout.

We'd love you to provide us with a testimonial. Testimonials will be used on various BES channels for new applicants to view and to promote our grants to the ecological community.

The award of a BES grant has been amazing. BES have provided me with the opportunity to carry out my own research, funding critical equipment and field trips to remote locations in Western Australia. The entire grants process with BES was extremely streamlined from the application process to claiming the awards and through the support offered to publicize my research. BES are truly a supportive community that promote excellent research, science with impact, and support for early career researchers through their grant schemes. Massive thank you to the BES for supporting my research!