



Thermal limits and preferences of large branchiopods (Branchiopoda: Anostraca and Spinicaudata) from temporary wetland arid zone systems

Murphy Tladi, Ryan J. Wasserman, Ross N. Cuthbert, Tatenda Dalu, Casper Nyamukondiwa

PII: S0306-4565(21)00165-0

DOI: <https://doi.org/10.1016/j.jtherbio.2021.102997>

Reference: TB 102997

To appear in: *Journal of Thermal Biology*

Received Date: 7 August 2020

Revised Date: 6 April 2021

Accepted Date: 16 May 2021

Please cite this article as: Tladi, M., Wasserman, R.J., Cuthbert, R.N., Dalu, T., Nyamukondiwa, C., Thermal limits and preferences of large branchiopods (Branchiopoda: Anostraca and Spinicaudata) from temporary wetland arid zone systems, *Journal of Thermal Biology* (2021), doi: <https://doi.org/10.1016/j.jtherbio.2021.102997>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2021 Published by Elsevier Ltd.

Murphy Tladi: Conceptualization, Supervision, Funding, Writing- Original draft preparation, Data collection, Data analyses

Ryan Wasserman: Conceptualization, Methodology, Supervision, Funding
Conceptualization Writing- Original draft preparation, Data analyses

Ross Cuthbert: Conceptualization, Methodology, Data analyses, Writing

Tatenda Dalu: Conceptualization, Methodology, Data analyses, Writing

Casper Nyamukondiwa: Conceptualization, Methodology, Supervision, Writing- Original draft preparation.

Thermal limits and preferences of large branchiopods (Branchiopoda: Anostraca and Spinicaudata) from temporary wetland arid zone systems

Murphy Tladi¹, Ryan J Wasserman^{2,1}, Ross N. Cuthbert³, Tatenda Dalu⁴, Casper Nyamukondiwa¹

¹ Department of Biological Sciences and Biotechnology, Botswana International University of Science and Technology, Palapye, Botswana

²Department of Zoology and Entomology, Rhodes University, Makhanda, South Africa

³GEOMAR Helmholtz-Zentrum für Ozeanforschung Kiel, 24105 Kiel, Germany

⁴ Aquatic Systems Research Group, School of Biology and Environmental Sciences, University of Mpumalanga, Nelspruit 1200, South Africa

**Corresponding author e-mail: r.wasserman@ru.ac.za*

Abstract

Large branchiopods are specialist crustaceans adapted for life in temporary, thermally dynamic wetland ecosystems. Certain large branchiopod species are, however, restricted to specific temporary wetland types, exemplified by their physico-chemical and hydroperiod characteristics. Here, we contrasted the thermal preference and critical thermal maxima (CT_{max}) and minima [CT_{min}] of southern African anostracans and spinicaudatans found exclusively in either temporary rock-pool or pan wetland types. We hypothesised that environment of origin would be a good predictor of thermal preference and critical thermal limits. To test this, *Branchiopodopsis tridens* (Anostraca) and *Leptestheria brevirostris* (Spinicaudata) were collected from rock-pool habitats, while *Streptocephalus cafer* (Anostraca) and a *Gondwanalimnadia* sp. (Spinicaudata) were collected from pan habitats. In contrast to our hypothesis, taxonomic relatedness was a better predictor of CT_{max} and temperature preference than environment of origin. Spinicaudatans were significantly more tolerant of high temperatures than anostracans, with *L. brevirostris* and *Gondwanalimnadia* sp. median CT_{max} values of 45.1 °C and 44.1 °C, respectively, followed by *S. cafer* (42.8 °C) and *B. tridens* (41.4 °C). Neither environment or taxonomic relatedness were good predictors of CT_{min} trends, with *B. tridens* (0.9 °C) and *Gondwanalimnadia* sp. (2.1 °C) having the lowest median CT_{min} values, followed by *L. brevirostris* (3.4 °C) and *S. cafer* (3.6 °C). On the contrary, temperature preferences differed according to taxa, with spinicaudatans significantly preferring higher temperatures than anostracans. *Leptestheria brevirostris* and *Gondwanalimnadia* sp. both spent most time at temperatures 30-32 °C, *S. cafer* at 18-20 °C and *B. tridens* at 21-23 °C. Constrained thermal traits reported here suggest that

the studied anostracans might be more susceptible to projected climatic warming than the spinicaudatans, irrespective of habitat type, however, these taxa may also compensate through phenotypic plasticity.

Keywords: Botswana, *Branchiopodopsis*, critical thermal limits, *Gondwanalimnadia*, *Leptestheria*, *Streptocephalus*, thermal preference.

Introduction

The density of temporary depression wetlands in the southern African region is among the highest worldwide (Goudie and Wells, 1995; Hamer and Brendonck, 1997). However, even within these unique systems, there are a variety of distinct temporary wetland habitat types which include vernal and rock pools among others (Calhoun et al., 2017). Habitat heterogeneity is a driver of biological diversity and is important for the maintenance of species (Tews et al., 2004; Stein et al., 2014; Fine, 2015). On an evolutionary time-scale, abiotic features determine biological inhabitant characteristics for fitness, while at the ecological time-scale, these features drive biological community dynamics (Carroll et al., 2007). However, climate change is expected to affect temporary wetlands in a number of ways, including shifts in acute and chronic temperature dynamics (Meehl and Tebaldi, 2004; Stillman, 2019; Xu et al., 2020) as well as inundation patterns associated with shifting rainfall and evaporation dynamics (Kusangaya et al., 2014). Temperature is one of the main environmental factors driving ectotherm fitness (Sanders et al., 2007; Stein et al., 2014). As such, there has been increasing interest from ecologists on elucidating how changes in climate will likely affect invertebrate biodiversity (e.g. Parmesan and Yohe, 2003; Parmesan, 2006; Thuiller, 2007; Deutsch et al., 2018). Lower and upper thermal activity limits of invertebrates are significant predictors of organismal performance, fitness, biogeography and overall survival and are thus often used for predicting ectotherm responses to shifting environments (Chown and Nicolson, 2004; Calosi et al., 2010; Deutsch et al., 2018; Arribas et al., 2012). However, most empirical studies have focused on terrestrial (Addo-Bediako et al., 2000; Dillon et al., 2010; Hoffman et al.,

2013; Deustch et al., 2018) and marine (Stillman and Somero 2000; Stillman, 2003; Gunderson et al., 2016) organisms. The few studies on thermal regimes of freshwater systems in the region were carried out in river systems (Dallas, 2008; Dallas and Ketley, 2011). Only little information is available on the thermal physiology of temporary wetland specialist fauna in the region (see Lagerspetz and Vainio, 2006).

Determining the thermal activity limits of organisms is fundamental for biological and autecological investigations, providing insight on fitness and habitat suitability (Huey and Stevenson, 1979; Stillman and Somero, 2000; Stillman, 2003; Chown and Nicolson, 2004; Andersen et al., 2015). Thermal activity limits can provide insight on critical temperature thresholds and preferred temperatures of organisms, aiding in the understanding of population distribution and even sink and source dynamics (Dzialowski, 2005; Calosi et al., 2008; 2010; Sunday et al., 2012). It is also useful for predicting how environmental changes may alter species spatio-temporal population phenologies, abundance and dynamics (Kingsolver, 1989; Chown and Nicolson, 2004; Robinet and Roques, 2010). Tolerance has been quantified using either a static method, time of collapse at a constant stressful temperature, or a dynamic method, where the end point temperature of collapse is measured by increasing or decreasing temperature at a constant ramping rate (Rezende et al., 2014). Lower and upper thermal limits investigations typically explore critical activity endpoints e.g. critical thermal minima (CT_{min}) and maxima (CT_{max}) (Lutterschmidt and Hutchison, 1997; Terblanche et al., 2011; Andersen et al., 2015), while others explore temperature preferences (e.g. Dillon et al., 2009; Hering et al., 2009). Critical thermal limits assessment determines the activity ranges upon which an organism operates optimally

and those that are potentially stressful to lethal (Loeschcke and Hoffmann, 2007). They are relatively easy to measure under laboratory settings but correlate extreme well with invertebrate species distributions (Andersen et al., 2015). These have often been investigated using static and more ecologically relevant dynamic protocols (Chown and Nicolson, 2004; Terblanche et al., 2007; 2011). The preferred temperatures, however, show those temperatures that are better suited for population recruitment success, as well as the optimal temperatures for organism rearing (Chown and Nicolson, 2004). As shown by Huey and Kingsolver (1993), the temperatures at which populations can perform optimally are positively correlated to the upper thermal limits of the same populations.

Large branchiopods are specialist temporary wetland crustaceans occupying aquatic habitats that rely on rain, snow melt, underground springs and even condensation (Blaustein and Schwartz, 2001). The large branchiopods are comprised of clam shrimps (Spinicaudata, Laevicaudata, and Cyclestherida), fairy shrimps (Anostraca) and tadpole shrimps (Notostraca) (Brendonck et al., 2008). These organisms have adapted for life in temporary aquatic ecosystems through the development of rapid life cycles and the production of dormant eggs (Brendonck, 1996). Usually, these habitats remain dry for variable periods during the year and have unpredictable inundation patterns (Vanschoenwinkel et al., 2009; Martin et al., 2016). As such, individuals that emerge from dormant eggs following inundation need to develop rapidly, attain sexual maturity and produce eggs. The group typically produces resting stage eggs (cysts) that can remain in sediment for many years before hatching (Wang and Rogers, 2018).

Temporary wetland types are diverse and are characterized by different physico-chemical conditions associated with their underlying geology and nature of their hydroperiods (Williams, 2002; Carrino-Kyker and Swanson, 2007). Indeed, certain large branchiopod species are restricted to certain temporary wetland types (Hamer and Martens, 1998; Helm, 1998; Timms, 2006). As such, the characteristics of different wetland types likely have evolutionary implications for inhabitants (e.g. Van Buskirk and Steiner, 2009). In arid subtropical Botswana, small rock pools on rocky outcrops and pans (clay-lined ponds) are common (Buxton et al., 2020). These environments typically contain large branchiopods, although the species that occur in these two environments are often discrete. Given the differences in size, depth and underlying geology of these temporary wetlands, it is likely that they are characterized by different thermal dynamics. However, it remains unknown whether the thermal profiles of specialist large branchiopod species differ according to their respective wetland habitat types, or whether they are constrained according to taxonomic relatedness. In related organisms, environmental history has large consequences for key thermal traits (see Nyamukondiwa and Terblanche, 2010). This means that organisms often beneficially adapt to their habitat environment, synonymous with the beneficial acclimation hypothesis which suggest that acclimation in an environment induces organisms to develop traits that help them survive better in that same environment (see e.g. Leroi et al., 1994; Wilson and Franklin, 2002; Sgrò et al., 2016).

The ecology of large branchiopods and temporary wetlands have been intensively studied (Samraoui et al., 2006; Mabidi et al., 2016; Bird et al. 2019). However, there

remain pervasive gaps in the knowledge of thermal profiles of most large branchiopods. Thermal profiling could aid in understanding the threat that climate change poses on large branchiopods. Climate models project that if mitigation measures would fail, parts of southern Africa are expected to be drier in future (Engelbrecht et al., 2015; Maure et al., 2018). Given that temporary wetland habitats are ecologically among the most extreme aquatic environments (Mahoney et al., 1990), they may be particularly susceptible to such changes. Shifting climates, for example increase mean temperatures and temperature variability which may affect large branchiopod fitness (Bartolini et al., 2013). For example, both stressful high and low temperatures affect key activity and life history traits e.g. locomotion, mating, molting and development (Azra et al., 2018; Tang et al., 2020). Change in inundation patterns may also negatively interrupt the life cycle of large branchiopods. This may cause habitats to dry up before emerged individuals attain sexual maturity or may compromise the cyst-bank in the sediment through protracted dry periods.

Given the broad significance of large branchiopods' thermal fitness in explaining their responses to climate change, we contrasted the thermal profiles of select anostracans and spinicaudatans found exclusively in temporary rock-pool and pan wetland types. We hypothesized that the rock-pool specialist large branchiopods would have higher thermal activity limits and wider temperature preference than pan specialist large branchiopods, as smaller water bodies (rock pools) both heat and cool more variably and rapidly than larger water bodies (pans) (de la Fuente and Meruane, 2017). Indeed, the climate variability hypothesis predicts similar relationships, whereby a positive relationship exists between thermal tolerance traits (critical thermal limits) and range of

temperatures experienced by organisms (Gutiérrez-Pesquera et al., 2016). Given the presence of representatives of the select species groups in each habitat type, we could assess if habitat or taxonomic relatedness is a better predictor of thermal fitness. Although based on only a few species, the information from this study will help bridge the knowledge gap on large branchiopods thermal biology and motivate further works. Thermal activity thresholds are also important and may help mechanistic models looking at how climate change will affect large branchiopods and may help improve understanding of optimal conditions for activity.

Materials and Methods

Study site and pond temperature monitoring

The study was conducted in the Central District of Botswana, between the towns of Sherwood (22°56'3.78"S, 27°53'28.52"E) and Palapye (22°32'59.68"S, 27° 7'59.66"E) (Fig. 1). The study region is classified as arid, with a high mean annual temperature of 28.5 °C and a low total rainfall of 443.8 mm (Kenabatho et al., 2012; Akinyemi and Abiodun, 2019). The region typically receives rainfall between October and April (Batisani and Yarnal, 2010). For the first component of the study, two major wetland types were categorized, rock pools and pans, each containing Anostraca and Spinicaudata representatives. Five such rock pools and five pans were selected for thermal characterization over their hydroperiods (Table S1), of which some were also used for the collection of animals (component 2: see section below for details). For this first component, programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) were used for the monitoring of temperatures in

the wetlands. Loggers (0.5°C accuracy) were set to 1 h sampling frequencies and deployed in the centre of each wetland and allowed to record temperatures between December 2019 and February 2020.

Species selection and collection

Preliminary surveillance showed anostracan *Branchiopodopsis tridens* Daday, 1910 and spinicaudatan *Leptestheria brevirostris* Barnard, 1924 were found in rock pools. In turn, the anostracan *Streptocephalus cafer* (Lovén, 1847) and spinicaudatan *Gondwanalimnadia* sp. were consistently found in pans between Palapye and Sherwood. The second component of the study comprised lab-based experiments. For the experimental components, animals were collected during the austral summer rainy season in 2019 and 2020. From rock pools and pans, adult large branchiopods were collected by gently pulling sweep nets through the water. A square sweep net (20 cm x 10 cm; 500 µm mesh) was used to sample rock pools, while a round sweep net (diameter: 30 cm; 1 mm mesh) was used to sample the pans. Captured animals were gently transferred from the sweep nets into 5 L containers, filled with source water, following protocols by Martin et al. (2016). Since *Leptestheria brevirostris* was only found in one pond (22°35'55.6"S, 27°07'51.6"E), all other species used in the experiments were collected from one pond each where possible, or from wetlands in very close proximity to one another. This was done to reduce, potential conspecific diversity effects associated with meta-populations across different wetlands as much as possible. *Branchiopodopsis tridens* were collected from rock pool site (22°35'46.1"S, 27°07'16.5"E), while *Streptocephalus cafer* were collected from a pan site

(22°52'16.0"S, 27°47'42.7"E). The presently unidentified *Gondwanalimnadia* sp. (Tladi et al., 2020) were collected from two pans in close proximity to one another (22°52'16.0"S, 27°47'42.7"E; 22°49'45.9"S, 27°37'18.1"E), as individual numbers were relatively low in both ponds (Fig. 1). In the laboratory, samples were maintained in shallow containers (100 cm × 70 cm) using water (strained through a 500 µm filter) collected from their respective source wetlands, in climate chambers (HPP 260, Memmert GmbH + Co.KG, Germany) set at 28 °C (± 1 °C) under a 12:12 light:dark photocycle. These were kept at low but optimal densities of 5 organisms per liter to prevent stressful effects of overcrowding confounding our thermal activity assays (Sørensen and Loeschcke, 2001). All experiments were done within 2 days of specimen collection. Upon completion of each experiment, individuals were immediately transferred to 70% ethanol for identification verification. All collection, handling and disposal of the invertebrates were done following standardised university approved protocols.

Critical thermal limits

Individuals were placed in 10 isolated chambers (test tubes) within double-jacketed chambers (organ pipe) connected to a programmed water bath (Lauda Eco Gold, Lauda DR.R. Wobser GMBH and Co. KG, Germany) such that the programmed water bath regulates temperature changes in the isolated chambers (as in Sinclair et al., 2015; Machekano et al., 2020) (Fig. S1). The water bath was filled with a 1:1 water:propylene glycol ratio, which was circulated through the system to maintain uniform temperatures within the test tubes. An additional chamber was used for temperature verification within

the system using digital thermometer (Fluke 53/54IIB, Fluke Cooperation, USA) (Fig. S1). The test tubes were filled with 50 ml of species-specific source wetland water. The water bath was programmed to have a 10 minutes temperature equilibration time at 28 °C before increasing temperature for CT_{max} or decreasing for CT_{min} at a ramping rate of 0.25 °C/minute (Verberk et al., 2018). Critical thermal limits depend on methodological context and can vary with starting temperature, ramping, and organismal environmental history (acclimation) and others (Terblanche et al., 2007). An equilibration timing of 10 minutes is sufficient to ensure all organisms have the same body temperature (Stevenson 1985). On the other hand, the start temperature was selected as the highest temperature other than incubation temperature that did not affect the activity of all species, per preliminary results. This enabled changes in activity to be monitored easily by looking at the reference start temperature which was higher than the incubation temperature. The samples were then examined every 0.5 minutes for cessation of motion and response to mild mechanical stimulus. New individuals were used for each replicate. Critical thermal limits were defined as the temperature at which the animal lost coordinated muscle function in response to physical stimulation (Lutterschmidt and Hutchison, 1997; Nyamukondiwa and Terblanche, 2010; Salachan and Sørensen, 2017). For each species, the same critical thermal limits experimental procedures were followed. The sample sizes for each species, according to environmental availability, were as follows: *B. tridens* (CT_{min} n = 46, CT_{max} n = 54), *S. cafer* (CT_{min} n = 50, CT_{max} n = 56), *Gondwanalimnadia* sp. (CT_{min} n = 50, CT_{max} n = 50) and *L. brevistoris* (CT_{min} n = 49, CT_{max} n = 54).

Thermal preference

A thermal gradient stage was connected to two programmed water baths, one at high- and the other at a low temperature. The high temperature-water bath was set at 50 °C and the low temperature-water bath at 5 °C to regulate the temperature gradient on the stage. A three lane (700 × 71 mm per lane) PVC half square-pipe water holding stage was placed on top of the thermal gradient stage with each lane holding 500 ml species-specific source water (Fig. S2). The temperature setting of the two water baths enabled the establishment of a thermal gradient from ≈ 10 to ≈ 40 °C in each of the lanes. A single individual was placed in each lane at ≈ 25 °C and allowed to acclimate for 30 minutes. The animals were then observed for an hour, with temperature recorded at their position in the lanes every minute using a digital temperature thermometer (Fluke 53/54IIB, Fluke Cooperation, USA). Initially, ten experimental runs for each species were carried out so that a sample size of 30 was obtained, with observations used to determine the temperatures preferred by the species. Data from trials where individuals lost equilibrium or stopped swimming for periods of longer than five minutes were treated as compromised and excluded. A total of 14 individuals were ultimately used for analysis for *S. cafer*, 20 replicates were used for *B. tridens*, while 22 and 17 replicates were used for *Gondwanalimnadia* sp. and *L. brevirostris*, respectively.

Data analysis

Wetland Thermal Profiles

In order to directly compare temperatures of wetlands, temporal synchronisation was deemed necessary. For the period of 18–24 January 2020, all the study wetlands

contained water at the same time, and as such, temperature data from this period was used for comparison between wetland types. From the hourly temperature data, daily habitat maximum (HT_{\max}), minimum (HT_{\min}) and average (HT_{mean}) values were determined for each of the 7 days from each data logger. Data loggers were successfully retrieved from all five rock pools, however data loggers from one pan were missing. As such, temperature measurements of $n = 5$ were available for the rock pools and $n = 4$ for the pans. Differences in HT_{\max} , HT_{\min} , their range and HT_{mean} , between wetland types, were analysed using separate linear mixed effects models with individual wetlands included as a random effect to account for repeated measures over time (Bates et al., 2015). In other words, each temperature variable was modelled separately with wetland type (rock pools and pans) as an explanatory variable. Diagnostic plots confirmed data conformed to parametric assumptions.

Critical thermal limits

Differences in CT_{\max} and CT_{\min} across the different species were analysed using separate Kruskal-Wallis tests followed by Dunn tests post-hoc (Ogle et al., 2020), as residuals violated assumptions of parametric testing. Accordingly, the two models model included species (four levels) as an explanatory variable for CT_{\max} or CT_{\min} .

Thermal preference

Occurrences related to thermal preferences were analysed using zero-inflated generalised linear mixed models assuming a negative binomial distribution (Brooks et al., 2017). Model diagnostics were checked using simulated residuals (Hartig, 2020).

Here, occupancy counts were summed for each replicate and examined as a function of temperature and species, and their interaction. Individual experimental subjects were included as a random effect to account for repeated measures and inter-individual variation within species. Temperatures were classified into discrete 3 °C bands and considered categorically in the model. As such, the model considered counts of species occurrence within each temperature band as a function of species and temperature, and their interaction. Tukey tests were used for post-hoc pairwise comparisons of significant effects. All statistical analyses were performed in R v.4.0.2 (R Core Team, 2020).

Results

Wetland Thermal Profiles

Differences in HT_{max} between rock pools and pans were significant ($F_{1,7} = 42.99$, $p < 0.001$), with rock pools on average reaching 6.0 °C higher maximal temperatures than pans (Fig. 2). Furthermore, HT_{mean} between rock pools and pans also differed significantly ($F_{1,7} = 9.98$, $p < 0.05$), with rock pools 1.8 °C warmer than pans overall. However, there were not significant differences in HT_{min} between rock pools and pans ($F_{1,7} = 0.79$, $p > 0.05$). A general pattern was observed in that the coefficients of variance were higher in rock pools than in pans for both the dry period and hydroperiod, and the minimum-maximum range was indeed significantly greater ($F_{1,7} = 47.46$, $p < 0.001$) (Fig. S3).

Critical Thermal Limits

The CT_{max} differed significantly across species ($\chi^2 = 144.16$, $df = 3$, $p < 0.001$) (Fig. 3a). Generally, CT_{max} was highest in *L. brevirostris* (highest heat tolerance) followed by

Gondwanalimnadia sp. then *S. cafer* and lastly with the lowest CT_{max} , *B. tridens*. *Leptestheria brevirostris* exhibited significantly the highest CT_{max} median (45.1 °C), and was significantly greater than all other species (all $p < 0.01$). *Gondwanalimnadia* sp. followed, with a median CT_{max} of 44.1 °C that significantly exceeded *S. cafer* (42.8 °C) and *B. tridens* (41.4 °C) (both $p < 0.001$). *Streptocephalus cafer* CT_{max} also significantly exceeded *B. tridens* ($p < 0.001$).

The CT_{min} also differed significantly among species ($\chi^2 = 64.31$, $df = 3$, $p < 0.001$) (Fig. 3b). Generally, CT_{min} was lowest in *B. tridens* followed by *Gondwanalimnadia* sp. then *L. brevirostris* and lastly with the highest CT_{min} (least cold tolerance), *S. cafer*. *Branchiopodopsis tridens* had significantly lowest CT_{min} (median = 0.9 °C) (highest cold tolerance) compared to *L. brevirostris* (median = 3.4 °C) and *S. cafer* (median = 3.6 °C) (both $p < 0.001$), but not lower than *Gondwanalimnadia* sp. (median = 2.1 °C) ($p > 0.05$). In turn, *Gondwanalimnadia* sp. was significantly more cold tolerant (lower CT_{min}) than *L. brevirostris* and *S. cafer* (both $p < 0.001$), with those two species having a more similar CT_{min} statistically ($p > 0.05$).

Thermal preference

Thermal occurrences among species differed significantly owing to a significant two-way 'temperature × species' interaction term ($\chi^2 = 114.58$, $df = 27$, $p < 0.001$). Preferences of *S. cafer* peaked between 18–20 °C, *B. tridens* between 21–23 °C, whilst both *Gondwanalimnadia* sp. and *L. brevirostris* peaked at 30–32 °C (Fig. 4). *Leptestheria brevirostris* was the only species to occupy high temperatures > 38 °C, yet was reciprocally relatively rarely detected < 15 °C. *Streptocephalus cafer* was significantly

more prevalent at 15–20 °C than *L. brevirostris*, whilst the converse was true at temperatures above 30 °C (all $p < 0.05$). *Streptocephalus cafer* was also significantly more prevalent than *B. tridens* at 15–17 °C, and *Gondwanalimnadia* sp. greater than *L. brevirostris* at this temperature (both $p < 0.05$). Further, *S. cafer* was significantly less prevalent than *Gondwanalimnadia* sp. at 30–32 °C ($p < 0.01$). *Leptestheria brevirostris* had significantly higher occurrence than *B. tridens* and *S. cafer* at 33–35 °C, and all species at above 36 °C (all $p < 0.05$) (Fig. 4).

Discussion

In contrast to our hypothesis, the present study showed that wetland type was not a consistent determinant of thermal fitness of the few selected large branchiopods. Rock pools showed greater temperature fluctuations and recorded both higher and lower temperatures than pans. However, taxonomic grouping was a strong predictor of heat tolerance and thermal preference trends among the four species, with the spinicaudatans exhibiting significantly greater heat tolerance (CT_{max}) and higher temperature preferences than the anostracans, irrespective of wetland type. Critical thermal maxima and preference experiments were in consonance, among the four species, with spinicaudatans tending to occur at elevated temperatures (30–32 °C) compared to anostracans (18–23 °C). However, CT_{min} values were neither according to taxonomic grouping nor environment type, with anostracan species comprising both the least (*S. cafer*) and most (*B. tridens*) cold-tolerant taxa. Within these taxonomic generalities in thermal responses, species-specific differences were also apparent. The rock pool anostracan *B. tridens* exhibited significantly lower CT_{max} compared to the pan

368 anostracan *S. cafer*. The spinicaudatans similarly showed significant differences
369 between their respective heat tolerances, with the CT_{max} for the rock-pool species *L.*
370 *brevirostris* higher than that of the pan taxon *Gondwanalimadia* sp. Whilst peak
371 occurrences were similar between spinicaudatans, *L. brevisrostris* exhibited the
372 significantly greatest occurrences at highest temperatures, and was the only taxon to
373 occur above 38 °C, and which seldom occurred below 15 °C. The findings of the
374 present study contribute novel insights into thermal tolerances and preferences of
375 understudied temporary wetland groups, and thus identifies species which may be most
376 vulnerable to localised pressures as temperatures shift. Overall, all species here have a
377 working thermal range of approximately ~15–40 °C. On the other hand, land surface
378 temperatures are expected to increase beyond 40 °C in future, especially when
379 mitigation measures against climate change fail (Engelbrecht et al., 2015). This
380 represents a potential threat to the fitness of these aquatic organisms in the face of
381 climate change. This threat may be more pronounced in rock pools than pans since
382 rock pools already experience temperatures closer to 40 °C regularly. However,
383 organisms often cope to stressful temperatures through shifting their phenotypes (Sgrò
384 et al., 2016) or through behavioural microhabitat selection (Pincebourde and Woods,
385 2020). The role of these mechanisms in compensating for large branchiopods' fitness
386 under stressful environments is unknown and warrant future investigation.

387 Several factors might have an influence in the observed thermal fitness traits among
388 taxa. Critical thermal limits in aquatic species are known to be limited by several factors
389 (see e.g. Terblanche et al., 2007) including phenotypic plasticity, which is constrained
390 by the genome (Chown and Nicolson, 2004; Sgrò et al., 2016). This limits capacities for

intraspecific tolerance to be different among populations of the same species. Furthermore, insect thermal fitness is highly subtle and constrained by a diversity of other factors including species, age, sex, nutrition, ontogeny, environmental history, and others (Chown and Nicolson, 2004; Bowler and Terblanche, 2008; Nyamukondiwa and Terblanche, 2010). Moreover, critical thermal limits measured here also depend on methodological context. Thus factors such as ramping rate, starting temperature and acclimation temperatures may all have effects on critical activity limits (Terblanche et al., 2007). Overall, these thermal tolerance results suggest that across both rock pool and pan habitats, the studied anostracans are likely to be affected before spinicaudatans by temperature increases associated with climate change. However, anostracans are widely distributed, ranging from extreme cold to extreme hot environments. As such, the reason why anostracans were more vulnerable to warming remains unknown and warrants further investigation. We speculate here, with caveats that anostracans may be more vulnerable because of (1) inhabiting environments close to their thermal limits and (2) that they may not be able to remodel their thermal phenotypes suffice to buffer against climate change effects (see Stillman 2003; van Heerwaarden et al., 2016). In particular, increasing incidences of heat waves (Thuiller, 2007; Tewksbury et al., 2008; Stillman, 2019; Xu et al., 2020) could result in potential population extirpation of vulnerable taxa owing to rapid acute temperature effects, should they exceed thermal maxima. These effects may be particularly adverse in small habitats (i.e., rock pools) where there is a lower potential for refugia from warming effects. Small rock-pools have lower thermal inertia, they warm quicker and warmer waters have lesser oxygen than colder waters (Willmer et al., 2005). Thus, warming

waters may constrain other metabolic processes through oxygen limitation as well as constraining thermal tolerance. Furthermore, the limited capacity for genetic change in large branchiopods, owing to the hatching of eggs from different generations in each hydroperiod (Simovich and Hathaway, 1997), might limit future capacities to adapt to changing climates.

Further work is required to elucidate potential for population-level differences among large branchiopods in discrete wetland habitats, as well as impacts of thermal regime on resting egg hatchability (Al-Tikrity and Grainger, 1990; Tladi et al., 2020). Thermal tolerances and preferences might exhibit population-level differences within species, according to the thermal regimes attributed to different habitat types. The results may additionally be influenced by the organism's thermal history (Crickenberger et al., 2020) and carry-over effects (O'Connor et al., 2014; Dickson et al., 2017). This calls for improvement of experimental methodology e.g. using high throughput assays to measure thermal fitness traits coupled with time analysis software to more accurately predict temperature preference (McMahon et al., 2008; Andreassen, 2019; Awde et al., 2020). Nonetheless, the results suggest that, even where taxa have adapted to different wetland types, large branchiopods thermal fitness appears to be relatively similar within their taxonomic grouping.

Our results suggest that for temporary aquatic habitats, resilience to higher temperatures may be somewhat grounded in taxonomic grouping, with the studied spinicaudatans having significantly higher heat tolerance and preference than anostracans in the region. However, low temperature tolerance was not readily distinguishable between taxonomic groups, given species-specificity in responses.

However, much larger sample sizes are needed before taxa-wide assertions can be made. Anostracans are more widely distributed, across regions that attain very warm and cold conditions, than Spinicaudatans (Thiéry, 1996; Brtek and Mura 2000). However, large branchiopods are largely eurythermal, given that they have evolved for life in small aquatic environments typically characterized by large diurnal temperature fluctuations. As such, the results of this study need to be interpreted with caution as these findings may not be reflective of anostracans and spinicaudatans more broadly. Further work should also seek to elucidate population-level differences in temperature tolerances and preferences in these and other temporary wetland biota, to better predict adaptabilities over time as well as future community composition under changing climates. Furthermore, future work should explore the role of behavioral microhabitat selection and phenotypic plasticity in buffering climate change associated effects on large branchiopod thermal fitness and ecology.

Acknowledgements

We acknowledge the support from Botswana International University of Science and Technology (BIUST) by providing infrastructure and funding to MT, CN and RJW. Gratitude is extended to the Department of Physics and Astronomy (BIUST) for equipment use and to D. Christopher Rogers for assistance in large branchiopod identification. The Ministry of Environment, Natural Resources Conservation and Tourism (Botswana) is thanked for the issuing of a research permit (ENT 8/ 36/4XXXXII [14]). RC acknowledges funding from the Alexander von Humboldt Foundation.

Declarations of interest:

None.

Journal Pre-proof

References

- Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2000. Thermal tolerance, climatic variability and latitude. *Proc Royal Soc London. B: Biol. Sci.* 267, 739–745.
- Akinyemi, F.O., Abiodun, B.J., 2019. Potential impacts of global warming levels 1.5 °C and above on climate extremes in Botswana. *Clim. Change.* 154, 387–400.
- Al-Tikrity, M.R., Grainger, J.N.R. 1990. The effect of temperature and other factors on the hatching of the resting eggs of *Tanytarsus stagnalis* (L.) (crustacea, anostraca). *J. Therm. Biol.* 15, 87–90.
- Andersen, J.L., Manenti, T., Sørensen, J.G., MacMillan, H.A., Loeschcke, V., Overgaard, J., 2015. How to assess *Drosophila* cold tolerance: chill coma temperature and lower lethal temperature are the best predictors of cold distribution limits. *Func. Ecol.* 29, 55–65.
- Andreassen, A.H., 2019. Development of an automated annular arena and thermal preference of zebrafish selected for thermal tolerance. Norwegian University of Science and Technology.
- Arribas, P., Velasco, J., Abellán, P., Sánchez-Fernández, D., Andújar, C., Calosi, P., Millán, A., Ribera, I., Bilton, D.T., 2021. Dispersal ability rather than ecological tolerance drives differences in range size between lentic and lotic water beetles (Coleoptera: Hydrophilidae). *J. Biogeogr.* 39, 984-994.
- Awde, D.N., Fowler, T.E., Pérez-Gálvez, F., Garcia, M.J., Teets, N.M., 2020. High-Throughput Assays of Critical Thermal Limits in Insects. *JoVE*, 160, e61186.
- Azra, M.N., Chen, J.C., Ikhwanuddin, M., Abol-Munafi, A.B., 2018. Thermal tolerance and locomotor activity of blue swimmer crab *Portunus pelagicus* instar reared at different temperatures. *J. Therm. Biol.* 74, 234–240.
- Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* 67, 1–48.

- 490 Batisani, N., Yarnal, B., 2010. Rainfall variability and trends in semi-arid Botswana:
491 implications for climate change adaptation policy. *Appl. Geogr.* 30, 483-489.
- 492 Bartolini, F., Barausse, A., Pörtner, H.O., Giomi, F., 2013. Climate change reduces
493 offspring fitness in littoral spawners: A study integrating organismic response and
494 long-term time-series. *Glob. Chang Biol.* 19, 373–386.
- 495 Bird, M.S., Mlambo, M.C., Wasserman, R.J., Dalu, T., Holland, A., Day, J.A., Villet, M.,
496 Bilton, D., Brendonck, L., Barber-James, H.M., 2019. Deeper knowledge of shallow
497 waters: reviewing the invertebrate fauna of southern African temporary wetlands.
498 *Hydrobiologia.* 827, 89–121.
- 499 Blaustein, L., Schwartz, S.S., 2001. Why study ecology in temporary pools? *Isr. J. Zool.*
500 47, 303–312.
- 501 Bowler, K., Terblanche, J.S., 2008. Insect thermal tolerance: what is the role of
502 ontogeny, ageing and senescence? *Biol. Rev.* 83, 339–355.
- 503 Brendonck, L., 1996. Diapause, quiescence, hatching requirements: what we can learn
504 from large freshwater branchiopods (Crustacea: Branchiopoda: Anostraca,
505 Notostraca, Conchostraca). *Hydrobiologia* 320, 85–97.
- 506 Brendonck, L., Rogers, D. C., Olesen, J., Weeks, S., Hoeh, W. R., 2008. Global
507 diversity of large branchiopods (Crustacea: Branchiopoda) in freshwater.
508 *Hydrobiologia* 595, 167–176.
- 509 Brooks, M.E., Kristensen, F., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen,
510 A., Skaug, H.J., Machler, M., Bolker, B.M., 2017. glmmTMB balances speed and
511 flexibility among packages for zero-inflated generalized linear mixed modeling. *R J.* 9,
512 378–400.
- 513 Brtek, J. and Mura, G., 2000. Revised key to families and genera of the Anostraca with
514 notes on their geographical distribution. *Crustaceana*, pp.1037-1088.
- 515 Buxton, M., Cuthbert, R.N., Dalu, T., Nyamukondiwa, C., Dick, J.A., Wasserman, R.J.,
516 2020. Temporary wetland predator diversity favours larval mosquito control. *Biol.*
517 *Control.* 144, 104216.

- Calhoun, A.J., Mushet, D.M., Bell, K.P., Boix, D., Fitzsimons, J.A., Isselin-Nondedeu, F.,
2017. Temporary wetlands: challenges and solutions to conserving a
'disappearing' ecosystem. *Biol. Conserv.* 211, 3–11.
- Calosi, P., Bilton, D. T., Spicer, J. I., 2008. Thermal tolerance, acclimatory capacity and
vulnerability to global climate change. *Biol. Lett.* 4, 99–102.
- Calosi, P., Bilton, D.T., Spicer, J.I., Votier, S.C., Atfield, A., 2010. What determines a
species' geographical range? Thermal biology and latitudinal range size relationships
in European diving beetles (Coleoptera: Dytiscidae). *J. Anim. Ecol.* 79, 194–204.
- Carrino-Kyker, S.R., Swanson, A.K., 2007. Seasonal physicochemical characteristics of
thirty northern Ohio temporary pools along gradients of GIS-delineated human land-
use. *Wetlands* 27, 749–760.
- Carroll, S.P., Hendry, A.P., Reznick, D.N., Fox, C.W., 2007. Evolution on ecological
time-scales. *Funct. Ecol.* 21, 387–393.
- Chown, S.L., Nicolson, S.W., 2004. *Insect Physiological Ecology: Mechanisms and
Patterns*. Oxford University Press, New York.
- Crickenberger, S., Hui, T.Y., Yuan, F.L., Bonebrake, T.C., Williams, G.A., 2020.
Preferred temperature of intertidal ectotherms: broad patterns and methodological
approaches. *J. Therm. Biol.* 87, 102468.
- Dallas, H., 2008. Water temperature and riverine ecosystems: An overview of
knowledge and approaches for assessing biotic responses, with special reference to
South Africa. *Water Sa*, 34, 393-404.
- Dallas, H.F., Ketley, Z.A., 2011. Upper thermal limits of aquatic macroinvertebrates:
comparing critical thermal maxima with 96-LT50 values. *J. Therm. Biol.* 36, 322–327.
- de la Fuente, A. and Meruane, C., 2017. Spectral model for long-term computation of
thermodynamics and potential evaporation in shallow wetlands. *Water Resour. Res.*,
53, 7696-7715.

- 544 Deutsch, C.A., Tewksbury, J.J., Tigchelaar, M., Battisti, D.S., Merrill, S.C., Huey, R.B.,
 545 Naylor, R.L., 2018. Increase in crop losses to insect pests in a warming climate.
 546 Science 361, 916–919.
- 547 Dickson, L.B., Jiolle, D., Minard, G., Moltini-Conclois, I., Volant, S., Ghoulane, A.,
 548 Bouchier, C., Ayala, D., Paupy, C., Moro, C.V., Lambrechts, L., 2017. Carryover
 549 effects of larval exposure to different environmental bacteria drive adult trait variation
 550 in a mosquito vector. Sci. Adv. 3, e1700585.
- 551 Dillon, M.E., Wang, G., Garriety, P.A., Huey, R.B., 2009. Thermal preference in
 552 *Drosophila*. J. Therm. Biol. 34, 109–119.
- 553 Dillon, M.E., Wang, G., Huey, R.B., 2010. Global metabolic impacts of recent climate
 554 warming. Nature 467, 704.
- 555 Dzialowski, E.M., 2005. Use of operative temperature and standard operative
 556 temperature models in thermal biology. J. Therm. Biol. 30, 317–334.
- 557 Engelbrecht, F., Adegoke, J., Bopape, M.J., Naidoo, M., Garland, R., Thatcher, M.,
 558 McGregor, J., Katzfey, J., Werner, M., Ichoku, C., Gatebe, C., 2015. Projections of
 559 rapidly rising surface temperatures over Africa under low mitigation. Environ. Res.
 560 Lett. 10, 085004.
- 561 Fine, P.V., 2015. Ecological and evolutionary drivers of geographic variation in species
 562 diversity. Annu Rev Ecol Evol Syst. 46, 369–392.
- 563 Forster, J., Hirst, A.G., Atkinson, D., 2012. Warming-induced reductions in body size are
 564 greater in aquatic than terrestrial species. Proc Natl Acad Sci. 109, 19310–19314.
- 565 Goudie, A.S., Wells, G.L., 1995. The nature, distribution and formation of pans in arid
 566 zones. Earth-Sci. Rev. 38, 1–69.
- 567 Graham, T.B., Wirth, D., 2008. Dispersal of large branchiopod cysts: potential
 568 movement by wind from potholes on the Colorado Plateau. Hydrobiologia 600, 17–
 569 27.

- Gunderson, A.R., Armstrong, E.J., Stillman, J.H., 2016. Multiple stressors in a changing world: the need for an improved perspective on physiological responses to the dynamic marine environment. *Ann Rev Mar Sci.* 8, 357–378.
- Gutiérrez-Pesquera, L.M., Tejedo, M., Olalla-Tárraga, M.Á., Duarte, H., Nicieza, A., Solé, M., 2016. Testing the climate variability hypothesis in thermal tolerance limits of tropical and temperate tadpoles. *J. Biogeogr.* 43, 1166–1178.
- Hamer, M.L., Brendonck, L., 1997. Distribution, diversity and conservation of Anostraca (Crustacea: Branchiopoda) in southern Africa. In *Studies on Large Branchiopod Biology and Conservation*. Springer, Dordrecht.
- Hamer, M.L., Martens, K., 1998. The large Branchiopoda (Crustacea) from temporary habitats of the Drakensberg region, South Africa. *Hydrobiologia* 384, 151–165.
- Hartig, F., 2020. DHARMA: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models. R package version 0.3.2.0.
- Helm, B.P., 1998. Biogeography of eight large branchiopods endemic to California. In *Ecology, Conservation, and Management of Vernal Pool Ecosystems—Proceedings from a 1996 Conference*. California Native Plant Society, Sacramento, California, 124–139.
- Hering, D., Schmidt-Kloiber, A., Murphy, J., Lücke, S., Zamora-Munoz, C., López-Rodríguez, M.J., Huber, T., Graf, W., 2009. Potential impact of climate change on aquatic insects: a sensitivity analysis for European caddisflies (Trichoptera) based on distribution patterns and ecological preferences. *Aquat Sci.* 71, 3–14.
- Hoffmann, A.A., Chown, S.L., Clusella-Trullas, S., 2013. Upper thermal limits in terrestrial ectotherms: how constrained are they? *Funct Ecol.* 27, 934–949.
- Huey, R.B., Kingsolver, J.G., 1993. Evolution of Resistance to High Temperature in Ectotherms. *Am. Nat.* 142, s21–s46.
- Huey, R.B., Stevenson, R.D., 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *Am. Zool.* 19, 357–366.

- 597 Kenabatho, P.K., Parida, B.P., Moalafhi, D.B., 2012. The value of large-scale climate
598 variables in climate change assessment: The case of Botswana's rainfall. *Phys.*
599 *Chem. Earth* 50, 64-71.
- 600 Kingsolver, J.G., 1989. Weather and the population dynamics of insects: integrating
601 physiological and population ecology. *Physiol. Zool* 62, 314–334.
- 602 Kusangaya, S., Warburton, M.L., Van Garderen, E.A., Jewitt, G.P., 2014. Impacts of
603 climate change on water resources in southern Africa: A review. *Phys. Chem. Earth*
604 67, 47–54.
- 605 Lagerspetz, K.Y., Vainio, L.A., 2006. Thermal behaviour of crustaceans. *Biol. Rev.* 81,
606 237–258.
- 607 Leroi, A.M., Bennett, A.F., Lenski, R.E., 1994. Temperature acclimation and competitive
608 fitness: an experimental test of the beneficial acclimation assumption. *Proc. Natl.*
609 *Acad. Sci.* 91, 1917–1921.
- 610 Loeschcke, V., Hoffmann, A.A., 2007. Consequences of heat hardening on a field fitness
611 component in *Drosophila* depend on environmental temperature. *Am. Nat.* 169, 175–
612 183.
- 613 Lutterschmidt, W.I., Hutchison, V.H., 1997. The critical thermal maximum: history and
614 critique. *Can. J. Zool.* 75, 1561–1574.
- 615 Mabidi, A., Bird, M.S., Perissinotto, R., Rogers, D.C., 2016. Ecology and distribution of
616 large branchiopods (Crustacea, Branchiopoda, Anostraca, Notostraca, Laevicaudata,
617 Spinicaudata) of the Eastern Cape Karoo, South Africa. *ZooKeys.*, 15.
- 618 Machekano, H., Mutamiswa, R., Singano, C., Joseph, V., Chidawanyika, F.,
619 Nyamukondiwa, C., 2020. Thermal resilience of *Prostephanus truncatus* (Horn): Can
620 we derive optimum temperature-time combinations for commodity treatment? *J.*
621 *Stored Prod. Res.* 86, 101568.
- 622 Mahoney, D.L., Mort, M.A., Taylor, B.E., 1990. Species richness of calanoid copepods,
623 cladocerans and other branchiopods in Carolina bay temporary ponds *Am. Midl. Nat.*
624 244–258.

- 625 Martin, J.W., Rogers, D.C., Olesen, J., 2016. Collecting and processing branchiopods.
626 J. Crust. Biol. 36, 396–401.
- 627 Maúre, G., Pinto, I., Ndebele-Murisa, M., Muthige, M., Lennard, C., Nikulin, G., Dosio,
628 A. and Meque, A., 2018. The southern African climate under 1.5 C and 2 C of global
629 warming as simulated by CORDEX regional climate models. Environ. Res. Lett, 13,
630 p.065002.
- 631 McMahon, T.E., Bear, E.A., Zale, A.V., 2008. Use of an annular chamber for testing
632 thermal preference of westslope cutthroat trout and rainbow trout. J. Freshw. Ecol.
633 23, 55–63.
- 634 Meehl, G.A., Tebaldi, C., 2004. More intense, more frequent, and longer lasting heat
635 waves in the 21st century. Science 305, 994–997.
- 636 Nyamukondiwa, C., Terblanche, J.S., 2010. Within-generation variation of critical
637 thermal limits in adult Mediterranean and Natal fruit flies *Ceratitis capitata* and
638 *Ceratitis rosa*: thermal history affects short-term responses to temperature. Physiol.
639 Entomol. 35, 255–264.
- 640 O'Connor, C.M., Norris, D.R., Crossin, G.T., Cooke, S.J., 2014. Biological carryover
641 effects: linking common concepts and mechanisms in ecology and evolution.
642 Ecosphere 5, 1–11.
- 643 Ogle, D.H., Wheeler, P., Dinno, A., 2020. FSA: Fisheries Stock Analysis. R package
644 version 0.8.30.
- 645 Parmesan, C., Yohe, G., 2003. A globally coherent fingerprint of climate change
646 impacts across natural systems. Nature 421: 37–42.
- 647 Parmesan, C., 2006. Ecological and evolutionary responses to recent climate change.
648 Annu. Rev. Ecol. Evol. Syst. 37, 637–669.
- 649 Pincebourde, S., Woods, H.A., 2020. There is plenty of room at the bottom:
650 microclimates drive insect vulnerability to climate change. Curr. Opin. Insect. Sci. In
651 press

- 652 R Core Team, 2020. R: A language and environment for statistical computing. R
653 Foundation for Statistical Computing, Vienna, Austria.
- 654 Rezende, E.L., Castañeda, L.E. and Santos, M., 2014. Tolerance landscapes in thermal
655 ecology. *Funct. Ecol.* 28, 799-809.
- 656 Robinet, C., Roques, A., 2010. Direct impacts of recent climate warming on insect
657 populations. *Integr. Zoo?* 5, 132–142.
- 658 Salachan, P.V., Sørensen, J.G., 2017. Critical thermal limits affected differently by
659 developmental and adult thermal fluctuations. *J. Exp. Biol.* 220, 4471–4478.
- 660 Samraoui, B., Chakri, K., Samraoui, F., 2006. Large branchiopods (Branchiopoda:
661 Anostraca, Notostraca and Spinicaudata) from the salt lakes of Algeria. *J. Limnol.* 65,
662 83–88.
- 663 Sanders, N.J., Lessard, J.P., Fitzpatrick, M.C., Dunn, R.R., 2007. Temperature, but not
664 productivity or geometry, predicts elevational diversity gradients in ants across spatial
665 grains. *Glob. Ecol. Biogeogr.* 16, 640–649.
- 666 Sgrò, C.M., Terblanche, J.S., Hoffmann, A.A., 2016. What can plasticity contribute to
667 insect responses to climate change?. *Annu. Rev. Entomol.* 61, 433–451.
- 668 Simovich, M.A., Hathaway, S.A., 1997. Diversified bet-hedging as a reproductive
669 strategy of some ephemeral pool anostracans (Branchiopoda). *J. Crust. Biol.* 17, 38–
670 44.
- 671 Sinclair, B.J., Alvarado, E.C.L., Ferguson, E.L. 2015. An invitation to measure insect
672 cold tolerance: Methods, approaches, and workflow. *J. Ther. Biol.* 53, 180-97.
- 673 Sørensen, J.G., Loeschcke, V., 2001. Larval crowding in *Drosophila melanogaster*
674 induces Hsp70 expression, and leads to increased adult longevity and adult thermal
675 stress resistance. *J. Insect Physiol.* 47, 1301–07.
- 676 Stein, A., Gerstner, K., Kreft, H., 2014. Environmental heterogeneity as a universal
677 driver of species richness across taxa, biomes and spatial scales. *Ecol. Lett.* 17,
678 866–880.

- 679 Stillman, J.H., 2003. Acclimation capacity underlies susceptibility to climate change.
680 Science 301, 65–65.
- 681 Stillman, J.H., 2019. Heat waves, the new normal: summertime temperature extremes
682 will impact animals, ecosystems, and human communities. Physiology (Bethesda)
683 34, 86–100.
- 684 Stillman, J.H., Somero, G.N., 2000. A comparative analysis of the upper thermal
685 tolerance limits of eastern Pacific porcelain crabs, genus *Petrolisthes*: influences of
686 latitude, vertical zonation, acclimation, and phylogeny. *Physiol. Biochem. Zool.* 73,
687 200–208.
- 688 Sunday, J.M., Bates, A.E., Dulvy, N.K., 2012. Thermal tolerance and the global
689 redistribution of animals. *Nat. Clim. Chang.* 2, 686–690.
- 690 Tang, Y., Chen, Z.Q., Lin, Y.F., Chen, J.Y., Ding, G.H., Ji, X., 2020. The combined
691 effects of temperature and aromatase inhibitor on metamorphosis, growth,
692 locomotion, and sex ratio of tiger frog (*Hoplobatrachus rugulosus*) tadpoles. *PeerJ* 8,
693 e8834.
- 694 Terblanche, J.S., Deere, J.A., Clusella-Trullas, S., Janion, C., Chown, S.L., 2007.
695 Critical thermal limits depend on methodological context. *Proc. Royal Soc. B.* 274,
696 2935–2943.
- 697 Terblanche, J.S., Hoffmann, A.A., Mitchell, K.A., Rako, L., le Roux, P.C., Chown, S.L.,
698 2011. Ecologically relevant measures of tolerance to potentially lethal temperatures.
699 *J. Exp. Biol.* 214, 3713–3725.
- 700 Tewksbury, J.J., Huey, R.B., Deutsch, C.A., 2008. Putting the heat in tropical animals.
701 Science 320, 1296–1297.
- 702 Tews, J., Brose, U., Grimm, V., Tielbörger, K., Wichmann, M.C., Schwager, M., Jeltsch,
703 F., 2004. Animal species diversity driven by habitat heterogeneity/diversity: the
704 importance of keystone structures. *J. Biogeogr.* 31, 79–92.

- Thiéry, A., 1996. Large Branchiopods (Crustacea: Anostraca, Notostraca, Spinicaudata, Laevicaudata) from temporary inland waters of the Arabian Peninsula. *Fauna of Saudi Arabia*, Vol. 15., pp.37-98.
- Thuiller, W., 2007. Biodiversity: climate change and the ecologist. *Nature*. 448, 550–552.
- Timms, B.V., 2006. The large branchiopods (Crustacea: Branchiopoda) of gnammas (rock holes) in Australia. *J R Soc West Aust*. 89, 163.
- Tladi, M., Dalu, T., Rogers, D.C., Nyamukondiwa, C., Parbhu, S.P., Teske, P.R., Emami-Khoyi, A., Wasserman, R.J., 2020. The complete mitogenome of the fairy shrimp *Streptocephalus cafer* (Lovén, 1847) (Crustacea: Branchiopoda: Anostraca) from an ephemeral pond in Botswana, southern Africa. *Mitochondrial DNA B*. 5, 623–625.
- Tladi, M., Nyamukondiwa, C., Cuthbert, R., Wasserman, R.J. (2020). Emergent effects of light and temperature on hatching success of *Streptocephalus cafer* resting eggs. *Austral Ecol*. 45, 1062–1066.
- Van Buskirk, J., Steiner, U.K., 2009. The fitness costs of developmental canalization and plasticity. *J. Evol. Biol*. 22, 852–860.
- Van Heerwaarden, B., Kellerman, V., Sgrò, C.M. 2016. Limited scope for plasticity to increase upper thermal limits. *Funct. Ecol*. 30, 1947–1956.
- Vanschoenwinkel, B., Hulsmans, A., De Roeck, E., De Vries, C., Seaman, M., Brendonck, L., 2009. Community structure in temporary freshwater pools: Disentangling the effects of habitat size and hydroregime. *Freshw. Biol*. 54, 1487–1500.
- Verberk, W.C., Leuven, R.S., van der Velde, G., Gabel, F., 2018. Thermal limits in native and alien freshwater peracarid Crustacea: the role of habitat use and oxygen limitation. *Funct Ecol*. 32, 926–936.

- 731 Verberk, W.C., Overgaard, J., Ern, R., Bayley, M., Wang, T., Boardman, L., Terblanche,
732 J.S., 2016. Does oxygen limit thermal tolerance in arthropods? A critical review of
733 current evidence. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* 192, 64–78.
- 734 Wang, C.C., Rogers, D.C., 2018. Bet hedging in stochastic habitats: an approach
735 through large branchiopods in a temporary wetland. *Oecologia* 188, 1081–1093.
- 736 Williams, D.D., 2002. Temporary water crustaceans: biodiversity and habitat loss. In
737 *Modern Approaches to the Study of Crustacea*, Springer, Boston, MA, pp. 223-233.
- 738 Willmer, P., Stone, G., Johnston, I., 2009. *Environmental physiology of animals*, 2nd ed.
739 Blackwell, Malden, MA.
- 740 Xu, C., Kohler, T.A., Lenton, T.M., Svenning, J.C., Scheffer, M., 2020. Future of the
741 human climate niche. *Proc. Natl. Acad. Sci.* 117, 11350–11355.

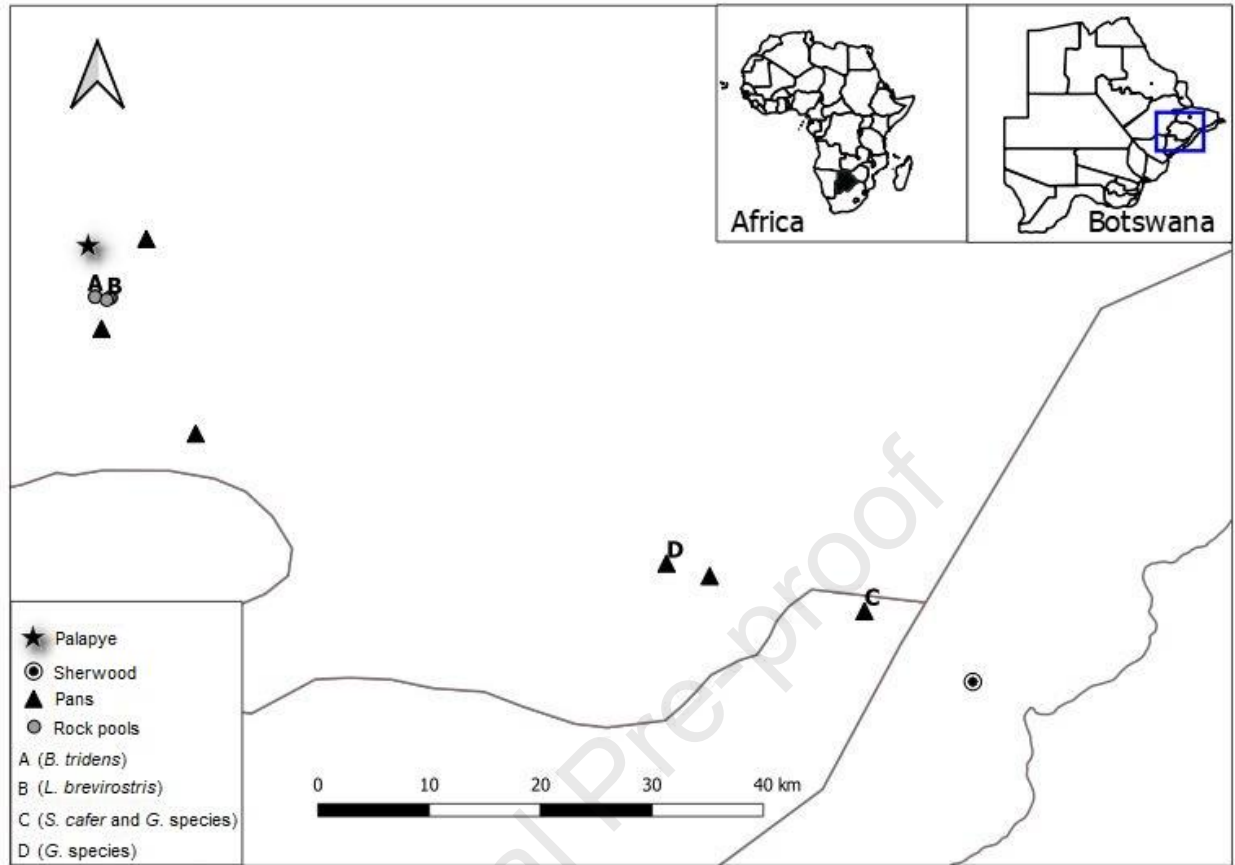


Figure 1. Locations of the rock-pools and pans in which, temperature loggers were deployed in central district, Botswana. For the lab-based experiments, *Branchiopodopsis tridens* was collected from rock-pool A and *Leptestheria brevirostris* from rock-pool B, while *Streptocephalus cafer* was collected from pan C and *Gondwanalimnadia* sp. from pans C & D

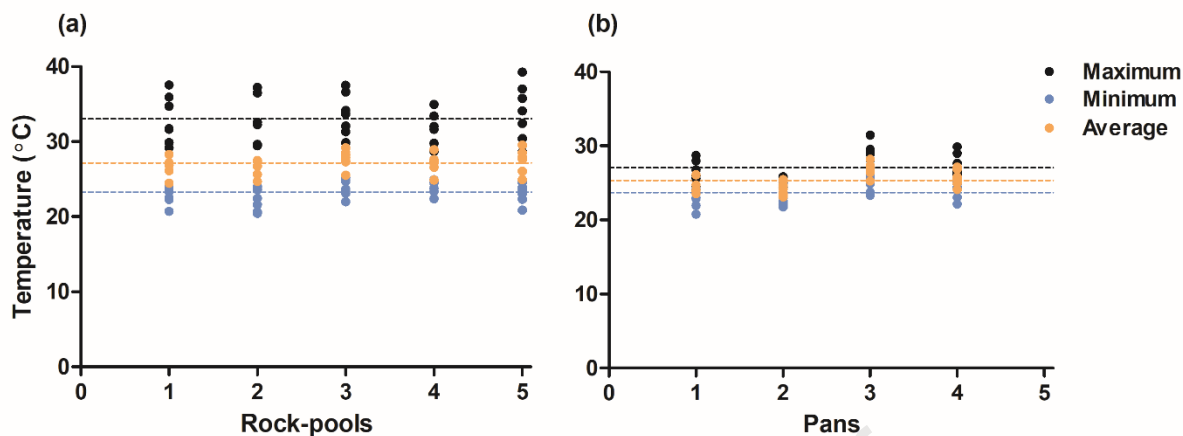


Figure 2. Daily habitat maximum (HT_{max}), minimum (HT_{min}) and average (HT_{mean}) values from a) rock-pools and b) pans. Values were derived between 18-23 January 2020 when all wetlands were inundated at the same time, facilitating direct comparison. Raw data are points. Hashed lines are mean values of all data points.

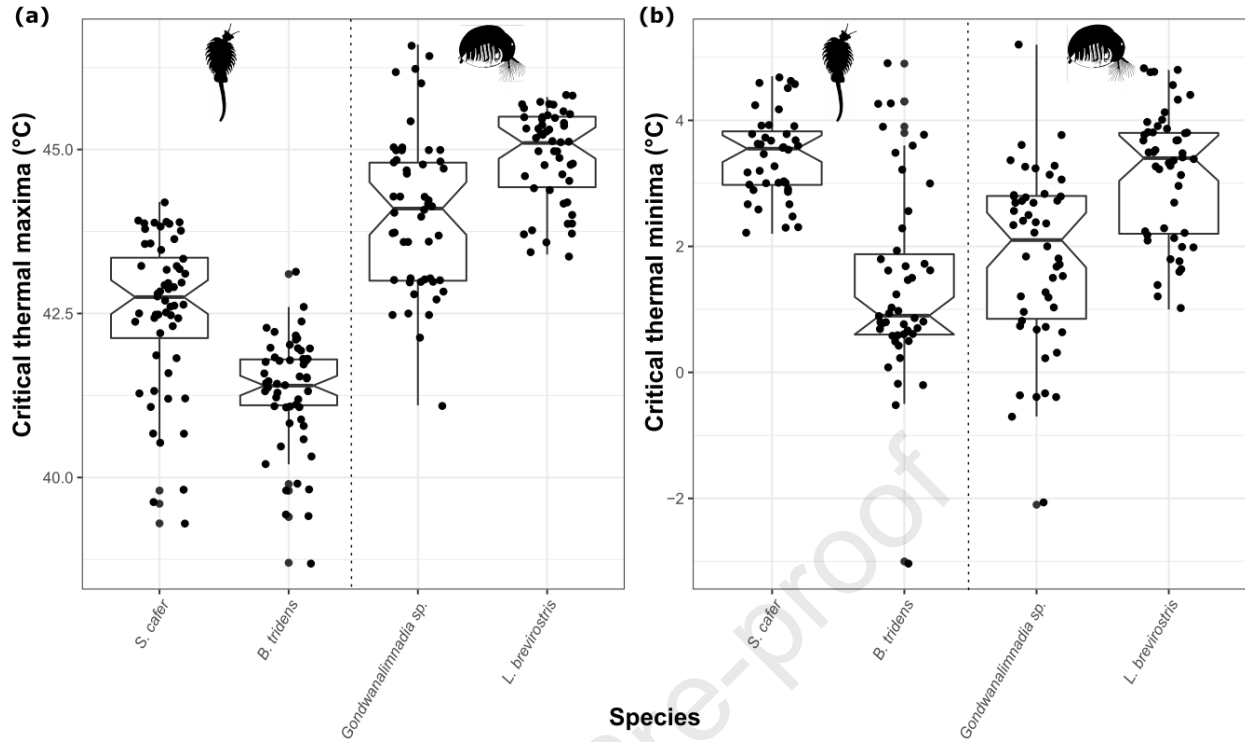


Figure 3. Box plots showing (a) critical thermal maxima of *Streptocephalus cafer* ($n = 56$), *Branchiopodopsis tridens* ($n = 54$), *Gondwanalimnadia* sp. ($n = 50$) and *Leptestheria brevirostris* ($n = 54$) (°C) and (b) critical thermal minima (°C) of *Streptocephalus cafer* ($n = 50$), *Branchiopodopsis tridens* ($n = 46$), *Gondwanalimnadia* sp. ($n = 50$) and *Leptestheria brevirostris* ($n = 49$). Jittered points are raw data. In the boxplots, the horizontal bar displays the median, the box gives the interquartile ranges and the whiskers show the largest and smallest values up to $1.5 \times$ interquartile range.

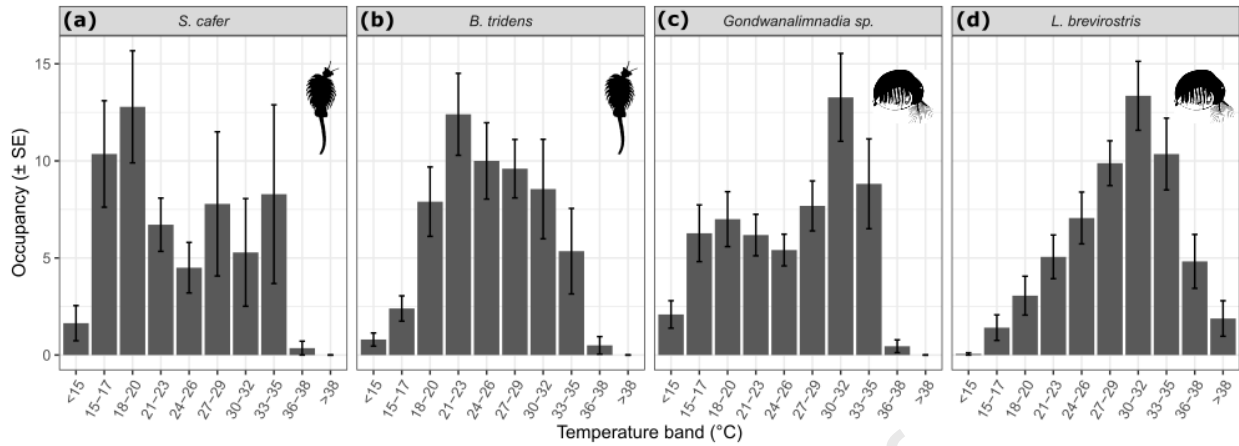
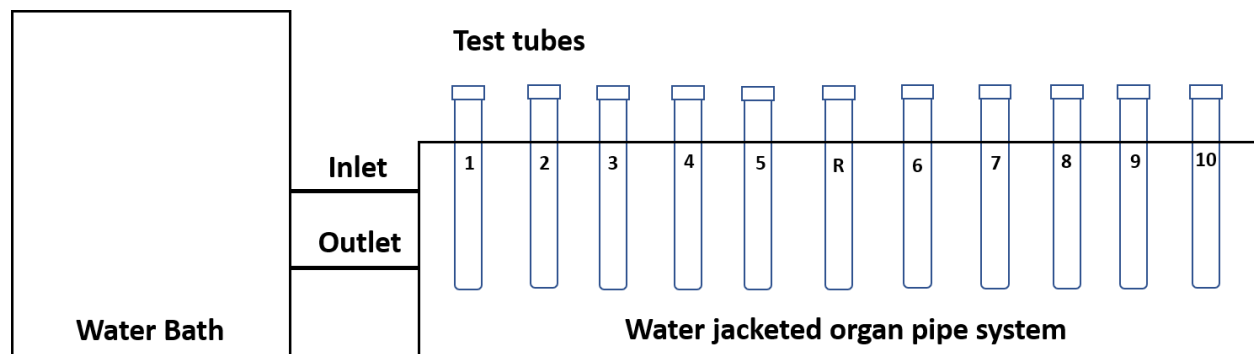


Figure 4. Thermal preference occurrences of *Streptocephalus cafer*, *Branchiopodopsis tridens*, *Gondwanalimnadia* sp. and *Leptestheria brevisrostris* across temperature bands. Medians are shown alongside standard errors (SE).

Electronic Supplementary Material

Table S1. Locations of the rock pools and pans used for thermal characterization during hydroperiods, as well as large branchiopod species encountered in each wetland. Programmable data logger (0.5°C accuracy) were set to 1 h sampling frequencies and deployed, on the bottom in the centre of each wetland, and allowed to record temperatures between December 2019 and February 2020. Wetlands used to sample animals for the experimental components of the study are outlined (A,B,C,D) and correspond with Figure 1. *B. tridens* = *Branchiopodopsis tridens*, *L. brevisrostris* = *Leptestheria brevisrostris*, *S. cafer* = *Streptocephalus cafer*.

Wetland	GPS	Dimensions when full: length (m) × width (m)	Studied species present	Wetland code (see Fig. 1)
Rockpool 1	22°35'48.4"S 27°08'05.5"E	4.2 × 4.0	<i>B. tridens</i>	
Rockpool 2	22°35'49.6"S 27°07'59.6"E	5.8 × 5.5	<i>B. tridens</i>	
Rockpool 3	22°35'45.8"S 27°07'15.8"E	3.4 × 2.6	<i>B. tridens</i>	
Rockpool 4	22°35'46.1"S 27°07'16.5"E	2.4 × 2.6	<i>B. tridens</i>	A
Rockpool 5	22°35'55.6"S 27°07'51.5"E	1.5 × 0.5	<i>B. tridens</i> , <i>L. brevisrostris</i>	B
Pan 1	22°37'26.4"S 27°07'35.3"E	50 × 50	<i>S. cafer</i>	
Pan 2	22°49'45.9"S 27°37'18.1"E	14 × 10	<i>S. cafer</i> , <i>Gondwanalimnadia</i> sp.	D
Pan 3	22°52'16.0"S 27°47'42.7"E	76 × 54	<i>S. cafer</i> , <i>Gondwanalimnadia</i> sp.	C
Pan 4	22°42'56.8"S 27°12'32.4"E	142 × 1.5	<i>S. cafer</i> , <i>Gondwanalimnadia</i> sp.	
Pan 5	22°50'25.5"S 27°39'34.2"E	55 × 21	<i>S. cafer</i> , <i>Gondwanalimnadia</i> sp.	



784 **Figure S1.** Organ pipe design for the critical thermal limit experiment. The programmed
785 water bath (20L) regulates the heat in the test tubes through circulation of temperature
786 controlled 1:1 water:propylene glycol through the jacket system. Ten replicate test tubes
787 (1-10) house the test animals, while the reference test tube (R) is used to monitor
788 temperature within the test tubes.

a)

Cold Water Bath (5°C)	~10°C	Lane 1	~40°C	Warm Water Bath (50°C)
	~10°C	Lane 2	~40°C	
	~10°C	Lane 3	~40°C	

b)

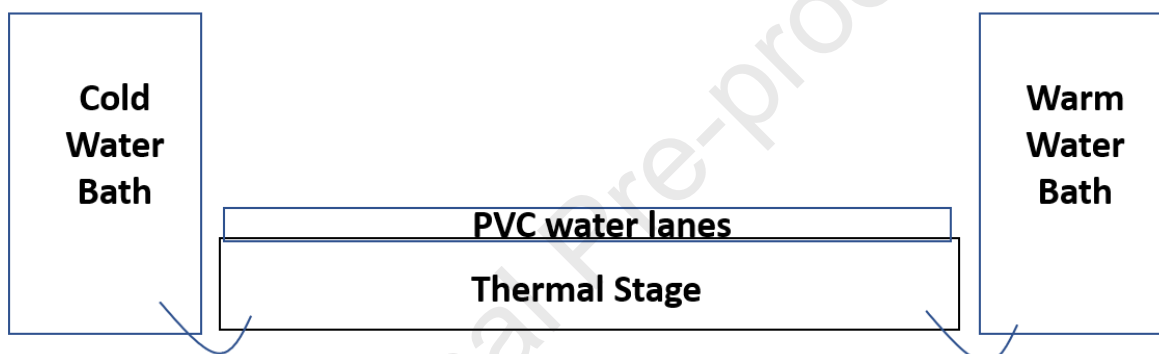


Figure S2. Schematic representation of the thermal stage set-up showing a) aerial view of the 3-laned PVC pipes, each holding water, and b) lateral view of the PVC lanes on top of the metal thermal gradient stage, fed by cold water on the left and warm water on the right from respective water baths.

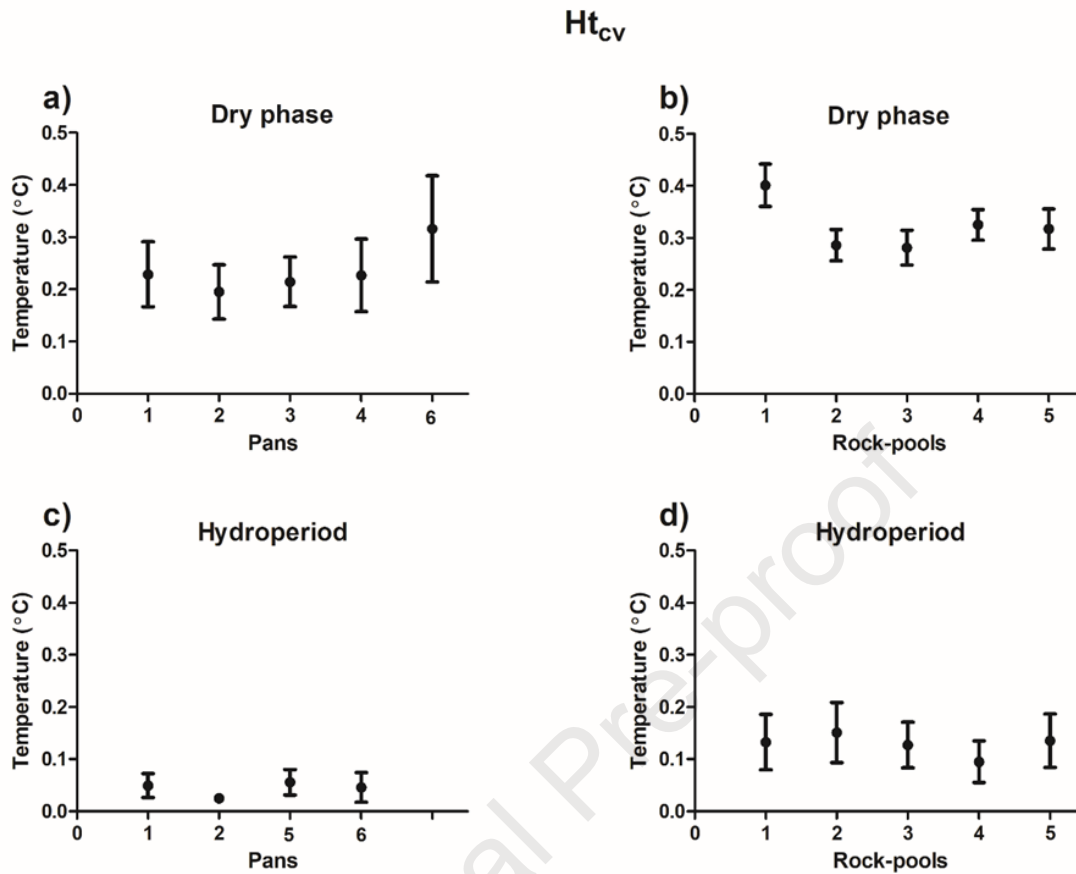


Figure S3. Mean (\pm standard deviation) daily habitat coefficient of variation in temperatures (Ht_{cv}) across five pans (a and c) and rock-pools (b and d) in the Palapye region, during a synchronised dry phase (2-7 November 2019) and hydroperiod (18-24 January 2020).

Highlights

- Thermal biology of rock-pool and pan specialist branchiopods were contrasted
- wetland type was not a good predictor of branchiopod thermal preference/limits
- spinicaudatans preferring higher temperatures than anostracans
- spinicaudatans were more tolerant of high temperatures than anostracans
- anostracans may be more susceptible to projected climatic warming