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

3

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13

14

15 **Abstract**

16 Large branchiopods are specialist crustaceans adapted for life in temporary, thermally
17 dynamic wetland ecosystems. Certain large branchiopod species are, however,
18 restricted to specific temporary wetland types, exemplified by their physico-chemical
19 and hydroperiod characteristics. Here, we contrasted the thermal preference and critical
20 thermal maxima (CT_{max}) and minima [CT_{min}] of southern African anostracans and
21 spinicaudatans found exclusively in either temporary rock-pool or pan wetland types.
22 We hypothesised that environment of origin would be a good predictor of thermal
23 preference and critical thermal limits. To test this, *Branchiopodopsis tridens* (Anostraca)
24 and *Leptestheria brevirostris* (Spinicaudata) were collected from rock-pool habitats,
25 while *Streptocephalus cafer* (Anostraca) and a *Gondwanalimnadia* sp. (Spinicaudata)
26 were collected from pan habitats. In contrast to our hypothesis, taxonomic relatedness
27 was a better predictor of CT_{max} and temperature preference than environment of origin.
28 Spinicaudatans were significantly more tolerant of high temperatures than anostracans,
29 with *L. brevirostris* and *Gondwanalimnadia* sp. median CT_{max} values of 45.1 °C and 44.1
30 °C, respectively, followed by *S. cafer* (42.8 °C) and *B. tridens* (41.4 °C). Neither
31 environment or taxonomic relatedness were good predictors of CT_{min} trends, with *B.*
32 *tridens* (0.9 °C) and *Gondwanalimnadia* sp. (2.1 °C) having the lowest median CT_{min}
33 values, followed by *L. brevirostris* (3.4 °C) and *S. cafer* (3.6 °C). On the contrary,
34 temperature preferences differed according to taxa, with spinicaudatans significantly
35 preferring higher temperatures than anostracans. *Leptestheria brevirostris* and
36 *Gondwanalimnadia* sp. both spent most time at temperatures 30-32 °C, *S. cafer* at 18-
37 20 °C and *B. tridens* at 21-23 °C. Constrained thermal traits reported here suggest that

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

41

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

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46 **Introduction**

47 The density of temporary depression wetlands in the southern African region is among
48 the highest worldwide (Goudie and Wells, 1995; Hamer and Brendonck, 1997).
49 However, even within these unique systems, there are a variety of distinct temporary
50 wetland habitat types which include vernal and rock pools among others (Calhoun et al.,
51 2017). Habitat heterogeneity is a driver of biological diversity and is important for the
52 maintenance of species (Tews et al., 2004; Stein et al., 2014; Fine, 2015). On an
53 evolutionary time-scale, abiotic features determine biological inhabitant characteristics
54 for fitness, while at the ecological time-scale, these features drive biological community
55 dynamics (Carroll et al., 2007). However, climate change is expected to affect
56 temporary wetlands in a number of ways, including shifts in acute and chronic
57 temperature dynamics (Meehl and Tebaldi, 2004; Stillman, 2019; Xu et al., 2020) as
58 well as inundation patterns associated with shifting rainfall and evaporation dynamics
59 (Kusangaya et al., 2014). Temperature is one of the main environmental factors driving
60 ectotherm fitness (Sanders et al., 2007; Stein et al., 2014). As such, there has been
61 increasing interest from ecologists on elucidating how changes in climate will likely
62 affect invertebrate biodiversity (e.g. Parmesan and Yohe, 2003; Parmesan, 2006;
63 Thuiller, 2007; Deutsch et al., 2018). Lower and upper thermal activity limits of
64 invertebrates are significant predictors of organismal performance, fitness,
65 biogeography and overall survival and are thus often used for predicting ectotherm
66 responses to shifting environments (Chown and Nicolson, 2004; Calosi et al., 2010;
67 Deutsch et al., 2018; Arribas et al., 2012). However, most empirical studies have
68 focused on terrestrial (Addo-Bediako et al., 2000; Dillon et al., 2010; Hoffman et al.,

69 2013; Deustch et al., 2018) and marine (Stillman and Somero 2000; Stillman, 2003;
70 Gunderson et al., 2016) organisms. The few studies on thermal regimes of freshwater
71 systems in the region were carried out in river systems (Dallas, 2008; Dallas and Ketley,
72 2011). Only little information is available on the thermal physiology of temporary wetland
73 specialist fauna in the region (see Lagerspetz and Vainio, 2006).

74 Determining the thermal activity limits of organisms is fundamental for biological and
75 autecological investigations, providing insight on fitness and habitat suitability (Huey
76 and Stevenson, 1979; Stillman and Somero, 2000; Stillman, 2003; Chown and Nicolson,
77 2004; Andersen et al., 2015). Thermal activity limits can provide insight on critical
78 temperature thresholds and preferred temperatures of organisms, aiding in the
79 understanding of population distribution and even sink and source dynamics
80 (Dzialowski, 2005; Calosi et al., 2008; 2010; Sunday et al., 2012). It is also useful for
81 predicting how environmental changes may alter species spatio-temporal population
82 phenologies, abundance and dynamics (Kingsolver, 1989; Chown and Nicolson, 2004;
83 Robinet and Roques, 2010). Tolerance has been quantified using either a static
84 method, time of collapse at a constant stressful temperature, or a dynamic method,
85 where the end point temperature of collapse is measured by increasing or decreasing
86 temperature at a constant ramping rate (Rezende et al., 2014). Lower and upper
87 thermal limits investigations typically explore critical activity endpoints e.g. critical
88 thermal minima (CT_{\min}) and maxima (CT_{\max}) (Lutterschmidt and Hutchison, 1997;
89 Terblanche et al., 2011; Andersen et al., 2015), while others explore temperature
90 preferences (e.g. Dillon et al., 2009; Hering et al., 2009). Critical thermal limits
91 assessment determines the activity ranges upon which an organism operates optimally

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

102
103 Large branchiopods are specialist temporary wetland crustaceans occupying aquatic
104 habitats that rely on rain, snow melt, underground springs and even condensation
105 (Blaustein and Schwartz, 2001). The large branchiopods are comprised of clam shrimps
106 (Spinicaudata, Laevicaudata, and Cycletherida), fairy shrimps (Anostraca) and tadpole
107 shrimps (Notostraca) (Brendonck et al., 2008). These organisms have adapted for life in
108 temporary aquatic ecosystems through the development of rapid life cycles and the
109 production of dormant eggs (Brendonck, 1996). Usually, these habitats remain dry for
110 variable periods during the year and have unpredictable inundation patterns
111 (Vanschoenwinkel et al., 2009; Martin et al., 2016). As such, individuals that emerge
112 from dormant eggs following inundation need to develop rapidly, attain sexual maturity
113 and produce eggs. The group typically produces resting stage eggs (cysts) that can
114 remain in sediment for many years before hatching (Wang and Rogers, 2018).

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

135

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

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

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

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

169

170 **Materials and Methods**

171 **Study site and pond temperature monitoring**

172 The study was conducted in the Central District of Botswana, between the towns of
173 Sherwood (22°56'3.78"S, 27°53'28.52"E) and Palapye (22°32'59.68"S, 27° 7'59.66"E)
174 (Fig. 1). The study region is classified as arid, with a high mean annual temperature of
175 28.5 °C and a low total rainfall of 443.8 mm (Kenabatho et al., 2012; Akinyemi and
176 Abiodun, 2019). The region typically receives rainfall between October and April
177 (Batisani and Yarnal, 2010). For the first component of the study, two major wetland
178 types were categorized, rock pools and pans, each containing Anostraca and
179 Spinicaudata representatives. Five such rock pools and five pans were selected for
180 thermal characterization over their hydroperiods (Table S1), of which some were also
181 used for the collection of animals (component 2: see section below for details). For this
182 first component, programmable data logger probes and software (HOBOWare Pro,
183 version 3.7.16, Massachusetts, USA) were used for the monitoring of temperatures in

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

187

188 **Species selection and collection**

189 Preliminary surveillance showed anostracan *Branchiopodopsis tridens* Daday, 1910 and
190 spinicaudatan *Leptestheria brevirostris* Barnard, 1924 were found in rock pools. In
191 turn, the anostracan *Streptocephalus cafer* (Lovén, 1847) and spinicaudatan
192 *Gondwanalimnadia* sp. were consistently found in pans between Palapye and
193 Sherwood. The second component of the study comprised lab-based experiments. For
194 the experimental components, animals were collected during the austral summer rainy
195 season in 2019 and 2020. From rock pools and pans, adult large branchiopods were
196 collected by gently pulling sweep nets through the water. A square sweep net (20 cm x
197 10 cm; 500 µm mesh) was used to sample rock pools, while a round sweep net
198 (diameter: 30 cm; 1 mm mesh) was used to sample the pans. Captured animals were
199 gently transferred from the sweep nets into 5 L containers, filled with source water,
200 following protocols by Martin et al. (2016). Since *Leptestheria brevirostris* was only
201 found in one pond (22°35'55.6"S, 27°07'51.6"E), all other species used in the
202 experiments were collected from one pond each where possible, or from wetlands in
203 very close proximity to one another. This was done to reduce, potential conspecific
204 diversity effects associated with meta-populations across different wetlands as much as
205 possible. *Branchiopodopsis tridens* were collected from rock pool site (22°35'46.1"S,
206 27°07'16.5"E), while *Streptocephalus cafer* were collected from a pan site

207 (22°52'16.0"S, 27°47'42.7"E). The presently unidentified *Gondwanalimnadia* sp. (Tladi
208 et al., 2020) were collected from two pans in close proximity to one another
209 (22°52'16.0"S, 27°47'42.7"E; 22°49'45.9"S, 27°37'18.1"E), as individual numbers were
210 relatively low in both ponds (Fig. 1). In the laboratory, samples were maintained in
211 shallow containers (100 cm × 70 cm) using water (strained through a 500 µm filter)
212 collected from their respective source wetlands, in climate chambers (HPP 260,
213 Memmert GmbH + Co.KG, Germany) set at 28 °C (± 1 °C) under a 12:12 light:dark
214 photocycle. These were kept at low but optimal densities of 5 organisms per liter to
215 prevent stressful effects of overcrowding confounding our thermal activity assays
216 (Sørensen and Loeschcke, 2001). All experiments were done within 2 days of specimen
217 collection. Upon completion of each experiment, individuals were immediately
218 transferred to 70% ethanol for identification verification. All collection, handling and
219 disposal of the invertebrates were done following standardised university approved
220 protocols.

221

222 **Critical thermal limits**

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

230 the system using digital thermometer (Fluke 53/54IIB, Fluke Cooperation, USA) (Fig.
231 S1). The test tubes were filled with 50 ml of species-specific source wetland water. The
232 water bath was programmed to have a 10 minutes temperature equilibration time at 28
233 °C before increasing temperature for CT_{max} or decreasing for CT_{min} at a ramping rate of
234 0.25 °C/minute (Verberk et al., 2018). Critical thermal limits depend on methodological
235 context and can vary with starting temperature, ramping, and organismal environmental
236 history (acclimation) and others (Terblanche et al., 2007). An equilibration timing of 10
237 minutes is sufficient to ensure all organisms have the same body temperature
238 (Stevenson1985). On the other hand, the start temperature was selected as the highest
239 temperature other than incubation temperature that did not affect the activity of all
240 species, per preliminary results. This enabled changes in activity to be monitored easily
241 by looking at the reference start temperature which was higher than the incubation
242 temperature. The samples were then examined every 0.5 minutes for cessation of
243 motion and response to mild mechanical stimulus. New individuals were used for each
244 replicate. Critical thermal limits were defined as the temperature at which the animal lost
245 coordinated muscle function in response to physical stimulation (Lutterschmidt and
246 Hutchson, 1997; Nyamukondiwa and Terblanche, 2010; Salachan and Sørensen,
247 2017). For each species, the same critical thermal limits experimental procedures were
248 followed. The sample sizes for each species, according to environmental availability,
249 were as follows: *B. tridens* (CT_{min} n = 46, CT_{max} n = 54), *S. cafer* (CT_{min} n = 50, CT_{max} n
250 = 56), *Gondwanalimnadia* sp. (CT_{min} n = 50, CT_{max} n = 50) and *L. brevistoris* (CT_{min} n =
251 49, CT_{max} n = 54).

252

253 **Thermal preference**

254 A thermal gradient stage was connected to two programmed water baths, one at high-
255 and the other at a low temperature. The high temperature-water bath was set at 50 °C
256 and the low temperature-water bath at 5 °C to regulate the temperature gradient on the
257 stage. A three lane (700 × 71 mm per lane) PVC half square-pipe water holding stage
258 was placed on top of the thermal gradient stage with each lane holding 500 ml species-
259 specific source water (Fig. S2). The temperature setting of the two water baths enabled
260 the establishment of a thermal gradient from ≈ 10 to ≈ 40 °C in each of the lanes. A
261 single individual was placed in each lane at ≈ 25 °C and allowed to acclimate for 30
262 minutes. The animals were then observed for an hour, with temperature recorded at
263 their position in the lanes every minute using a digital temperature thermometer (Fluke
264 53/54IIB, Fluke Cooperation, USA). Initially, ten experimental runs for each species
265 were carried out so that a sample size of 30 was obtained, with observations used to
266 determine the temperatures preferred by the species. Data from trials where individuals
267 lost equilibrium or stopped swimming for periods of longer than five minutes were
268 treated as compromised and excluded. A total of 14 individuals were ultimately used for
269 analysis for *S. cafer*, 20 replicates were used for *B. tridens*, while 22 and 17 replicates
270 were used for *Gondwanalimnadia* sp. and *L. brevirostris*, respectively.

271

272 **Data analysis**

273 *Wetland Thermal Profiles*

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

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

288

289 *Critical thermal limits*

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

294

295 *Thermal preference*

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

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

307

308 **Results**

309 *Wetland Thermal Profiles*

310 Differences in HT_{\max} between rock pools and pans were significant ($F_{1,7} = 42.99$, $p <$
311 0.001), with rock pools on average reaching 6.0 °C higher maximal temperatures than
312 pans (Fig. 2). Furthermore, HT_{mean} between rock pools and pans also differed
313 significantly ($F_{1,7} = 9.98$, $p < 0.05$), with rock pools 1.8 °C warmer than pans overall.
314 However, there were not significant differences in HT_{\min} between rock pools and pans
315 ($F_{1,7} = 0.79$, $p > 0.05$). A general pattern was observed in that the coefficients of
316 variance were higher in rock pools than in pans for both the dry period and hydroperiod,
317 and the minimum-maximum range was indeed significantly greater ($F_{1,7} = 47.46$, $p <$
318 0.001) (Fig. S3).

319 *Critical Thermal Limits*

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

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

328 The CT_{min} also differed significantly among species ($\chi^2 = 64.31$, $df = 3$, $p < 0.001$) (Fig.
329 3b). Generally, CT_{min} was lowest in *B. tridens* followed by *Gondwanalimnadia* sp. then
330 *L. brevirostris* and lastly with the highest CT_{min} (least cold tolerance), *S. cafer*.
331 *Branchiopodopsis tridens* had significantly lowest CT_{min} (median = 0.9 °C) (highest cold
332 tolerance) compared to *L. brevirostris* (median = 3.4 °C) and *S. cafer* (median = 3.6 °C)
333 (both $p < 0.001$), but not lower than *Gondwanalimnadia* sp. (median = 2.1 °C) ($p >$
334 0.05). In turn, *Gondwanalimnadia* sp. was significantly more cold tolerant (lower CT_{min})
335 than *L. brevirostris* and *S. cafer* (both $p < 0.001$), with those two species having a more
336 similar CT_{min} statistically ($p > 0.05$).

337

338 *Thermal preference*

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

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

352

353 Discussion

354 In contrast to our hypothesis, the present study showed that wetland type was not a
355 consistent determinant of thermal fitness of the few selected large branchiopods. Rock
356 pools showed greater temperature fluctuations and recorded both higher and lower
357 temperatures than pans. However, taxonomic grouping was a strong predictor of heat
358 tolerance and thermal preference trends among the four species, with the
359 spinicaudatans exhibiting significantly greater heat tolerance (CT_{max}) and higher
360 temperature preferences than the anostracans, irrespective of wetland type. Critical
361 thermal maxima and preference experiments were in consonance, among the four
362 species, with spinicaudatans tending to occur at elevated temperatures (30–32 °C)
363 compared to anostracans (18–23 °C). However, CT_{min} values were neither according to
364 taxonomic grouping nor environment type, with anostracan species comprising both the
365 least (*S. cafer*) and most (*B. tridens*) cold-tolerant taxa. Within these taxonomic
366 generalities in thermal responses, species-specific differences were also apparent. The
367 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

391 intraspecific tolerance to be different among populations of the same species.
392 Furthermore, insect thermal fitness is highly subtle and constrained by a diversity of
393 other factors including species, age, sex, nutrition, ontogeny, environmental history, and
394 others (Chown and Nicolson, 2004; Bowler and Terblanche, 2008; Nyamukondiwa and
395 Terblanche, 2010). Moreover, critical thermal limits measured here also depend on
396 methodological context. Thus factors such as ramping rate, starting temperature and
397 acclimation temperatures may all have effects on critical activity limits (Terblanche et
398 al., 2007). Overall, these thermal tolerance results suggest that across both rock pool
399 and pan habitats, the studied anostracans are likely to be affected before
400 spinicaudatans by temperature increases associated with climate change. However,
401 anostracans are widely distributed, ranging from extreme cold to extreme hot
402 environments. As such, the reason why anostracans were more vulnerable to warming
403 remains unknown and warrants further investigation. We speculate here, with caveats
404 that anostracans may be more vulnerable because of (1) inhabiting environments close
405 to their thermal limits and (2) that they may not be able to remodel their thermal
406 phenotypes suffice to buffer against climate change effects (see Stillman 2003; van
407 Heerwaarden et al., 2016). In particular, increasing incidences of heat waves (Thuiller,
408 2007; Tewksbury et al., 2008; Stillman, 2019; Xu et al., 2020) could result in potential
409 population extirpation of vulnerable taxa owing to rapid acute temperature effects,
410 should they exceed thermal maxima. These effects may be particularly adverse in small
411 habitats (i.e., rock pools) where there is a lower potential for refugia from warming
412 effects. Small rock-pools have lower thermal inertia, they warm quicker and warmer
413 waters have lesser oxygen than colder waters (Willmer et al., 2005). Thus, warming

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

419 Further work is required to elucidate potential for population-level differences among
420 large branchiopods in discrete wetland habitats, as well as impacts of thermal regime on
421 resting egg hatchability (Al-Tikrity and Grainger, 1990; Tladi et al., 2020). Thermal
422 tolerances and preferences might exhibit population-level differences within species,
423 according to the thermal regimes attributed to different habitat types. The results may
424 additionally be influenced by the organism's thermal history (Crickenberger et al., 2020)
425 and carry-over effects (O'Connor et al., 2014; Dickson et al., 2017). This calls for
426 improvement of experimental methodology e.g. using high throughput assays to
427 measure thermal fitness traits coupled with time analysis software to more accurately
428 predict temperature preference (McMahon et al., 2008; Andreassen, 2019; Awde et al.,
429 2020). Nonetheless, the results suggest that, even where taxa have adapted to different
430 wetland types, large branchiopods thermal fitness appears to be relatively similar within
431 their taxonomic grouping.

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

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

450

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459

460 **Declarations of interest:**

461 None.

462

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464 **References**

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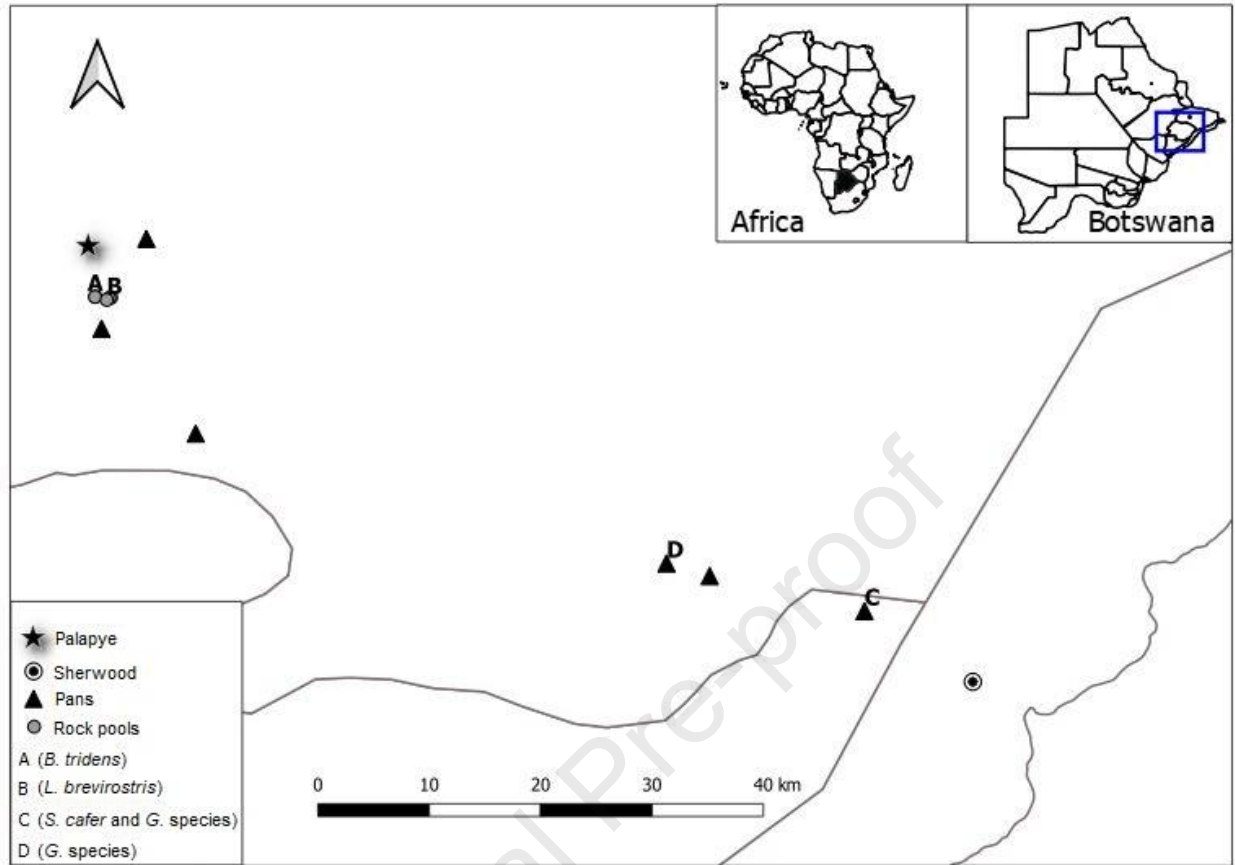
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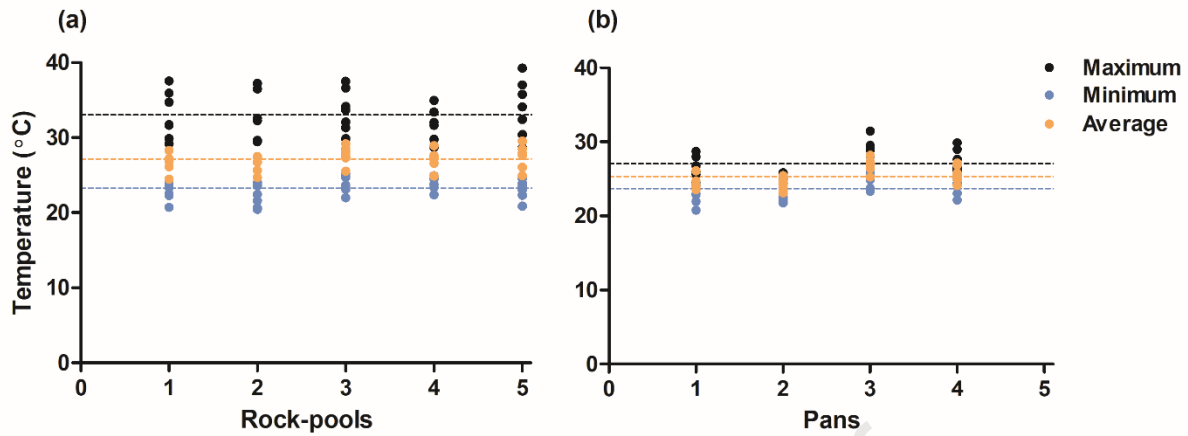


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743 **Figure 1.** Locations of the rock-pools and pans in which, temperature loggers were
 744 deployed in central district, Botswana. For the lab-based experiments,
 745 *Branchiopodopsis tridens* was collected from rock-pool A and *Leptestheria brevisrostris*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

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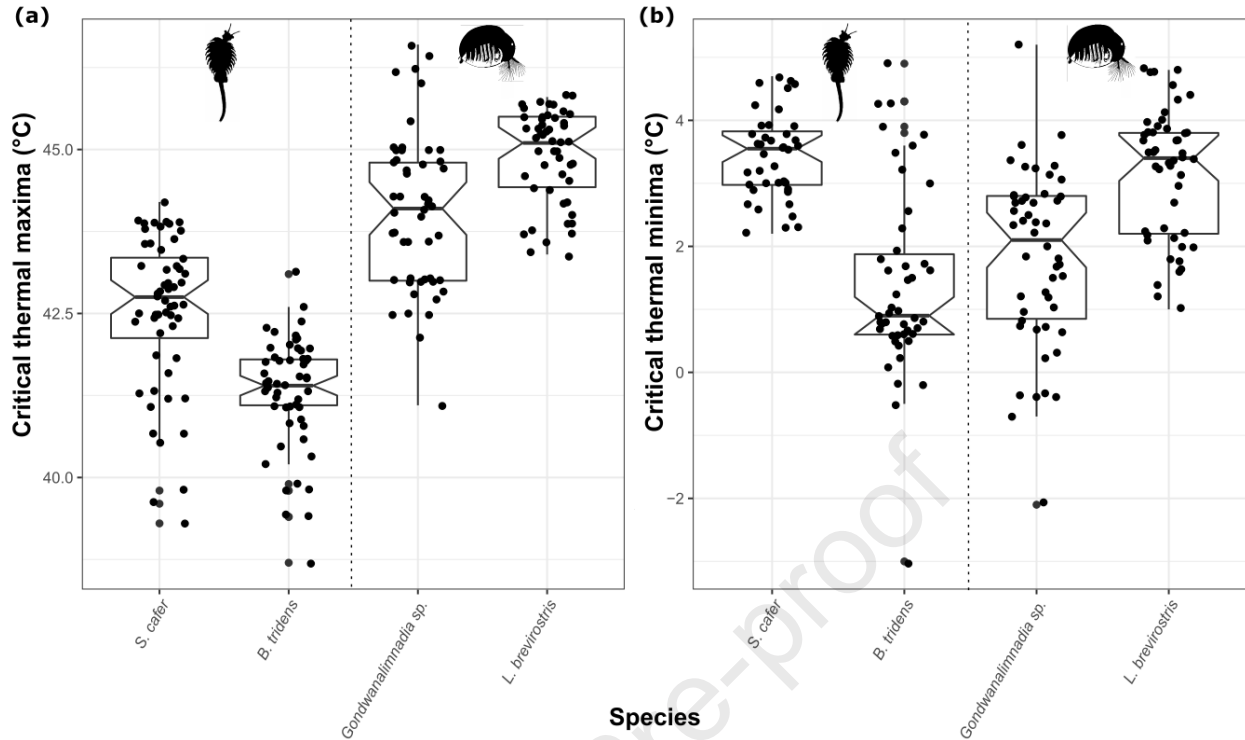


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751 **Figure 2.** Daily habitat maximum (HT_{max}), minimum (HT_{min}) and average (HT_{mean})
 752 values from a) rock-pools and b) pans. Values were derived between 18-23 January
 753 2020 when all wetlands were inundated at the same time, facilitating direct comparison.
 754 Raw data are points. Hashed lines are mean values of all data points.

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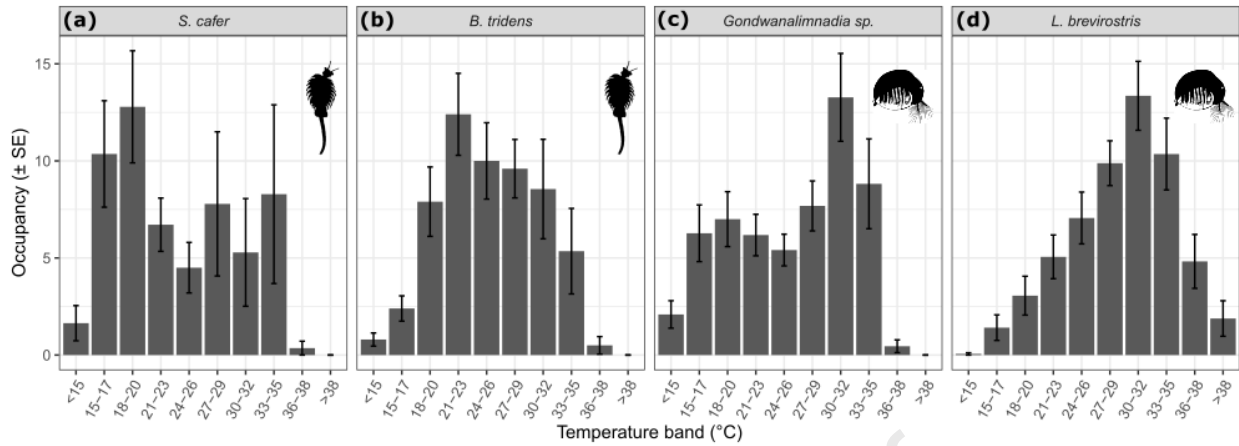
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758 **Figure 3.** Box plots showing (a) critical thermal maxima of *Streptocephalus cafer* ($n =$
 759 56), *Branchiopodopsis tridens* ($n = 54$), *Gondwanalimnadia* sp. ($n = 50$) and
 760 *Leptestheria brevirostris* ($n = 54$) ($^{\circ}\text{C}$)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

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

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772 Electronic Supplementary Material

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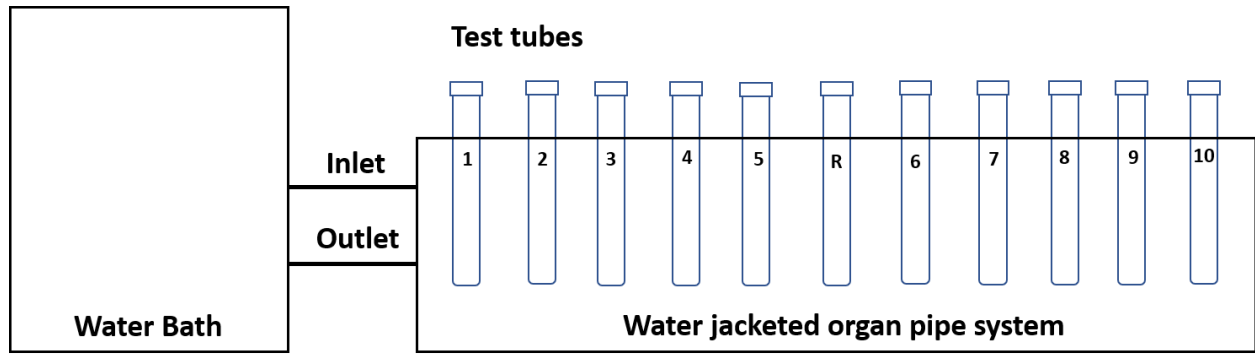
774 **Table S1.** Locations of the rock pools and pans used for thermal characterization during hydroperiods, as well as large
 775 branchiopod species encountered in each wetland. Programmable data logger (0.5°C accuracy) were set to 1 h sampling
 776 frequencies and deployed, on the bottom in the centre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

780

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.	

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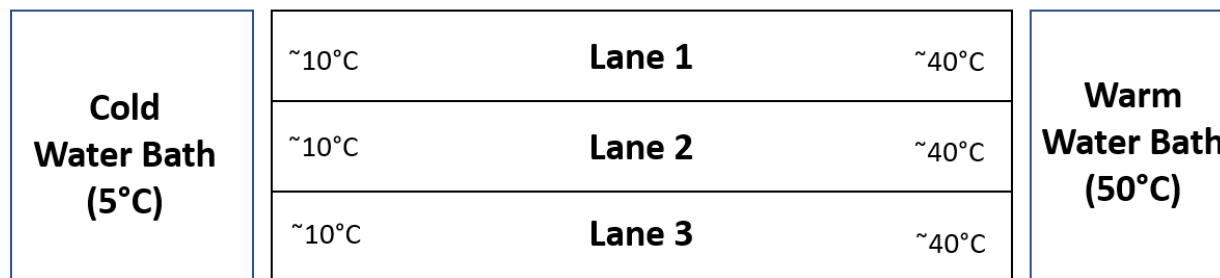


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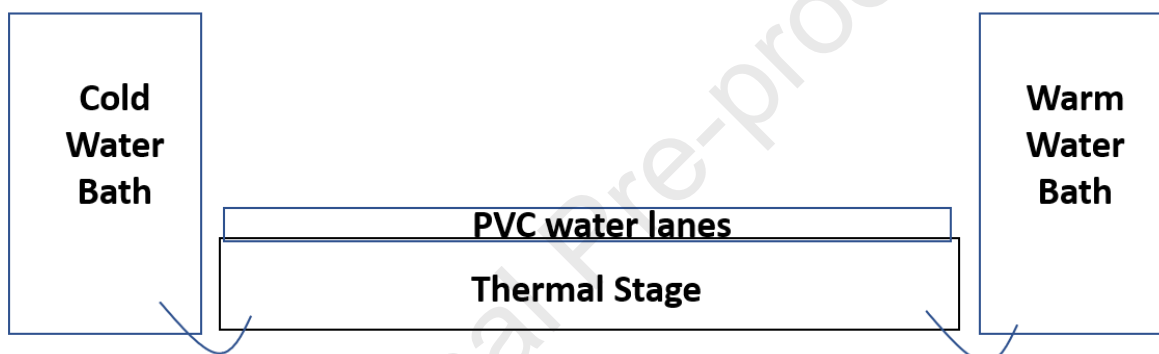
784 **Figure S1.** Organ pipe design for the critical thermal limit experiment. The programmed
785 water bath (20L) regulates the heat in the test tubes through circulation of temperature
786 controlled 1:1 water:propylene glycol through the jacket system. Ten replicate test tubes
787 (1-10) house the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

789

a)



b)

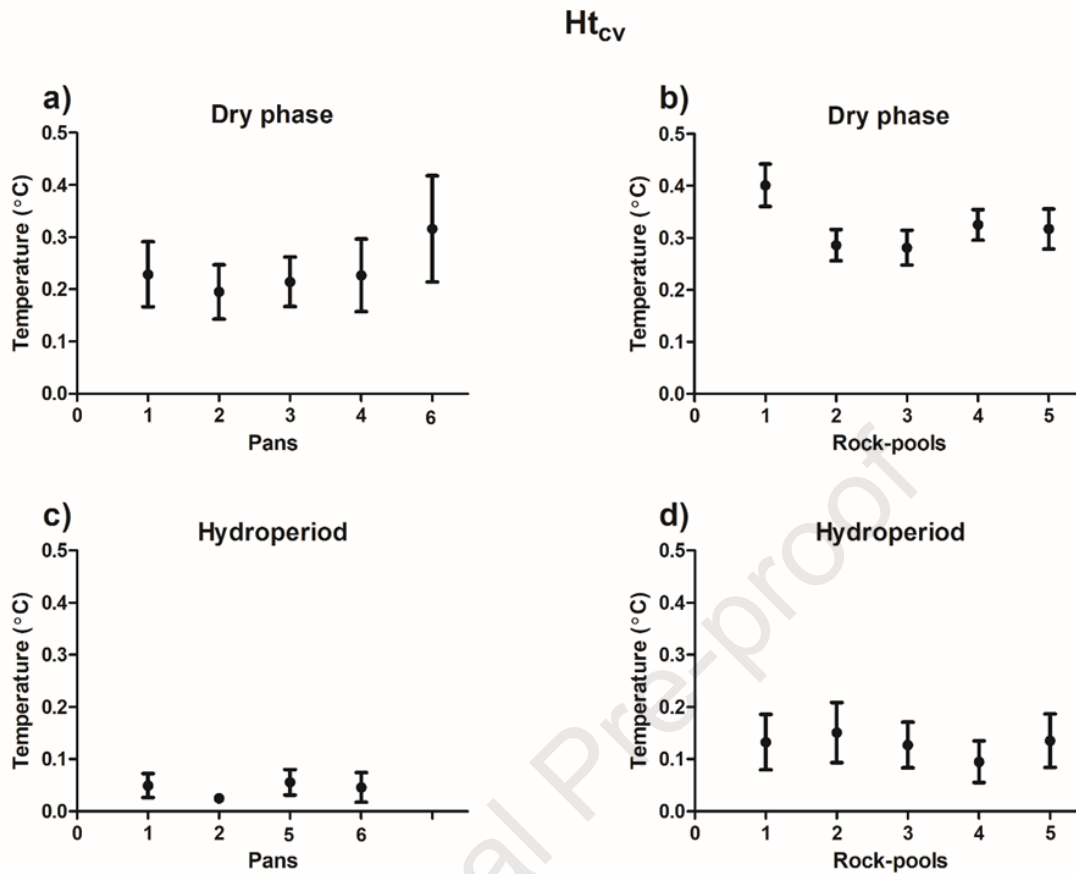


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791 **Figure S2.** Schematic representation of the thermal stage set-up showing a) aerial view
 792 of the 3-laned PVC pipes, each holding water, and b) lateral view of the PVC lanes on
 793 top of the metal thermal gradient stage, fed by cold water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

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797

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

802

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

Journal Pre-proof