



Bioecology of Large Branchiopods in Central District, Botswana

by

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Table of contents

Title page.....	i
Table of contents.....	ii
Declaration and copyright.....	iv
Certification	v
Preface.....	vi
Acknowledgements	vii
Dedication	viii
List of figures.....	ix
List of tables	xv
General abstract.....	1
Chapter 1	3
1.1 Temporary wetland ecology	4
1.2 Large branchiopods	7
1.3 Temporary wetland invertebrate research in Southern Africa	11
1.4 Threats to temporary wetlands in Southern Africa	13
1.5 Present study	14
Chapter 2	17
2.1 Introduction.....	20
2.2 Materials and methods	25
2.2.1 Study Site.....	25
2.2.2 Pond identification.....	27
2.2.3 Pond Temperature monitoring.....	29
2.2.4 Physico-chemical Characteristics.....	31
2.2.5 Data Analysis	31
2.3 Results.....	33
2.3.1 Wetland temperatures	33
2.3.2 Wetland physico-chemical characteristics.....	42
2.4 Discussion	44
Chapter 3	50
3.1 Introduction	52

3.2 Materials and methods	57
3.2.1 Species selection and collection	57
3.2.2 Critical thermal limits	58
3.2.3 Thermal preference	60
3.2.4 Data analysis.....	62
3.3 Results.....	62
3.3.1 Critical Thermal Limits.....	62
3.3.2 Thermal preference	64
3.4 Discussion	65
Chapter 4	70
4.1 Introduction.....	71
4.2 Materials and methods	74
4.2.1 Sample collection	74
4.2.2 Laboratory procedure	75
4.3 Results.....	78
4.4 Discussion	79
Chapter 5	82
5.1 Synthesis	83
5.2 Conclusions	85
List of references.....	89
Supplementary material	115

Declaration and copyright

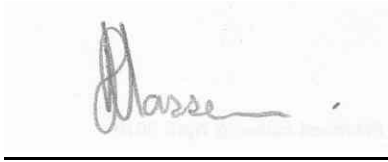
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Certification

The undersigned certifies that he has read and hereby recommends for acceptance by the Faculty of Science a dissertation/thesis titled: **Bioecology of Large Branchiopods in Central District, Botswana**, in fulfilment of the requirements for the degree of Master of Science in (Biological Sciences and Biotechnology) of the BIUST.



Prof. Ryan Wasserman
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Preface

This thesis comprises a general introduction (Chapter 1), followed by three data chapters (Chapters 2, 3 and 4) and a general synthesis and conclusion chapter (Chapter 5). The data chapters are stand-alone with regards to materials and methods, and as such, they are organised as scientific papers. The combined reference list at the end of the thesis has, however, ensured limited repetition. Some of the data chapters and associated supplementary materials are published or are under review (outlined below):

- Chapter 3: Tladi, M., Wasserman, R. J., Cuthbert, R. N., Dalu T., Nyamukondiwa, C. (under review). Thermal limits and preferences of branchiopods from temporary wetland arid zone systems. *Journal of Thermal Biology*.
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- Supplementary file (File S2): 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 Part B*, 5, 623-625.
- Supplementary file (File S3): Tladi, M., Emami-Khoyi, A., Dalu, T., Teske, P. R., Rogers, Jansen van Vuuren B., D. C., Nyamukondiwa, C., Wasserman, R. J. (submitted) The complete mitogenome of *Leptestheria brevirostris* Barnard, 1924, a rock-pool clam shrimp (Branchiopoda: Spinicaudata) from Central District, Botswana. *Mitochondrial DNA Part*

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Dedication

I would like to dedicate this research to my parents and siblings. They gave me the drive to complete this research.

List of figures

- Fig 1. 1:** A hypothetical schematic diagram outlining temporary wetland cycles between dry phases and wet phases (hydroperiods), highlighting the variable nature of the ecosystems. Hydroperiods refer to periods of inundation when the depressions hold water. Dry phases represent those periods between the hydroperiods, when the depressions hold no water.....5
- Fig 1. 2:** A schematic diagram outlining hypothetical temporary wetland community development over the course of a hydroperiod. Specialist taxa (including large branchiopods) hatch from dormant eggs in the sediment. Non-specialist taxa are typically capable of flight (and predatory) and invade these environments soon after inundation, contributing to within-pond diversity and trophic complexity 7
- Fig 1. 3:** Examples of large branchiopod a) and b) anostracans, c) a notostracan and d) a spinicaudatan, sampled during the present study.....9
- Fig 2. 1:** A map of study site showing the location of Palapye and Sherwood together with identified temporary wetlands in relation to Central District of Botswana in Southern Africa.....26
- Fig 2. 2:** Monthly total rainfall (mm) for the period October 2017 to February 2020 at the Palapye weather station (22°34'S 27°09'E; elevation = 908 Metres). Rainfall data was supplied by the Department of Meteorological Services of Botswana. Jan = January; Feb = February; Mar = March; Apr = April; Jun = June; Jul = July; Aug = August; Sep = September; Oct = October; Nov = November; Dec = December...27
- Fig 2. 3:** Examples of rock-pools on rocky outcrops in the Palapye region showing a rock-pool during a) a dry phase, b) a hydroperiod and c) and d) the landscapes in which these rocky outcrops and associated rock-pools occur.....28
- Fig 2. 4:** Examples of pans in the Palapye/ Sherwood region showing a pan during a) a dry phase, b) a hydroperiod and c) and d) landscapes in which these pans occur.....29

- Fig 2. 5:** Examples of daily temperature fluctuations within the wetland types for three example rock-pools (a and c) and pans (b and c), during both dry phases (a, b) and hydroperiods (c, d). The 24-hours example periods represent the hottest days recorded in each wetland type during the dry phase (07/11/2019) and hydroperiod (23/01/2020).....34
- Fig 2. 6:** Mean ($\pm$ standard deviation) daily habitat maximum temperatures ($H_{t_{max}}$) 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).....36
- Fig 2. 7:** Mean ($\pm$ standard deviation) daily habitat minimum temperatures ($H_{t_{min}}$) 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).....37
- Fig 2. 8:** Mean ($\pm$ standard deviation) daily habitat average temperatures ($H_{t_{mean}}$) 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).....38
- Fig 2. 9:** Mean ($\pm$ standard deviation) daily habitat coefficient of variation in temperatures ($H_{t_{cv}}$) 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).....39
- Fig 2. 10:** Cluster dendrogram of wetland types based on thermal characteristics ($H_{t_{max}}$, $H_{t_{min}}$, $H_{t_{mean}}$, $H_{t_{cv}}$), highlighting the differences between rock-pools and pans. The Euclidean distance differentiated the ponds according to the temperature variables calculated for the synchronized dry phase over the period 2-7 November 2019. Groups were identified based on levels of similarity on the Y-axis (Distance).....40
- Fig 2. 11:** Cluster dendrogram of wetland types based on thermal characteristics ($H_{t_{max}}$, $H_{t_{min}}$, $H_{t_{mean}}$, $H_{t_{cv}}$), highlighting the differences between rock-pools and pans. The

Euclidean distance differentiated the ponds according to the temperature variables calculated for the synchronized hydroperiod over the period 2-7 November 2019. Groups were identified based on levels of similarity on the Y-axis (Distance).....	42
Fig 2. 12: Cluster dendrogram of wetland types based on physico-chemical characteristics (electrical conductivity ($\mu\text{S}/\text{cm}$), total dissolved solids (mg/L) and turbidity (NTU), highlighting the differences between rock-pools and pans. The Euclidean distance differentiated the ponds according to the physico-chemical variables measured from the ponds during a hydroperiod around six days after the wetlands filled. Groups were identified based on levels of similarity on the Y-axis (Distance).....	44
Fig 3. 1: Locations of the rock-pools for the collection of <i>Branchiopodopsis tridens</i> (A) and <i>Leptestheria brevirostris</i> (B), and pans for the collection of <i>Streptocephalus cafer</i> (C) and <i>Gondwanalimnadia</i> sp. (C and D) in central district, Botswana.....	58
Fig 3. 2: Organ pipe design for the critical thermal limit experiment. The programmed water bath regulates the heat in the test tubes through circulation of temperature controlled 1:1 water:propylene glycol through the jacket system. Ten replicate test tubes (1-10) house the test animals, while the reference test tube (R) is used to monitor temperature within the test tubes.....	60
Fig 3. 3: 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 stage, fed by cold water on the left and warm water on the right from respective water baths.....	61
Fig 3. 4: Box plots showing (a) critical thermal maxima ($^{\circ}\text{C}$) and (b) critical thermal minima ($^{\circ}\text{C}$) of <i>Streptocephalus cafer</i> , <i>Branchiopodopsis tridens</i> , <i>Gondwanalimnadia</i> sp. and <i>Leptestheria brevirostris</i> . 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.....	64

Fig 3. 5: Thermal preference occurrences of <i>Streptocephalus cafer</i> , <i>Branchiopodopsis tridens</i> , <i>Gondwanalimnadia</i> sp. and <i>Leptestheria brevisrostris</i> across temperature bands. Medians are shown alongside standard errors (SE).....	65
Fig 4. 1: Nunc 96 well-plates were used for the experiment, whereby four replicate rows (A, B, C and D) of eight wells each were filled with distilled water per well-plate, holding five <i>Streptocephalus cafer</i> cysts each. The blue dye water was used for illustration purposes for this image.....	76
Fig 4. 2: Effects of temperature on fairy shrimp hatchability between two light regimes. A quadratic temperature term was included owing to non-linearities of the temperature effect. Point are raw data and shading represents 95 % confidence intervals.....	79
Fig S 1: Temperature records of a pan in Botswana International University of Science and Technology campus (22°37'26.4"S 27°07'35.3"E) (Pan 1). Programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) (0.5°C accuracy) were used for the monitoring of temperatures in the ponds at 1 hour time intervals throughout the sampling period. Loggers were placed in the deepest part of selected wetlands.....	115
Fig S 2: Temperature records of a pan along Palapye to Martins Drift road (22°49'45.7"S 27°37'17.9"E) (Pan 2). Programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) (0.5°C accuracy) were used for the monitoring of temperatures in the ponds at 1 hour time intervals throughout the sampling period. Loggers were placed in the deepest part of selected wetlands.....	116
Fig S 3: Temperature records of a pan along Palapye to Martins Drift road (22°52'16.0"S 27°47'42.7"E) (Pan 3). Programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) (0.5°C accuracy) were used for the monitoring of temperatures in the ponds at 1 hour time intervals throughout the sampling period. Loggers were placed in the deepest part of selected wetlands.....	117

- Fig S 4:** Temperature records of a pan along Palapye to Martins Drift road (22°42'56.8"S 27°12'32.4"E) (Pan 4). Programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) (0.5°C accuracy) were used for the monitoring of temperatures in the ponds at 1 hour time intervals throughout the sampling period. Loggers were placed in the deepest part of selected wetlands.....118
- Fig S 5:** Temperature records of a pan along Palapye to Martins Drift road (22°50'25.5"S 27°39'34.2"E) (Pan 5). Programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) (0.5°C accuracy) were used for the monitoring of temperatures in the ponds at 1 hour time intervals throughout the sampling period. Loggers were placed in the deepest part of selected wetlands.....119
- Fig S 6:** Temperature records of a pan along Palapye to Martins Drift road (22°32'43.1"S 27°09'56.8"E) (Pan 6). Programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) (0.5°C accuracy) were used for the monitoring of temperatures in the ponds at 1 hour time intervals throughout the sampling period. Loggers were placed in the deepest part of selected wetlands.....120
- Fig S 7:** Temperature records of a rock-pool in Botswana International University of Science and Technology campus (22°35'48.4"S 27°08'05.5"E) (Rock-pool 1). Programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) (0.5°C accuracy) were used for the monitoring of temperatures in the ponds at 1 hour time intervals throughout the sampling period. Loggers were placed in the deepest part of selected wetlands.....121
- Fig S 8:** Temperature records of a rock-pool in Botswana International University of Science and Technology campus (22°35'49.6"S 27°07'59.6"E) (Rock-Pool 2). Programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) (0.5°C accuracy) were used for the monitoring of temperatures in the ponds at 1 hour time intervals throughout the sampling period. Loggers were placed in the deepest part of selected wetlands.....122

- Fig S 9:** Temperature records of rock-pool in Botswana International University of Science and Technology campus (22°35'45.8"S 27°07'15.8"E) (Rock-Pool 3). Programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) (0.5°C accuracy) were used for the monitoring of temperatures in the ponds at 1 hour time intervals throughout the sampling period. Loggers were placed in the deepest part of selected wetlands.....123
- Fig S 10:** Temperature records of a rock-pool in Botswana International University of Science and Technology campus (22°35'46.1"S 27°07'14.6"E) (Rock-Pool 4). Programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) (0.5°C accuracy) were used for the monitoring of temperatures in the ponds at 1 hour time intervals throughout the sampling period. Loggers were placed in the deepest part of selected wetlands.....124
- Fig S 11:** Temperature records of a rock-pool in Botswana International University of Science and Technology campus (22°35'55.6"S 27°07'51.5"E) (Rock-Pool 5). Programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) (0.5°C accuracy) were used for the monitoring of temperatures in the ponds at 1 hour time intervals throughout the sampling period. Loggers were placed in the deepest part of selected wetlands.....125
- Fig S 12:** An example of the number of hydroperiods experienced per pond type; A) a pan and B) a rock-pool. Note that rock-pools, as smaller aquatic environments underlined with bedrock experience more, but shorter, hydroperiod events than pans.....126

List of tables

Table 2. 1: Locations of the rock-pools sampled for physico-chemical characterization. Temp = Temperture measurements recorded hourly using HOBO data loggers. Physico-chemical = total dissolved solids, electrical conductivity and turbidity measuements of the water. Dry phase and hydroperiod represent the different phases in which data were collected.....	30
Table 2. 2: Welch two sample t-test outputs testing differences in temperatures between pans and rock-pools during the synchronized dry phases (2-7 November 2019). Ht _{max} = habitat temperature daily maximum, Ht _{min} = habitat temperature daily minimum, Ht _{mean} = habitat temperature daily average, Ht _{cv} = habitat temperature daily coefficient of variation. t-crit- critical t value. Df = degrees of freedom.....	35
Table 2. 3: Welch two sample t-test outputs testing differences in temperatures between pans and rock-pools during the synchronized hydroperiod (18-24 January 2020). Ht _{max} = habitat temperature daily maximum, Ht _{min} = habitat temperature daily minimum, Ht _{mean} = habitat temperature daily average, Ht _{cv} = habitat temperature daily coefficient of variation. t-crit- critical t value. Df = degrees of freedom.....	41
Table 2. 4: Physico-chemical properties of pans and rock-pools. The physico-chemical data was obtained in hydroperiods in January and February 2020. t-crit =critical t value. Df = degrees of freedom.....	43
Table 4. 1: Temperature and light treatments used for testing the hatching success of S. cafer resting eggs in 96 well plates, placed within an incubator. H = hours. Lighting in the incubators during the 12 H light phase produced a measured visible light ranging 700 to 1000 lux.....	77
Table 4. 2: Linear model coefficients considering fairy shrimp hatchability as a function of light regime (2 levels), temperature (5 levels), and their interaction. Significant effects are emboldened.....	79

General abstract

The bioecology of large branchiopods in Central District, Botswana, was analysed to determine if genetic relatedness or environment of origin determined the thermal physiology of large branchiopods. Two temporary wetland types (Rock-pools and Pans) were identified and their thermal conditions characterised. Temperatures experienced in each wetlands type were measured for synchronised periods spanning both dry phases and hydroperiods of the temporary wetlands. Anostraca and Spinicaudata specialists from each temporary wetland were then identified and their thermal physiology assessed. Physiological thermal fitness of large branchiopods were measured using critical thermal limits and thermal preferences. Finally, the effect of light and temperature on dormant cysts of a dominant large branchiopod were assessed. *Streptocephalus cafer* dormant cyst hatching success was analysed using variable temperatures and light durations. The thermal properties of rock-pools and pans were grouped according to wetland type, highlighting the thermal differences between these different temporary wetland types. Rock-pools experienced high temperatures compared to pans in general. However, thermal physiologies of large branchiopods differed according to taxonomic orders in thermal limits. Spinicaudatans were tolerant to higher temperatures than anostracans. The results for thermal limits and thermal preference differed across species. The hatching success of *S. cafer* was optimal at 27 °C in 12hrs:12hrs light:dark regime. In conclusion, the thermal physiologies of large branchiopods appear to primarily determined by genetic relatedness with the environment of origin being secondary. While not part of the thesis itself, the opportunity to describe the complete mitochondrial DNA of the studied

species was also taken, to aid in resolving phylogenies for these understudied taxa. These results are presented as supplementary material (See files S1-3).

Chapter 1

General introduction



Plate 1: A Dry rock-pool, known to host branchiopods located in BIUST campus, Central District, Botswana. Indeed “Water is the true wealth in a dry land” - Wallace Stegner. (Photo: Murphy Tladi)

1.1 Temporary wetland ecology

Temporary wetlands are beneficial to both wildlife and human beings as they provide a variety of services that range from ecological to sociological (Dodds and Whiles, 2010; Dalu et al., 2017a). Described as among the most productive ecosystems in the world (Reddy and DeLaune, 2008), these environments are recognised globally as sanctuaries for fauna, as they provide food and breeding sites for a variety of taxa (Dodds and Whiles, 2010; Calhoun et al., 2017). Occurring globally in different climates where water from different sources stagnates temporarily (Williams, 1996), temporary wetlands exist in different forms depending on the nature of the geological depressions that facilitate the holding of water at the surface (Dodds and Whiles, 2010). These differences in underlying geological conditions, as well as numerous other environmental conditions such as depression dimensions, rainfall pattern and insolation dynamics result in temporary wetland variability (Reddy and DeLaune, 2008; Melly et al., 2017; Lanfranco and Briffa, 2019). Not only does this drive different temporary wetland types, but even within a particular wetland, dry phase and hydroperiod lengths and frequencies can be extremely variable (Fig. 1). As a result, temporary freshwater wetland habitats are considered to be among the most ecologically extreme of aquatic environments (Euliss and Mushet, 2004; Dodds and Whiles, 2010; Schwartz and Jenkins, 2000). While widespread, the abundance of temporary wetlands differs across the globe with some regions holding considerably more temporary wetlands than others (Goudie and Wells, 1995; Hamer and Brendonck, 1997). In arid and semi-arid regions, temporary wetlands are often common features of the landscape, and in certain instances are the dominant aquatic

habitat (Roshier et al., 2001; Euliss and Mushet, 2004; Waterkeyn et al., 2009; Melly et al., 2017; Bird et al., 2019). In Southern Africa, temporary wetlands are important aquatic environments. The region is largely semi-arid and arid, has one of the highest concentrations of temporary wetlands in the world (Goudie and Wells, 1995).

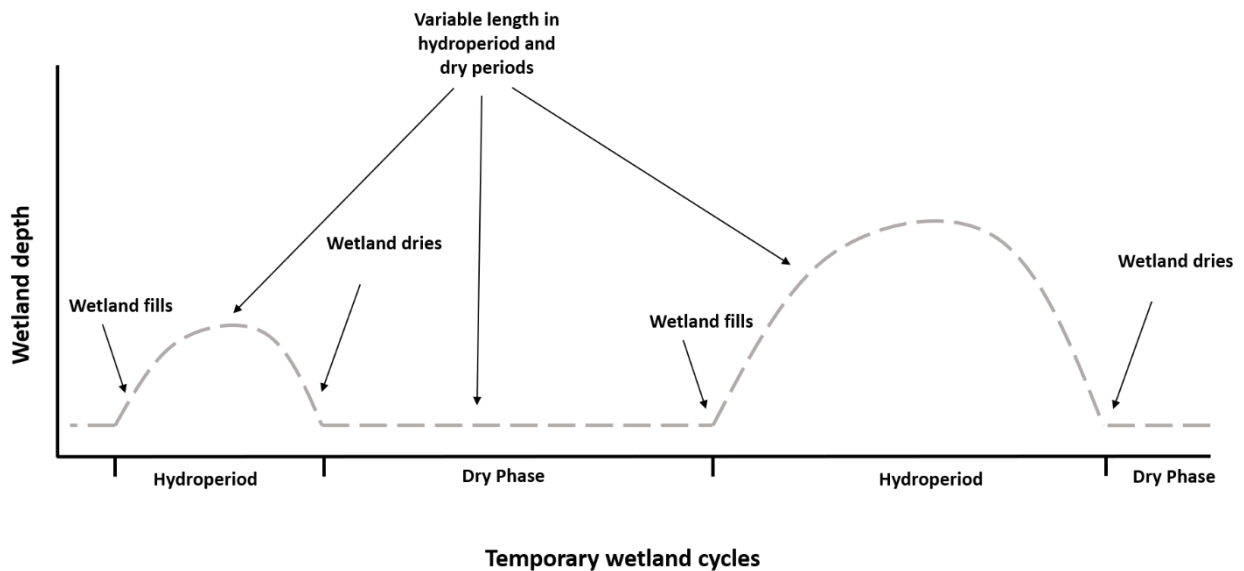


Fig 1. 1: A hypothetical schematic diagram outlining temporary wetland cycles between dry phases and wet phases (hydroperiods), highlighting the variable nature of the ecosystems. Hydroperiods refer to periods of inundation when the depressions hold water. Dry phases represent those periods between the hydroperiods, when the depressions hold no water. Constructed using Microsoft PowerPoint.

As unique aquatic habitats, temporary wetlands typically have unique biological communities. These communities comprise both specialist and non-specialist fauna (O'Neill and Thorpe, 2014). Specialist fauna are typically obligate temporary wetland species, while generalists utilise these environments facultatively (O'Neill and Thorpe, 2014; Bird et al., 2019). Community succession patterns within temporary wetlands

generally involve a pioneer community comprising specialist species that emerge from dormant eggs in the sediment (O'Neill and Thorpe, 2014; Wasserman et al., 2016a,b). Following inundation (at the start of the hydroperiod) dormant eggs hatch, giving rise to a pioneer community of temporary wetland taxa (Fig. 1.2). The pioneer community typically comprises primary consumers, that feed on the primary producers (phytoplankton and other microbes) that also emerge from cysts in the sediment (Dalu et al., 2017a; Celewicz et al., 2018). Crustaceans, including copepods, branchiopods and ostracods comprise this community, along with non-arthropod taxa such as planarians, annelids and molluscs (Jocque et al., 2010; Timms, 2012; Krodkiewska and Spyra, 2015; Bird et al., 2019). Over time, however, generalist species also start to invade these patches of aquatic habitat (Fig. 1.2) given their high levels of primary (phytoplankton) and secondary (specialist taxa) productivity levels (O'Neill and Thorpe, 2014; Dalu et al., 2017a). These faunae typically comprises insect predatory species (O'Neill and Thorpe, 2014), some of which invade as semi-aquatic adults (e.g. Notonectidae and Dyticidae), while others lay eggs that rapidly hatch to produce predatory early life stages (e.g. Odonata). As the wetlands reach the end of their hydroperiods, generalist taxa need to emigrate to survive. Many taxa migrate to permanent water bodies during drier periods, where temporary wetlands hold no water. Temporary wetland specialists, however, persist locally through the production of cysts (dormant eggs), which remain in the sediment over the variable dry period (Jocque et al., 2010).

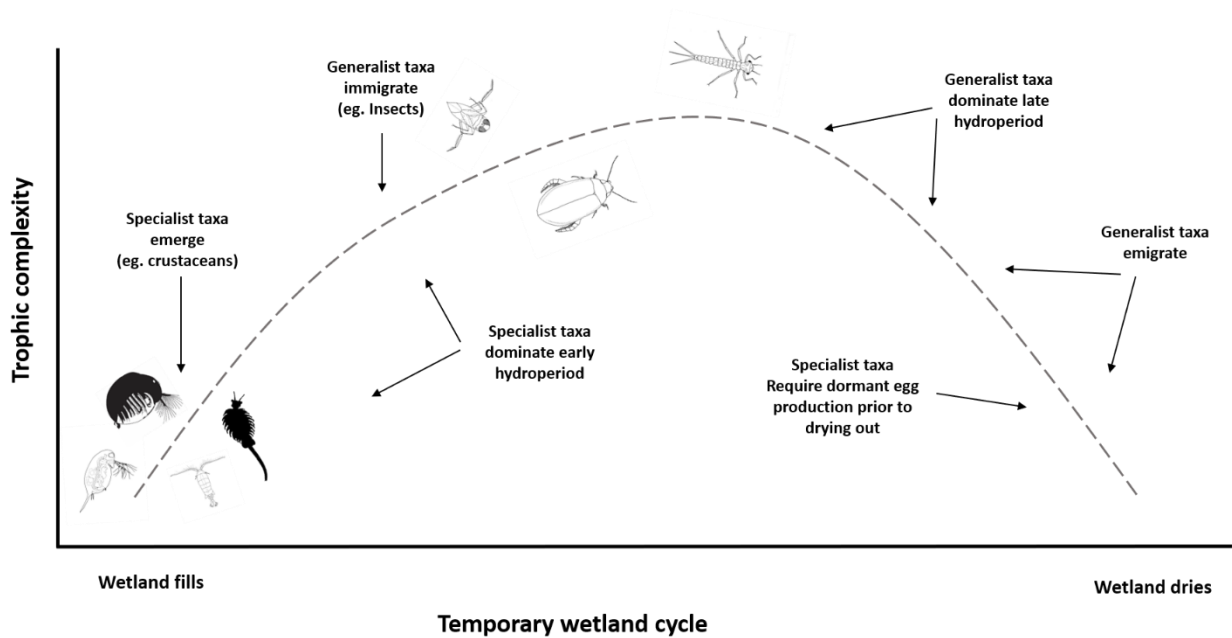


Fig 1. 2: A schematic diagram outlining hypothetical temporary wetland community development over the course of a hydroperiod. Specialist taxa (including large branchiopods) hatch from dormant eggs in the sediment. Non-specialist taxa are typically capable of flight (and predatory) and invade these environments soon after inundation, contributing to within-pond diversity and trophic complexity. Constructed using Microsoft PowerPoint.

While the patterns above are broadly relevant for many temporary wetland types, these environments are, however, physico-chemically diverse with discrete types of temporary wetlands having been described (Schwartz and Jenkins, 2000; Williams, 2005; Reddy and DeLaune, 2008). Differences in temporary wetland type associated with physico-chemical characteristics also have implications for the invertebrate communities found in these habitats, whereby certain specialist taxa can be restricted to certain temporary wetland types (Rains et al., 2008; Waterkeyn et al., 2008).

1.2 Large branchiopods

Branchiopoda, a group of freshwater crustaceans (Brendonck et al., 2008; Lindholm, 2014), comprises the cladocerans (water fleas), anostracans (fairy shrimp), lipostracans (now extinct), notostracans (tadpole shrimp) as well as laevicaudatans, spinicaudatans and cyclestherids (collectively known as clam shrimp). The enigmatic large branchiopods (fairy shrimp, tadpole shrimp and clam shrimp) are flagship invertebrates of temporary wetlands globally (Brendonck et al., 2008; Bird et al., 2019) (Fig. 2.3). The large branchiopods are well adapted to temporary wetland dynamics. Usually these habitats remain dry for most part of the year and have inundation patterns that are unpredictable (Vanschoenwinkel et al., 2010; Martin et al., 2019). As such, large branchiopods are characterised by rapid growth and attainment of sexual maturity to facilitate cyst production by adults, prior to the wetland drying out (Brendonck et al., 2000; Brendonck et al., 2008). Like seed banks in terrestrial landscapes, these cysts collect in the sediment and serve as an “egg bank” for future recruitment success following inundation events.

Brendonck et al. (2008) outlined that the cysts of many of large branchiopod species can withstand drought-conditions for decades, and potentially even centuries. Favorable environmental conditions such as temperature, photoperiod and more especially rainfall act as triggers for cysts to hatch (see chapter 4). However, only a fraction of the cysts hatch during each inundation period and cysts from different hydroperiod generations hatch in one hydroperiod which allows genetic stagnation as mutations in cysts from recent generations get corrected by those from older generations (Pinceel et al., 2013). Furthermore, the strategy of playing it safe by

hatching few eggs in each inundation is a bet-hedging strategy that ensures that the population survives for a long time (Brendonck and De Meester, 2003). The cysts also facilitate the dispersal of large branchiopods. Temporary wetlands are patchy environments and can be considered aquatic “islands” within terrestrial landscapes. Generalist fauna that utilise these environments do so through active dispersal mechanisms such as flight given that adult stages are semi-aquatic and capable of leaving the aquatic environment (Jocque et al., 2010). Branchiopods and other specialist crustaceans, however, are passively dispersed during the cyst stage, whereby cysts are transported between ponds through certain mechanisms (Boxshall and Defaye, 2008; Vanschoenwinkel et al., 2008). Cysts are transported by winds that scour eggs from the sediment during the dry phase (Parekh et al., 2014). They are also, however, transported during the hydroperiods by animals such as birds and even elephants that visit the wetlands for food or water and come into contact with the egg bank sediment (Vanschoenwinkel et al., 2011; Rogers, 2014). This process is known as zoochory.

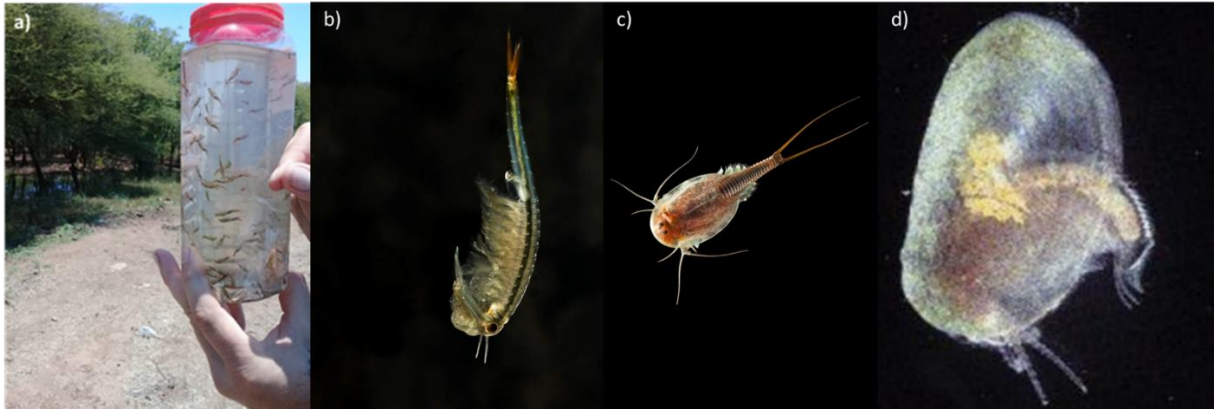


Fig 1. 3: Examples of large branchiopod a) and b) anostracans, c) a notostracan and d) a spinicaudatan, sampled during the present study. (Images b), c) and d) provided with permission for use, by Luc Brendonck)

Branchiopods are referred to as “living fossils” as their known fossils date back to as early as around 416 million years ago (Sun, 2016; Gueriau et al., 2016). Different orders of branchiopods tend to occur in the same habitats, and as such, individual temporary wetlands often contain more than more large branchiopod species (Thiéry, 1991). In this thesis we deal specifically with aspects of the thermal biology of co-occurring fairy shrimp (Anostracan) and clam shrimp (Spinicaudata) species. Anostracans are spread across the globe and are even found in the polar regions (Hawes, 2009; Rogers, 2015; Lindholm et al., 2016). They feed on planktonic organisms such as algae and yeasts in the water column (Brendonck, 1993). Anostracans can, however, be specific about the types of temporary wetlands in which they occur (Hamer and Brendonck, 1997). Spinicaudata is a suborder of order Diplostraca, commonly known as clam shrimps (Meyer-Milne et al., 2020). They tend to occupy and feed in the benthic portions of pools, hence they are often overlooked

(Brendonck et al., 2008; Munoz et al., 2008). They are thought to be omnivores with diets consisting of detritus, plankton and algae (Hethke et al., 2019). As with anostracans, certain spinicaudatan genera are restricted to certain temporary wetland types.

1.3 Temporary wetland invertebrate research in Southern Africa

Work conducted on temporary wetland invertebrate research in Southern Africa was recently reviewed by Bird et al. (2019), who structured the overview along taxonomic lines. This study outlined that there is much existing information regarding temporary wetlands in Southern Africa, citing over 280 published studies, the vast majority of which are regionally specific. From this review it is, however, evident that the large branchiopods have received the most research attention. This is not surprising given that large branchiopods are often referred to as the “flagship species” of temporary wetlands. However, the work conducted on large branchiopods in the region has largely been restricted to taxonomic and broad community ecological investigations (Bird et al., 2019 and references therein).

More generally the Bird et al. (2019) review outlined that temporary wetland species in Southern Africa have usually been identified using morphological characteristics using region-specific identification keys (Hamer, 1999; Martens, 2001). Furthermore, limited knowledge on distributions of the fauna have resulted in misleading conclusion about the endemism of some species in the region (van Damme et al., 2013). The relatively

minute fauna also appears to be complicated to collect and identify, which leaves them mostly unstudied (Bird et al., 2019). Regarding ecological investigations, investigations have assessed pollution dynamics in these environments (Zacharias et al., 2007; Bouahim et al., 2015; Mabidi et al., 2018), while others have looked at food webs (Dalu et al., 2016), phenological phenomena (Wasserman et al. 2018; Dalu et al., 2017b,c), cyst dispersal mechanisms (Brendonck et al., 2008; Boxshall and Defaye, 2008; Vanschoenwinkel et al., 2011; Parekh et al., 2014; Rogers, 2014) and meta-community dynamics (Vanschoenwinkel et al., 2010; Vanschoenwinkel et al., 2013). Surprisingly, other than a few studies on schistosome vector gastropods (Rubaba et al., 2016) and a few more recent studies on mosquito biological control potential of predatory copepods (Cuthbert et al., 2020 and references therein), the study of medically important vector invertebrates in temporary wetlands has been overlooked. A few studies outline that when compromised, temporary wetland communities shift, favoring certain species (Watson, 1958; Buxton et al., 2020a; Cuthbert et al., 2020). While it is yet to be quantified for the region, these studies suggest that in compromised temporary wetlands, specialist temporary wetland crustacean species are among the first to disappear and are replaced by generalist taxa, many of which are medically important and nuisance species such as mosquitoes (Dalu et al., 2017a; Cuthbert et al., 2020; Buxton et al., 2020a). The potential ecosystem services of in-tact temporary wetlands may, therefore, include the biological exclusion or medically important invertebrate disease vector species.

While not explicitly outlined in the Bird et al. (2019) review, the glaring gaps in the regional literature are those of eco-physiological and genetic investigations on temporary wetland specialists. Large branchiopods are no exception in this regard. Eco-physiological investigations on large branchiopods are particularly scant, with no studies having been conducted Southern Africa and very few having even been conducted globally (Eriksen and Brown, 1980; Qvenild et al., 2018). Given the life history of large branchiopods and their adaptations to temporary wetlands, eco-physiological information on adult and cyst stages would be useful to better understand the ecology of these organisms and the environmental drivers that facilitate persistence at local scales. This is particularly relevant in the Southern Africa region given the projections for climate change, which may have implications for the long-term persistence of extreme environment specialist species.

1.4 Threats to temporary wetlands in Southern Africa

Water resources in Southern Africa are vulnerable to anthropogenic and climate change effects (Kusangaya et al., 2014). Temporary wetlands are no exception. Temporary wetlands are relatively small in size and as such, are often overlooked, especially during the dry phases. In addition, government regulations do not inclusively protect temporary wetlands in the region where there appears to be competition among policies (Dalu et al., 2017a). This puts temporary wetlands in Southern Africa at a higher risk of degradation than other aquatic ecosystems.

Private landowners also favor land-use change of temporary wetlands for the benefits of their properties (Calhoun et al., 2017). They often encourage damming of temporary wetlands to increase the duration that they hold water so that they can continue using the wetland for watering. In many instances temporary wetlands are even converted into permanent wetlands in this way (Dalu et al., 2017a). However, this has a drastic effect on the community of temporary wetland fauna as they usually use these systems to avoid predators that inhabit permanent water bodies (Dalu et al., 2017a). Martens and de Moor, (1995) outlined the localized extinction of several ostracod fauna which resulted from conversion of temporary wetland into permanent water bodies. As such, there is a significant impact on the biodiversity of the region from damming of temporary wetlands. Furthermore, there is an increase pressure on temporary wetlands through agricultural practices. Livestock that frequently utilise temporary wetlands to meet water requirements can compromise these environments through increased nitrification associated with defecation and urination in and around the wetland (Buxton et al., 2020a). Excessive abstraction and direct removal of water by livestock might have implications in reducing the hydroperiods of temporary wetlands. This can result in abortive hatchings whereby hydroperiods are not sufficiently long enough for hatchlings to reach sexual maturity and reproduce, potentially resulting in localized extinction of temporary wetlands specialists.

1.5 Present study

The present study was conducted in the Central District Botswana and focused on aspects of the thermal biology of common large branchiopods in the region. Botswana is generally characterised by hot and dry climates. However, it is drier and cooler in the south west parts of the country while hotter and relatively wetter in the northern parts with variation gradient in-between. The region is, however, expected to experience variations in the precipitation patterns and temperature that may affect temporary wetlands (Kusangaya et al., 2014).

Direct and indirect effects of shifting temperature and rainfall on temporary wetland specialist remains largely unexplored. To address the major knowledge gap, this thesis deals with components of branchiopod thermal ecophysiology. The study focussed on the most commonly encountered branchiopod species, and the temporary wetlands in which they occurred. The work has, however, been contextualised within the broader eco-physiological context wherever possible, making the work more widely relevant. Included in the thesis is a fine-scale temporal description of the thermal characteristics of select wetland types. The study then contrasts the thermal biology of select adult branchiopods, relating the findings to the thermal characteristics of the specific wetland types from which the model taxa occur. Finally, cyst hatching dynamics, in relation to environmental conditions, were assessed for the most commonly encountered anostacan species. More specifically, the following aspects were investigated:

1. The thermal dynamics of rock-pool and pan temporary wetland types (Chapter 2).

2. The relevance of environment of origin (rock-pool or pan) and phylogenetic relatedness as a better predictor of thermal fitness for branchiopod taxa found in specific temporary wetland types (Chapter 3).
3. The role of temperature and light in mediating cyst hatching success following wetland inundation for a pan specialist Anostracan (Chapter 4).

The opportunity was also taken to contribute to the molecular knowledge gaps on large Branchiopods. As such, in addition to the autecological investigation of the four studied species, the mitochondrial DNA of these large branchiopods were also described, and in two instances, published. This information presented as supplementary material (files S 1-3) in the thesis.

Chapter 2

Physico-chemical characteristics of pans and rock-pools



Plate 2: Bless the rains down in Africa- sampling a recently inundated pan in Central District, Botswana (Photo: Ryan Wasserman).

Abstract

In order to attain *in situ* physico-chemical information on the environments in which temporary wetland specialist branchiopods inhabit, numerous rock-pools and pans were identified in the Palapye region, Botswana. The thermal profiles of these temporary wetlands were characterised during both the dry phase and hydroperiods. During synchronised dry phases and hydroperiod, daily habitat temperature- maximum ($H_{t_{max}}$), -minimum ($H_{t_{min}}$), -mean ($H_{t_{mean}}$) and coefficient of variation ($H_{t_{cv}}$) were recorded from temperature data loggers deployed in rock-pools and pans. Select physico-chemical features (Turbidity, Conductivity and Total Dissolved Solids) were also measured hydroperiods to further explore differences between rock-pool and pan temporary wetland types. Habitat temperature maximum ($H_{t_{max}}$) values were significantly higher ($p < 0.05$) in the rock-pools than the pans during both the dry phase and hydroperiod. Rock-pool and pan $H_{t_{max}}$ values were 54.0 ± 4.3 °C and 43.2 ± 0.8 °C during during the dry phase and 32.8 ± 1.1 °C and 27.1 ± 1.6 °C during the hydroperiod, respectively. Similar trends were evident for the $H_{t_{mean}}$ and $H_{t_{cv}}$ where differences between rock-pools and pans were significantly different ($p < 0.05$) in each case. Daily habitat temperature mean ($H_{t_{mean}}$) values in rock-pools dry phase and hydroperiod were 34.8 ± 0.8 °C and 27.1 ± 0.5 °C, respectively while from pans they were 31.1 ± 2.7 °C and 25.3 ± 1.8 °C, respectively. Daily habitat temperature coefficient of variation ($H_{t_{cv}}$) in rock-pools during the dry phase were 0.32 ± 0.05 , while during the hydroperiod they were 0.13 ± 0.02 . In the pans, $H_{t_{cv}}$ were lower than in rock-pools at 0.24 ± 0.05 during the dry phase and 0.04 ± 0.01 °C during the hydroperiod. There were, however, no significant differences ($p > 0.05$) in $H_{t_{min}}$ values between the two

pond types during the dry phase, or during the hydroperiod. Daily habitat temperature min (H_{tmin}) values in the rock-pools were $23.6 \pm 1.5^{\circ}\text{C}$ during the dry phase and $23.2 \pm 0.7^{\circ}\text{C}$ during the hydroperiod, while pan H_{tmin} values were $21.8 \pm 3.6^{\circ}\text{C}$ and $23.7 \pm 0.9^{\circ}\text{C}$ during the dry phase and hydroperiod, respectively. An Euclidean distance similarity matrix using H_{tmax} , H_{tmin} , H_{tmean} and H_{tcv} as variables, largely separated wetlands based on wetland type, irrespective of phase. Analysis on ponds' chemical properties showed pans were more turbid (334 ± 149.07 NTU) than rock-pools (0.17 ± 0.53 NTU). Similarly, pans had greater electrical conductivity (34.75 ± 25.5 $\mu\text{S}/\text{cm}$) and total dissolved solids (22.125 ± 16.17 mg/L) than rock-pools (5.4 ± 3.7 $\mu\text{S}/\text{cm}$ and 3.1 ± 2.5 mg/L, respectively). An Euclidean distance similarity matrix using NTU, EC, and TDS values as variables, similarly separated wetlands based on wetland type. These results highlight that specialist rock-pool and pan branchiopods are exposed to discrete physio-chemical conditions. Within the context of the thermal dynamics of rock-pools and pans, differences between the wetland types have likely implications for the thermal preferences and critical limits to activity for specialist branchiopod species that occur exclusively in either rock-pools or pans.

Keywords: temporary wetlands; thermal profiles; turbidity;

2.1 Introduction

Temporary wetlands are aquatic habitats that alternate between dry and wet phases (Schwartz and Jenkins, 2000). They have been recognised as global sanctuaries for wildlife and indigenous people (Ambelu et al., 2013). These temporary wetland habitats are usually not connected to a permanent source of water, but they have varying water sources and out flows (Mushet et al., 2015). They serve an important role in certain landscapes by replenishing groundwater, controlling floods, recycling nutrients and even removing toxins (Waterkeyn et al., 2008). Temporary wetlands include a variety of different water bodies with different underlying geology (Schwartz and Jenkins, 2000; Williams, 2005). This in return has an influence on the physio-chemical characteristics of temporary wetland types, with implications for the biological diversity of invertebrate communities that inhabit these environments (Rains et al., 2008; Waterkeyn et al., 2008).

Many temporary wetlands host specialist and generalist taxa that use these habitats obligately e.g. through synchronising their phenologies with the wet and dry cycles and facultatively by intermittently shifting environments (Wang and Chou, 2015). Many specialist taxa either produce dormant eggs during the hydroperiods (wet phases) that can withstand prolonged dry periods and hatch during subsequent inundation (Jocque et al., 2010). Certain widespread crustacean taxa (e.g. copepods and ostracods) have temporary wetland specialist species that exhibit such adaptations. Branchiopod crustaceans, however, are found exclusively in temporary wetland environments and have been described as flagship species for these unique aquatic ecosystems (Belk,

1998; Colburn, 2004). Generalist taxa that utilize these environments generally descend on temporary wetlands over the course of the variable hydroperiod, with wetland diversity generally increasing over time, largely as a result of immigration (O'Neill and Thorp, 2014). The utilisation of these environments by generalists is a result of the often-high levels of productivity in these ecosystems, which are exploited by invading taxa, including many predatory species such as birds (Brochet et al., 2010). Migration of animals such as birds from and between temporary wetlands has also been identified as an important form of dispersal of dormant eggs of the specialist inhabitants (Brochet et al., 2010). As a result, many temporary wetland specialist species that are widespread by zoochory, through propagule (dormant egg) dispersal, are common and presumably frequent. For example, the anostracan *Streptocephalus cafer* is widespread in Southern Africa, occurring in pans across numerous biogeographic zones in the region (Hamer and Brendonck, 1997). Similarly, *Branchiopodopsis wolffi* is widespread, occurring in rock-pools in relatively dense clusters across semi-arid Southern Africa (Hulsmans et al., 2007).

These wholly aquatic organisms are able to colonise new habitats given the dispersal of their propagules by generalist taxa that facultatively utilize temporary wetlands. However, propagule dispersal is presumably indiscriminate between wetlands and wetland types. While temporary wetlands are diverse in nature with specific wetland types characterised by a suite of environmental conditions many specialist branchiopods that inhabit these environments are only found in certain wetland types. For example, the fairy shrimp (Anostraca) *Streptocephalus cafer*, although widespread

in pans, are not found in rock-pool temporary wetlands (Hamer and Brendonck, 1997). Similarly, the closely related *Branchiopodopsis wolfi* (Anostraca) occurs throughout Southern Africa but is only found in rock-pool wetland types (Brendonck et al., 2000). In many instances rock-pools and pans are located in close proximity to each other. Presumably, propagules of *S. cafer* make their way into rock-pools, while *B. wolfi* make their way into pan temporary wetland types through indiscriminate dispersal mechanisms. The failure of *S. cafer* and *B. wolfi* to establish in rock-pools or pans respectively must, therefore, be a result of environmental mismatches, biotic effects or combinations of both.

The physio-chemical properties of temporary wetland types affect inhabitant invertebrate communities (Bird et al., 2019). This is a possible explanation as to why certain temporary wetland specialist species from the same order can only be found in some pond types but not others, even in the same geographical location (Hamer and Brendonck, 1997; Brendonck et al., 2000). Little work has, however, been done to identify specific environmental drivers that may contribute to these preclusion dynamics. While this type of knowledge is of fundamental interest, it also has implications for biodiversity in the Anthropocene. Changes in aquatic physico-chemistry in relation to human-mediated activities are increasingly evident (Calhoun et al., 2017) and are likely to be pronounced in regions where water resources are scarce, such as Southern Africa. In addition, shifting climates are projected to have an effect on different climatic regions (Engelbrecht et al., 2015; Calhoun et al., 2017) and the effects of such shifts will have an impact on aquatic ecosystems (Sim et al., 2013).

While these concerns are relevant for all aquatic ecosystems, temporary wetlands are thought to be among the most vulnerable of aquatic habitats-particularly from impacts associated with urbanisation, agriculture and climate change (Waterkeyn et al., 2008).

To contribute towards bridging the knowledge gap, this chapter focuses on physico-chemical characteristics of two abundant temporary wetland types in the Central District of Botswana, with particular emphasis on the thermal dynamics and other physico-chemical properties of these environments during the rainy season. Botswana is situated in a largely arid part of Southern Africa and expected to experience some of the worst shifts in precipitation and temperature variations in the world (Kusangaya et al., 2014; Engelbrecht et al., 2015). Given that temporary wetland habitats are among the most extreme aquatic environments (Mahoney et al., 1990), they are likely to be particularly susceptible to such changes. Within the context of their infauna, shifts in temperature means, extremes and variability will likely affect the ecology of these environments (Bartolini et al., 2013). Climate change will likely also increase dependence on temporary wetlands by farmers and indigenous people, thus exacerbating their threats.

As this thesis deals with aspects of the thermal ecology of large branchiopods in rock-pool and pan ecosystems, an in-depth description of the temperature dynamics of these different temporary wetland types was warranted. To the best of my knowledge, no previous studies have contrasted fine temporal scale temperature data in these

highly variable but different temporary wetland environments in sub-Saharan Africa. This is potentially problematic given that no long-term fine resolution data is available for baseline comparison for future studies. Given the climate change projections for the region, environmental data in temporary wetlands, as particularly vulnerable ecosystems should be prioritised. Temperature in water bodies is essential as a regulatory mechanism driving biochemical and physiological processes (Teoh et al., 2010; Burton and Einum, 2020), with implications for performance, behaviour and biotic interactions (Vucic-Pestic et al., 2011, Domenici et al., 2007). However, studies investigating thermal ecology require adequate and relevant temperature information on the environments in which organisms live. To this end, fine-scale temperature data was collected from a suite of rock-pool and pan ecosystems, with the goal of acquiring synchronised replicated data across the wetland types, to contrast dry phase and hydroperiod (wet phase) temperature dynamics. In particular, daily temperature maximum, minimum, average and relative variability (using coefficient of variation [CV]) data were contrasted over a synchronised dry phase and hydroperiod, respectively. It was hypothesised that during both dry phases and hydroperiods (wet phases), 1) mean daily temperatures would be similar between rock-pools and pan temporary wetland types, but that 2) rock-pools would experience higher maximum daily temperatures and 3) lower minimum daily conditions than pans given their smaller size and lower thermal inertia, facilitating 4) more variable (higher CV) daily conditions in rock-pools than in pans.

2.2 Materials and methods

2.2.1 Study Site

The study was conducted in austral summer (between November 2019 and March 2020) in the Central District of Botswana, between the towns of Sherwood (22°55'59.9"S 27°53'29.6"E) and Palapye (22°33'00.0"S 27°06'36.0"E) (Fig. 2.1). This region is classified as arid, with a high mean annual temperature of 22.7 °C and a low total rainfall of 351 mm (Batisani and Yarnal, 2010; Akinyemi, 2017; Akinyemi and Abiodun, 2019). The land cover in the region is characterised by mainly heavily grazed rangeland vegetation among canopies of various densities and heights (Alemaw et al., 2013). The underlying geology is a combination of sandstone, siltstone, mudstone, minor sandstone and quartzite (Ermanovics et al., 1978). The region typically receives rainfall between between October and April (Batisani and Yarnal, 2010; Oladelea et al., 2016), although there is considerable inter-annual variation in rainy season. Rainfall data from the Palapye weather station (22°34'S 27°09'E; elevation = 908 Metres), supplied by the Department of Meteorological Services of Botswana, highlight the months where the town received rainfall between October 2017 and February 2020 (Fig. 2.2).

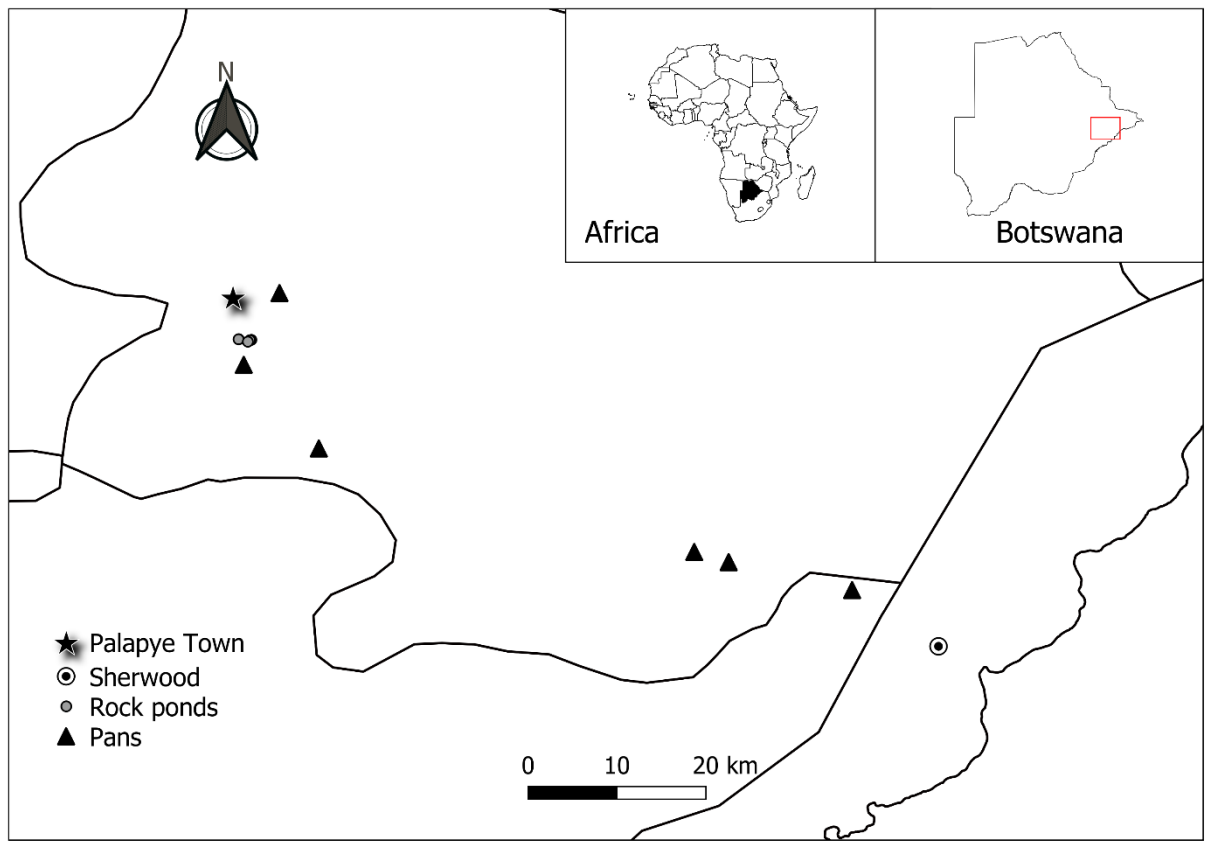


Fig 2. 1: A map of study site showing the location of Palapye and Sherwood together with identified temporary wetlands in relation to Central District of Botswana in Southern Africa.

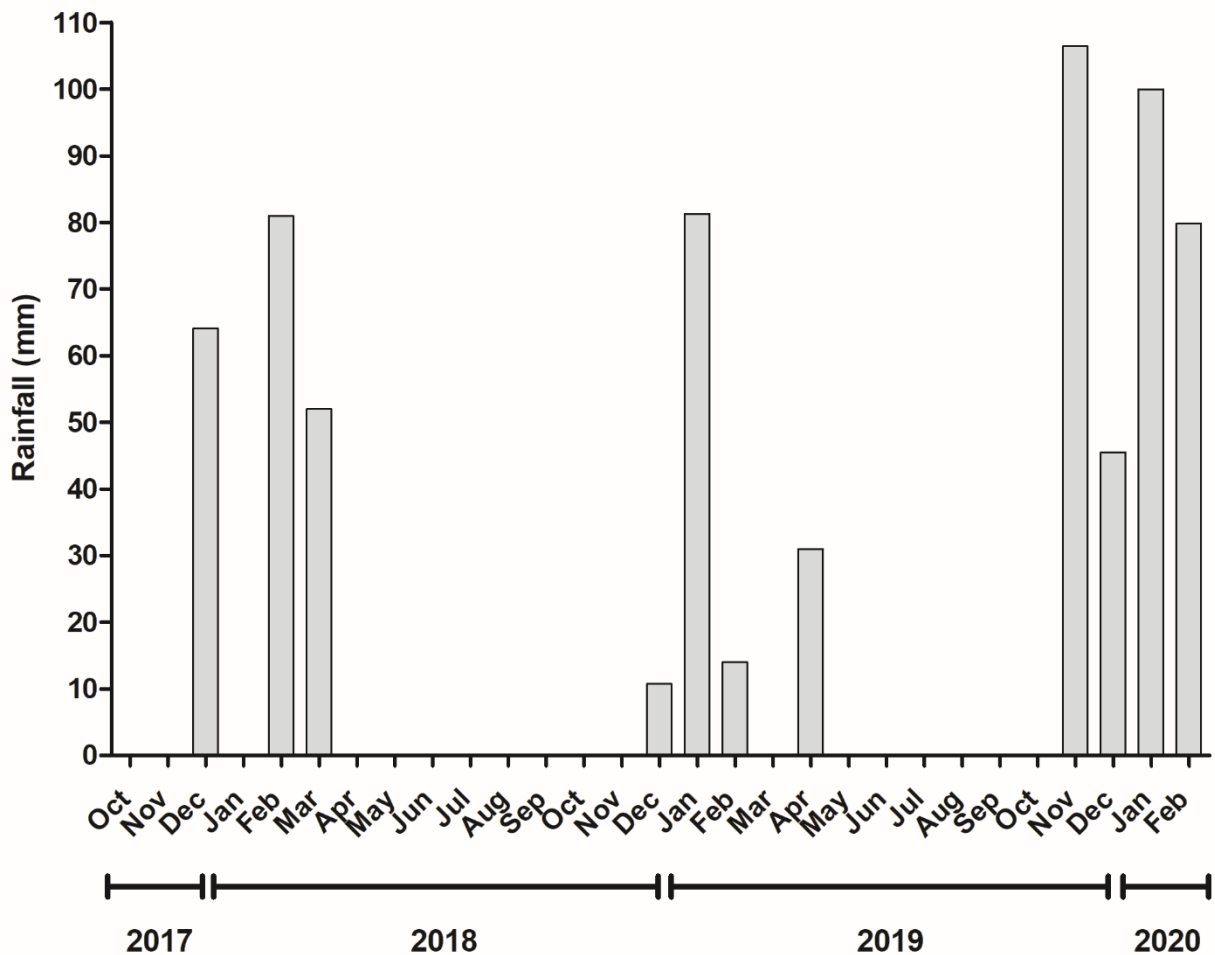


Fig 2. 2: Monthly total rainfall (mm) for the period October 2017 to February 2020 at the Palapye weather station (22°34'S 27°09'E; elevation = 908 Metres). Rainfall data was supplied by the Department of Meteorological Services of Botswana. Jan = January; Feb = February; Mar = March; Apr = April; Jun = June; Jul = July; Aug = August; Sep = September; Oct = October; Nov = November; Dec = December.

2.2.2 Pond identification

Ponds of each type were identified haphazardly between Sherwood and Palapye in Central District, Botswana. As part of a larger study on temporary wetland ecology, the ponds were identified after inundation following rainfall events in early 2018, for ease

of identification. Rocky outcrops on the outskirts of Palapye, were identified to hold numerous rock-pools (Fig. 2.3). As such, various rock-pools were identified for sample collection (see Chapter 3) and physico-chemical characterization and monitoring (Table 2.1). Similarly, pans (Fig. 2.4) were identified in the same vicinity, but between Palapye and Sherwood along the Martins Drift Road. All ponds selected for the study were observed containing populations of spinicaudatans, anostracans or both, among other crustaceans.

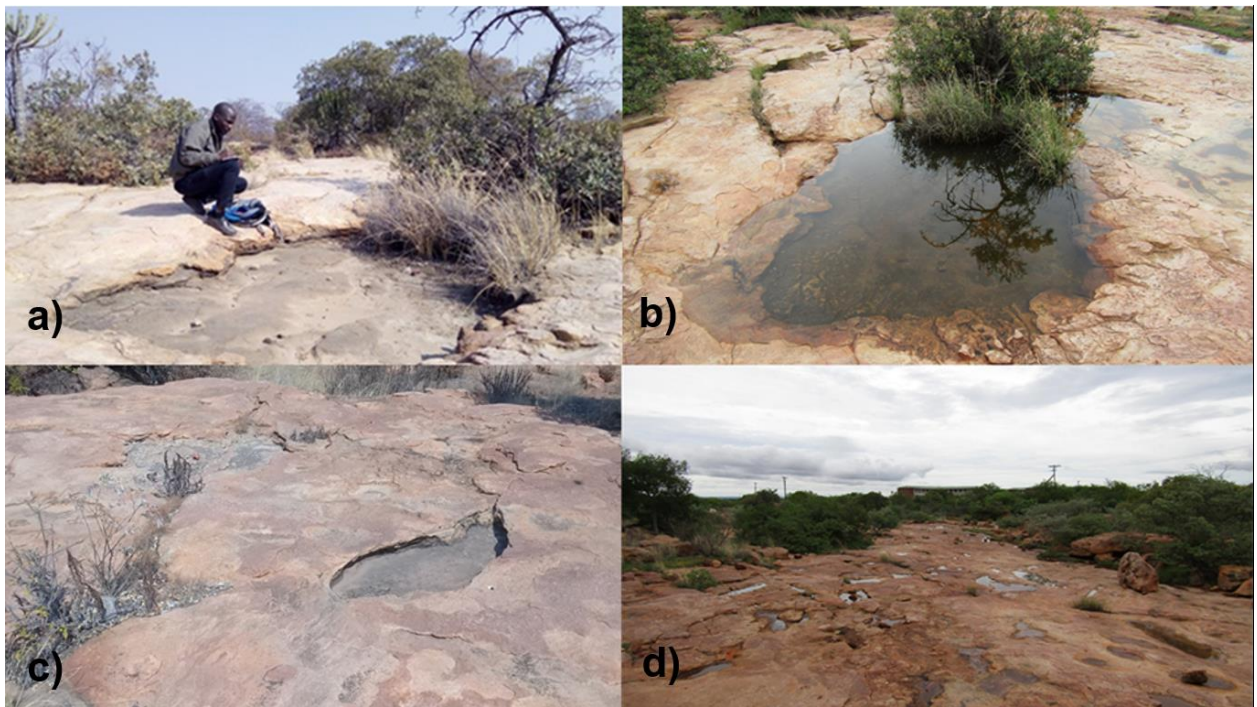


Fig 2. 3: Examples of rock-pools on rocky outcrops in the Palapye region showing a rock-pool during a) a dry phase, b) a hydroperiod and c) and d) the landscapes in which these rocky outcrops and associated rock-pools occur.

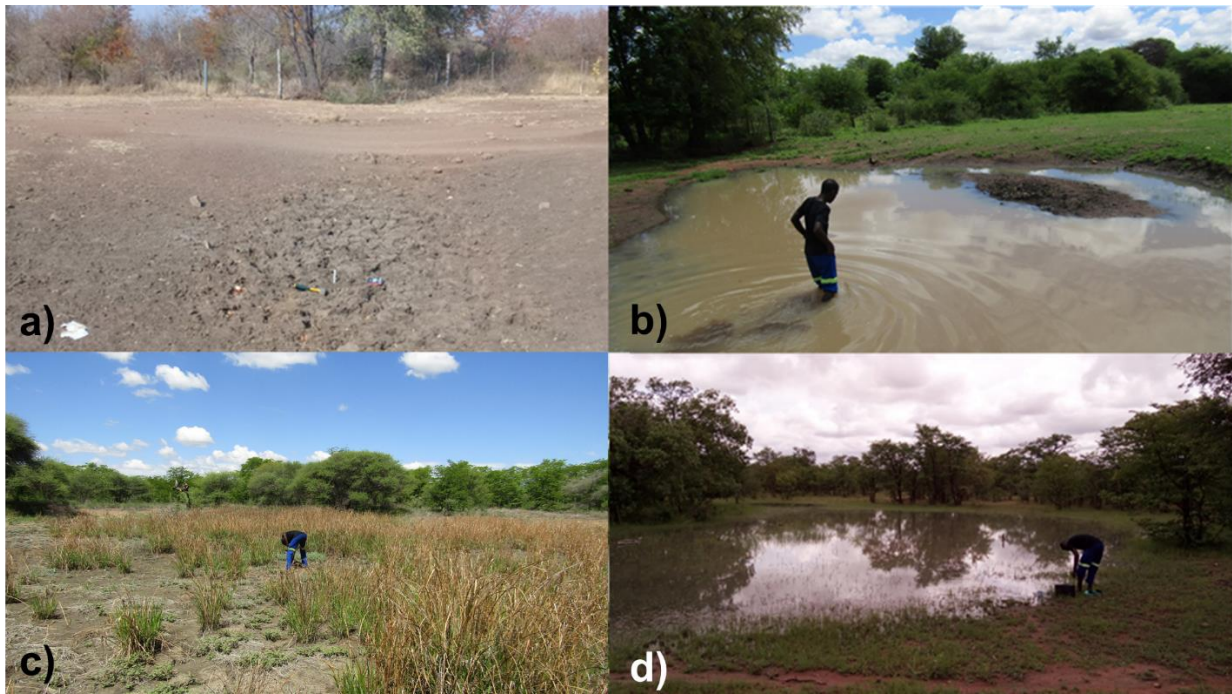


Fig 2. 4: Examples of pans in the Palapye/ Sherwood region showing a pan during a) a dry phase, b) a hydroperiod and c) and d) landscapes in which these pans occur.

2.2.3 Pond Temperature monitoring

Programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) were used for the monitoring of temperatures in the ponds. Loggers (0.5°C accuracy) were set to 1 h sampling frequencies and deployed in various rock-pools and pans. Loggers were placed in the deepest part of selected wetlands (Table 2.1) and allowed to record temperatures between November 2019 and February 2020. Data loggers were changed every 30 days and data recorded continuously from November 2019 to March 2020. Logger deployment spanned both dry and wet phases (hydroperiods) to allow comparison of both phases (dry and wet) across wetland types (pans and rock-pools).

Table 2. 1: Locations of the rock-pools sampled for physico-chemical characterization. Temp = Temperature measurements recorded hourly using HOBO data loggers. Physico-chemical = total dissolved solids, electrical conductivity and turbidity measurements of the water. Dry phase and hydroperiod represent the different phases in which data were collected.

Wetland	Co-ordinates	Dimensions when full (length (m) x width (m))	Data collected
Rock-pool 1	22°35'48.4"S 27°08'05.5"E	4.2 X 4.0	Temp (Dry phase and hydroperiod) Physico-chemical (hydroperiod)
Rock-pool 2	22°35'49.6"S 27°07'59.6"E	5.8 X 5.5	Temp (Dry phase and hydroperiod) Physico-chemical (hydroperiod)
Rock-pool 3	22°35'45.8"S 27°07'15.8"E	3.4 X 2.6	Temp (Dry phase and hydroperiod) Physico-chemical (hydroperiod)
Rock-pool 4	22°35'46.1"S 27°07'14.6"E	2.4 X 2.6	Temp (Dry phase and hydroperiod) Physico-chemical (hydroperiod)
Rock-pool 5	22°35'55.6"S 27°07'51.5"E	1.5 X 0.5	Temp (Dry phase and hydroperiod) Physico-chemical (hydroperiod)
Pan 1	22°37'26.4"S 27°07'35.3"E	50 X 50	Temp (Dry phase only)
Pan 2	22°49'45.7"S 27°37'17.9"E	14 X 10	Temp (Dry phase and hydroperiod) Physico-chemical (hydroperiod)
Pan 3	22°52'16.0"S 27°47'42.7"E	76 X 54	Temp (Dry phase and hydroperiod) Physico-chemical (hydroperiod)
Pan 4	22°42'56.8"S 27°12'32.4"E	142 X 1.5	Temp (Dry phase only) Physico-chemical (hydroperiod)
Pan 5	22°50'25.5"S 27°39'34.2"E	55 X 21	Temp (Dry phase and hydroperiod) Physico-chemical (hydroperiod)
Pan 6	22°32'43.1"S 27°09'56.8"E	208 X 61	Temp (Dry phase and hydroperiod)

2.2.4 Physico-chemical Characteristics

While the study and chapter focussed on the thermal dynamics of pans and rock-pools the physico-chemistry of the different wetland types were further characterised. All wetlands were measured at the same time, around six days after the wetlands filled with water, to allow for direct comparison. Electrical conductivity (EC) ($\mu\text{S}/\text{cm}$), total dissolved solids (TDS) (mg/L) and turbidity (NTU) were measured at midday. on one occasion. These variables were measured using the GPS aquameter-AP-1000 multi-probe (Aqua Read Ltd, UK). Briefly, the probe was held at midwater depth near the center of each water body and physico-chemical characteristics recorded. Care was taken to avoid disturbing water before taking the measurements.

2.2.5 Data Analysis

Long-term temperature data

The daily maximum, minimum and average temperatures (between 00:00 h and 24:00 h), were calculated for each pond, and used for general thermal descriptions. These daily temperature values were plotted in the software program GraphPad Prism (see Supplementary Figures 1-11 for full thermal profiles). Select data was, however, extracted for comparisons between wetland types during the rainy season. This was done for both a dry phase and a hydroperiod and was restricted to the warmest summer months (November-January), which coincided with rainy season. Data selection was constrained by mismatches between the variable hydroperiods and select data loggers that went missing. However, data was available for synchronised

dry phase for the period 2-7 November 2019 (pans $n = 5$; rock-pools $n = 5$), and for synchronised hydroperiods between 18-24 January 2020 (pans $n = 4$; rock-pools $n = 5$). The habitat temperature maximum (Ht_{max}), minimum (Ht_{min}), mean (Ht_{mean}) and coefficient of variation (CV: standard deviation/ mean) (Ht_{cv}) was calculated per day for these periods. Per pond, mean values of Ht_{max} , Ht_{min} , Ht_{mean} and Ht_{cv} over these periods were then used for comparison between pond types during respective phases. This data was tested for normality and homogeneity of variance using Shapiro-wilk test and Bartlett test, respectively. The differences in Ht_{max} , Ht_{min} , Ht_{mean} and Ht_{cv} between rock-pools and pans were either assessed using a Welch two sample t-test, or a Mann Whitney U test (R Core Team, 2019), depending on if the data met parametric test assumptions. This was done for both dry phase and hydroperiod data. Habitat temperature maximum (Ht_{max}), Ht_{min} , Ht_{mean} and Ht_{cv} were then also used as variable for further analyses in Primer version 6, whereby relative similarities between ponds were visualized through the constructing of separate dry phase and hydroperiod dendrograms. For this procedure, a Euclidean distance similarity matrix was generated for the data (11 water bodies comprised of 2 pond types, with Ht_{max} , Ht_{min} , Ht_{mean} and Ht_{cv} values for each pond). Group average hierarchical sorting was used to assess clusters in a dendrogram format. To illustrate the extent of daily fluctuations within the wetland types, hourly temperature was plotted for the hottest day phases and hydroperiods for each pond type (three examples each), respectively (Fig. 2.5).

Physico-chemical data

All physico-chemical data was tested for normality and homogeneity of variance and did not violate parametric assumptions. As such, Welch two sample t-tests were used to assess differences in EC, TDS and turbidity between rock-pools and ponds (R Core Team, 2019) (Table 2.2). Primer 6 was similarly used to determine the different chemical characteristics of different pond types. An Euclidean distance similarity matrix was generated for the data (9 water bodies comprised of 2 pond types, with EC, TDS, turbidity and mid-day temperature values for each pond). Clusters in a dendrogram format were also assessed using group average hierarchical sorting.

2.3 Results

2.3.1 Wetland temperatures

The rock-pools and pans were observed to be dry for much of the year. However, during the rainy seasons they were found to hold water for variable periods with pans holding water for considerably longer than rock-pools (for an example, see Supplementary Figure 12). Daily fluctuations in temperatures were, however, evident for both the dry and wet phases. During the dry phases daily temperatures peaked between 12:00-13:00 hours in the rock-pools and between 14:00-15:00 in the pans. Temperatures before and after these peak periods during the dry phases decreased relatively rapidly in both environments (Fig. 2.5). During the hydroperiods, however, peak temperatures shifted to later in the day to 14:00-15:00 in the rock-pools and 17:00-19:00 in the pans. The temperature peaks were not as well-defined during the

wet periods, with temperatures near the peak being similar to the maximum temperature.

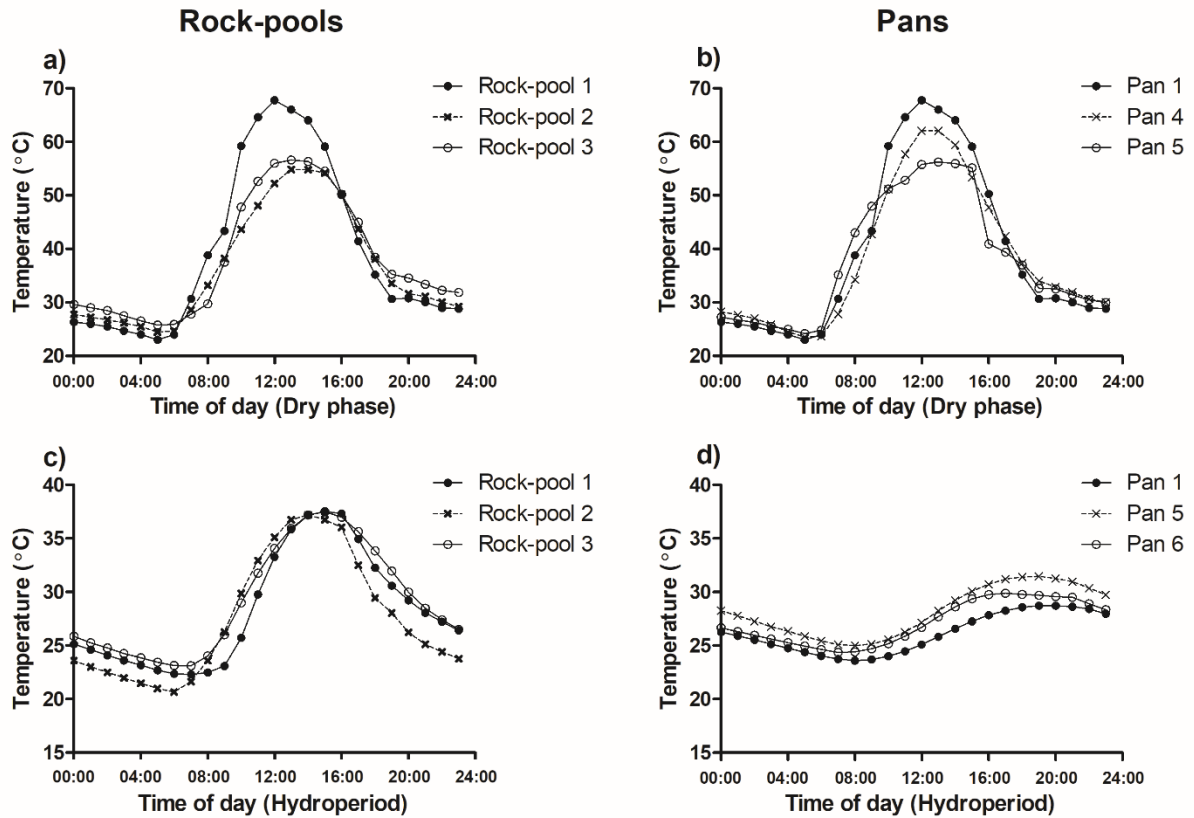


Fig 2. 5: Examples of daily temperature fluctuations within the wetland types for three example rock-pools (a and c) and pans (b and c), during both dry phases (a, b) and hydroperiods (c, d). The 24-hours example periods represent the hottest days recorded in each wetland type during the dry phase (07/11/2019) and hydroperiod (23/01/2020).

Habitat temperature maximum (Ht_{max}), Ht_{mean} and Ht_{cv} were significantly different ($p < 0.05$) between rock-pools and pans in the dry phase of the wetland cycle (Table 2.2). Habitat temperature minimum (Ht_{min}) was, however, not significant between pond types ($p > 0.05$). Rock-pools experienced the highest Ht_{max} (54.0 ± 4.5 °C) compared to pans (43.2 ± 0.8 °C) during the dry phase (Fig 2.6). This was also observed for the Ht_{mean}

rock-pools temperature (34.8 ± 0.8 °C) and pans temperature 31.1 ± 2.7 °C (Fig 2.8). The Ht_{cv} was also elevated in rock-pools (0.32 ± 0.05) compared to pans (0.23 ± 0.05) (Fig 2.9), highlighting the increased variability in temperature in the former wetland type. Habitat temperatures minimum was relatively the same for rock-pools 23.6 ± 1.5 °C and pans 21.8 ± 3.6 °C (Fig 2.7). The habitat temperatures experienced by ponds during the dry phase produced two groups in the cluster analysis, separated according to the pond types (Fig. 2.10). Rock-pools 1-5 (Group 2) clustered at a comparable wetland similarity level (distance ~ 9) to Pans 1, 2, 3, 4 and 6 (Group 1: distance ~ 10). However, in both wetland types a single outlier wetland (Rock-pool 1 and Pan 6) drove this level of overall similarity. Excluding these outliers, pans 1-4 had a surprisingly high level of similarity (distance ~ 2) compared to rock-pools 2-5 (distance ~ 5).

Table 2. 2: Welch two sample t-test outputs testing differences in temperatures between pans and rock-pools during the synchronized dry phases (2-7 November 2019). Ht_{max} = habitat temperature daily maximum, Ht_{min} = habitat temperature daily minimum, Ht_{mean} = habitat temperature daily average, Ht_{cv} = habitat temperature daily coefficient of variation. t-crit- critical t value. Df = degrees of freedom.

	<i>t</i> -crit	<i>Df</i>	<i>p</i>
Ht_{max}	-5.2243	4.2706	0.005342
Ht_{min}	-1.0454	5.4026	0.3403
Ht_{mean}	-2.9624	4.7152	0.03378
Ht_{cv}	-2.8689	7.9918	0.02089

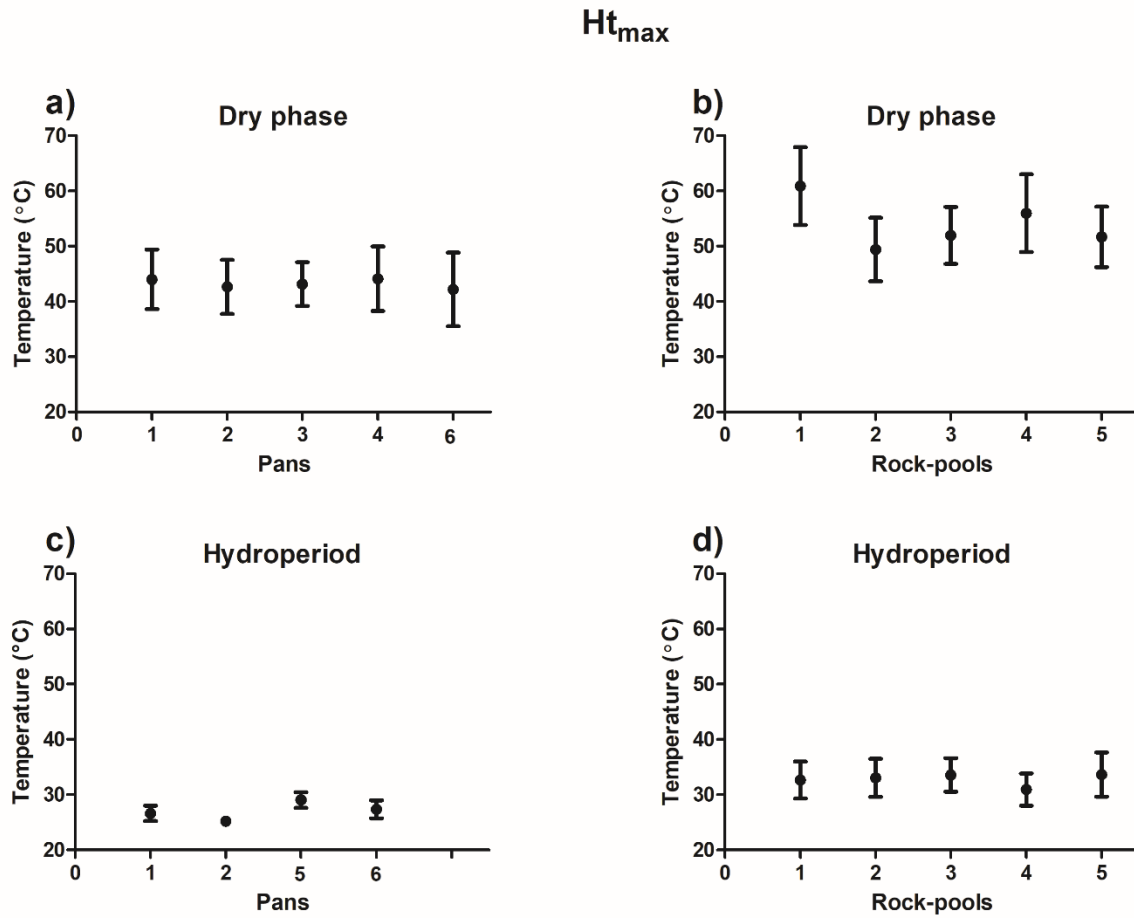


Fig 2. 6: Mean ($\pm$ standard deviation) daily habitat maximum temperatures (Ht_{max}) across five pans (a and c) and rock-pools (b and d) in the Palapye region, during a synchronised dry phase (2-7 November 2019) and hydroperiod (18-24 January 2020).

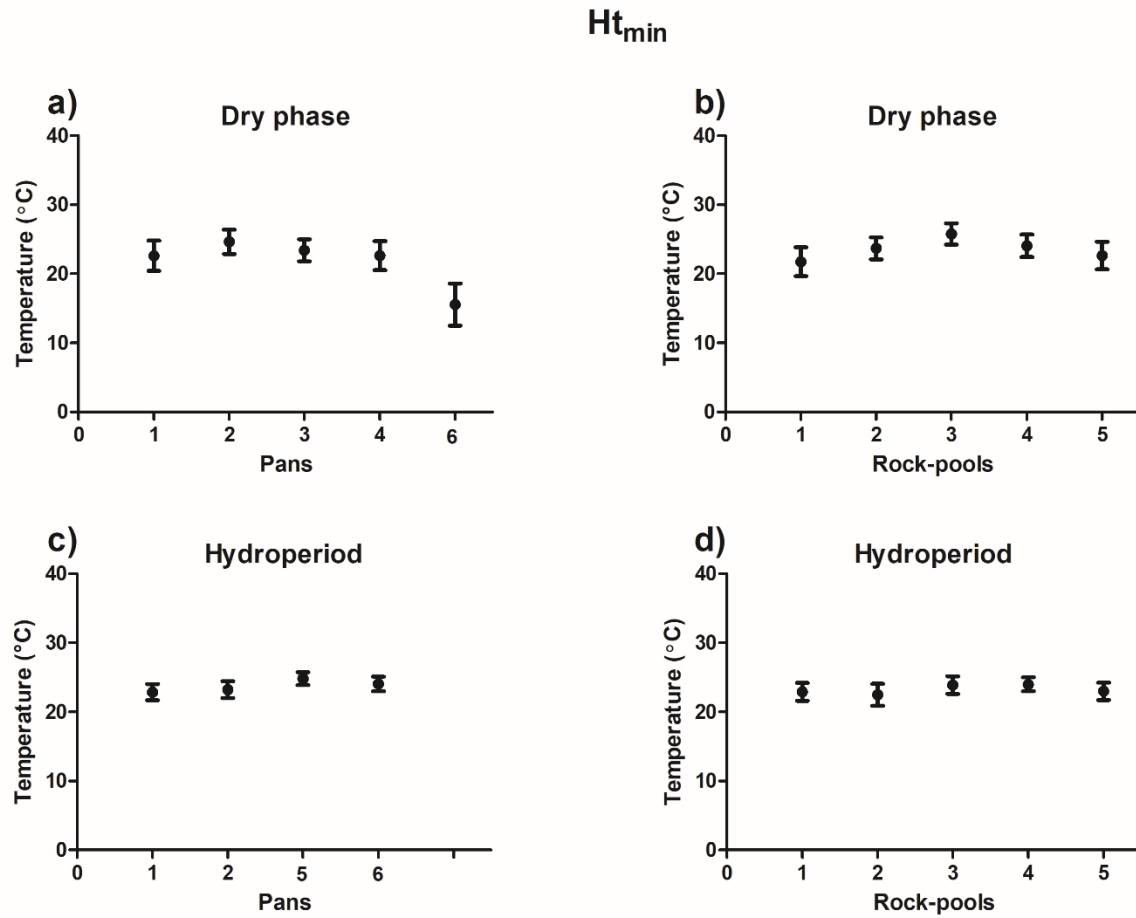


Fig 2. 7: Mean ($\pm$ standard deviation) daily habitat minimum temperatures ($H_{t_{min}}$) across five pans (a and c) and rock-pools (b and d) in the Palapye region, during a synchronised dry phase (2-7 November 2019) and hydroperiod (18-24 January 2020).

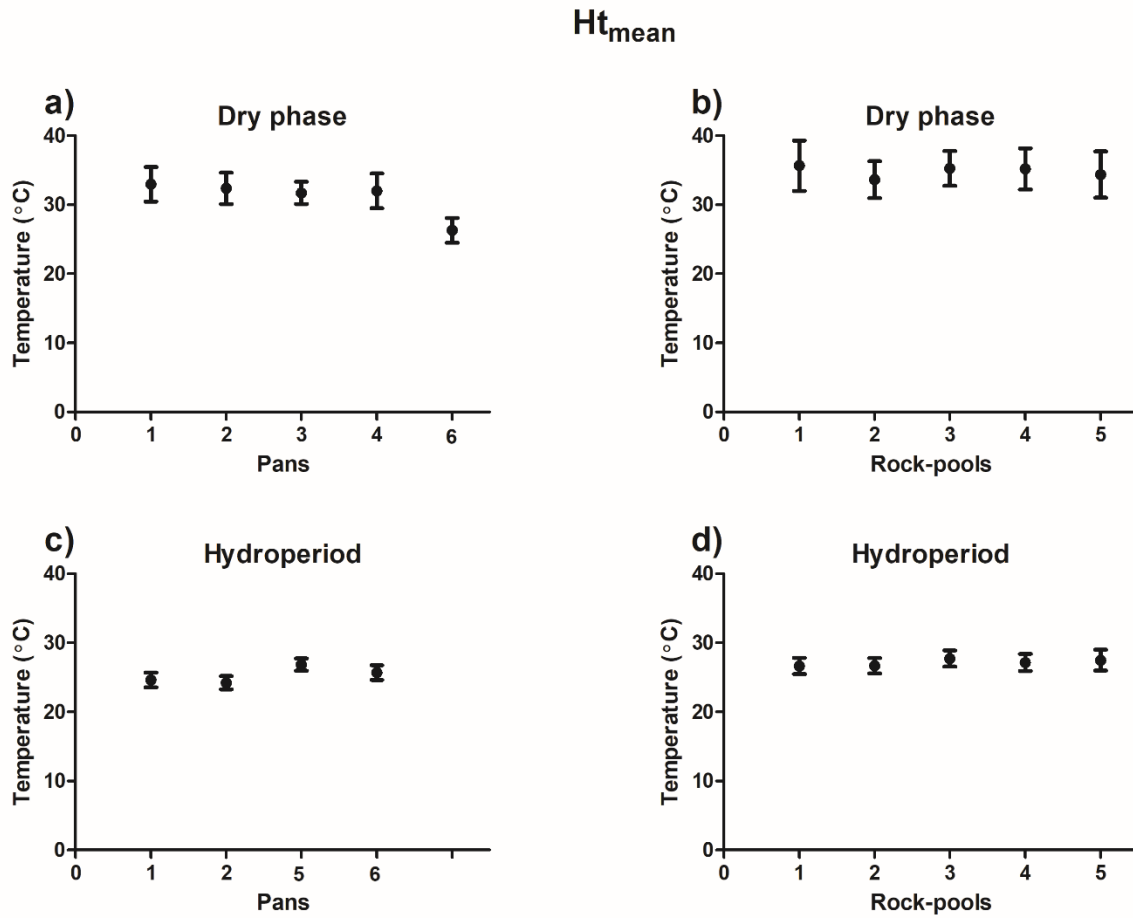


Fig 2. 8: Mean ($\pm$ standard deviation) daily habitat average temperatures (Ht_{mean}) across five pans (a and c) and rock-pools (b and d) in the Palapye region, during a synchronised dry phase (2-7 November 2019) and hydroperiod (18-24 January 2020).

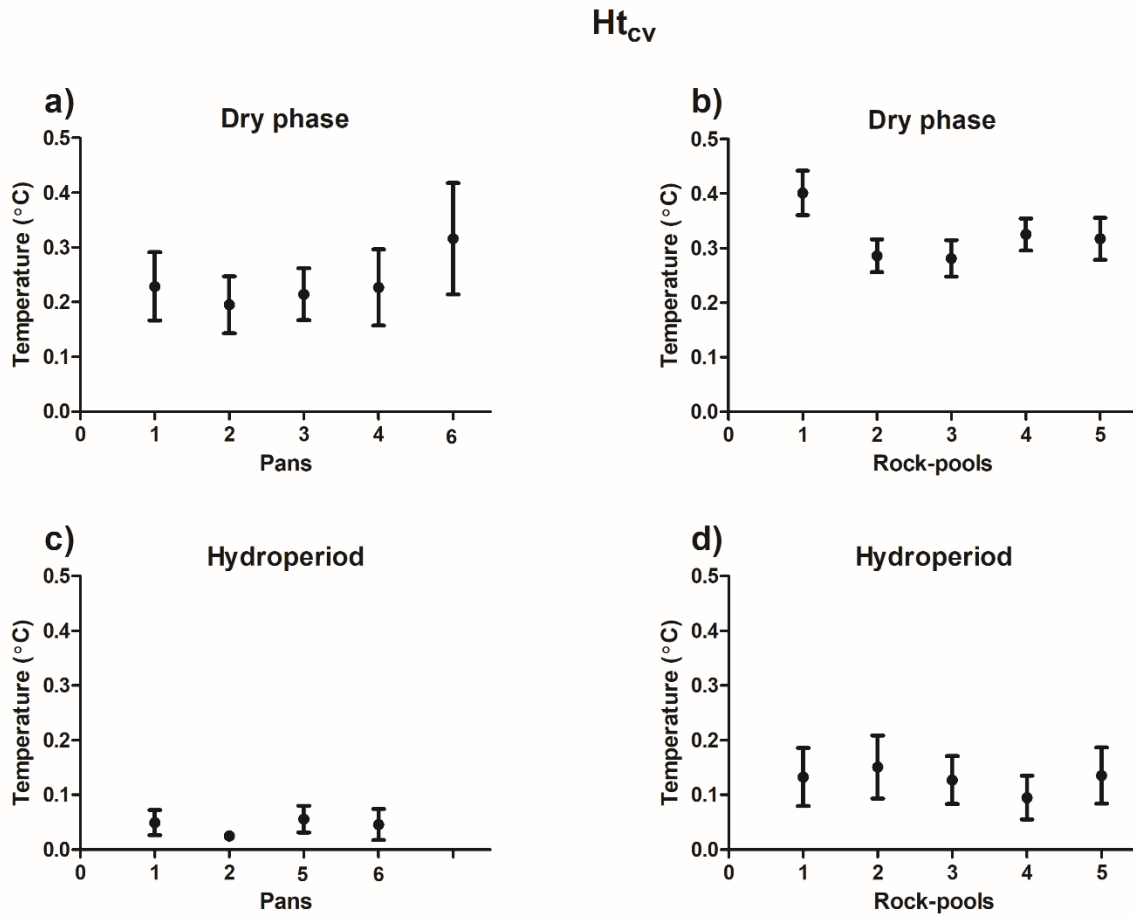


Fig 2. 9: Mean ($\pm$ standard deviation) daily habitat coefficient of variation in temperatures (Ht_{cv}) across five pans (a and c) and rock-pools (b and d) in the Palapye region, during a synchronised dry phase (2-7 November 2019) and hydroperiod (18-24 January 2020).

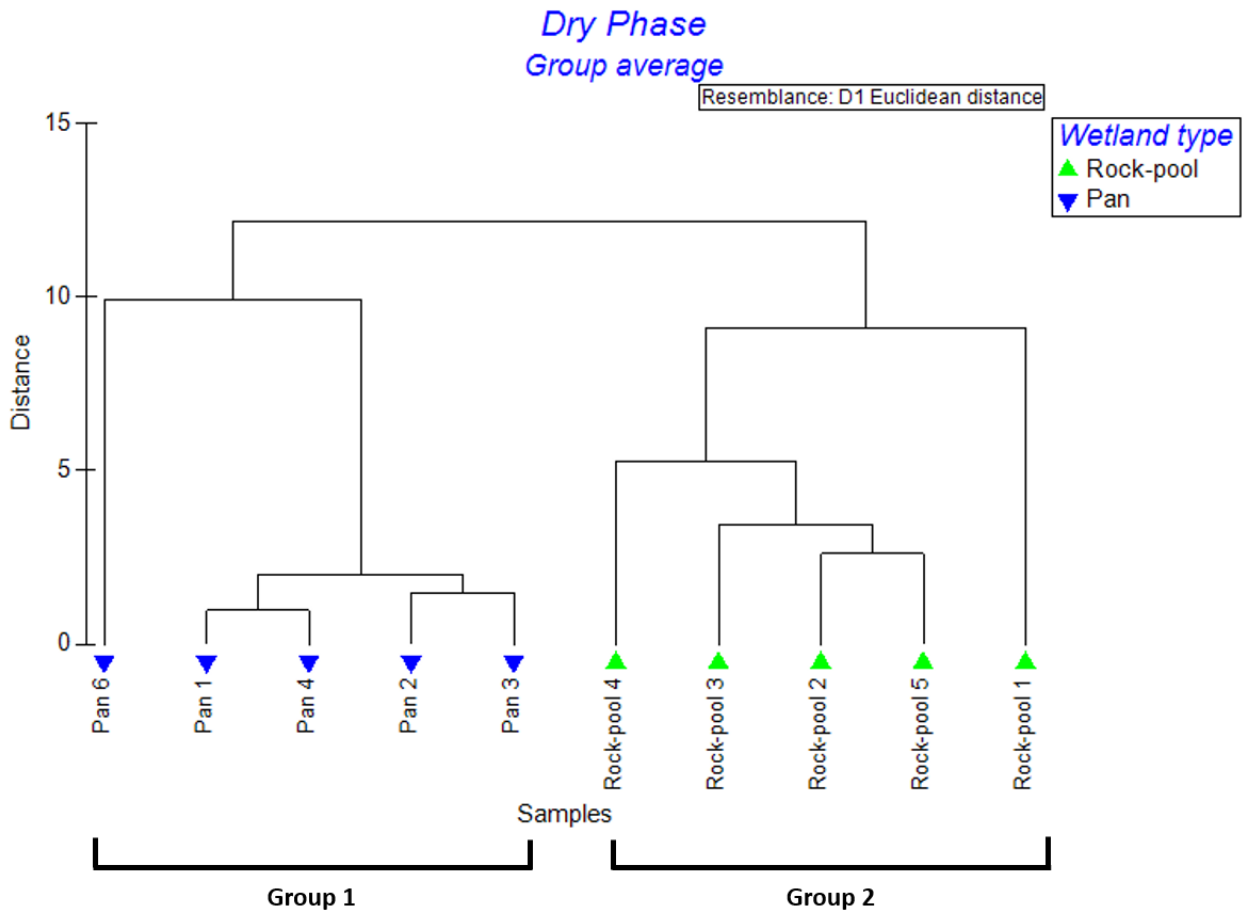


Fig 2. 10: Cluster dendrogram of wetland types based on thermal characteristics ($H_{t_{max}}$, $H_{t_{min}}$, $H_{t_{mean}}$, $H_{t_{cv}}$), highlighting the differences between rock-pools and pans. The Euclidean distance differentiated the ponds according to the temperature variables calculated for the synchronized *dry phase* over the period 2-7 November 2019. Groups were identified based on levels of similarity on the Y-axis (Distance).

Similar trends between wetland types were evident during the hydroperiod as were observed in the dry phase of ponds. Rock-pools had significantly higher ($p < 0.05$) $H_{t_{max}}$, $H_{t_{mean}}$ and $H_{t_{cv}}$ than pans, but statistically similar ($p > 0.05$) $H_{t_{min}}$ were observed between wetland types (Table 2.3). Rock-pools experienced the highest $H_{t_{max}}$ (32.8 ± 1.1 °C) compared to pans (27.1 ± 1.6 °C) during the hydroperiod phase (Fig 2.6). This was similarly observed for the $H_{t_{mean}}$ in rock-pools (27.1 ± 0.5 °C) and pans (25.3 ± 1.8

°C) (Fig 2.8.). The Ht_{cv} was also higher in rock-pools (0.13 ± 0.02) than in pans (0.04 ± 0.01) (Fig 2.9). Habitat temperature minimum of both ponds was relatively for both pond types with rock-pools experiencing Ht_{min} 23.2 ± 0.7 °C and pans Ht_{min} 23.7 ± 0.9 °C (Fig 2.7). At the Euclidean distance similarity level of 3, three groups were identified in the cluster analysis (Fig 2.11). Group 1 comprised exclusively pans (pans 1, 2 and 6), while Group 2 comprised Pan 5 and Rock-pool 4. Group 3 comprised exclusively rock-pools (rock-pools 1, 2, 3 and 5). The second group was more similar to the rock-pool group than the pan group (distance ~ 3.6). (Fig. 2.11)

Table 2. 3: Welch two sample t-test outputs testing differences in temperatures between pans and rock-pools during the synchronized hydroperiod (18-24 January 2020). Ht_{max} = habitat temperature daily maximum, Ht_{min} = habitat temperature daily minimum, Ht_{mean} = habitat temperature daily average, Ht_{cv} = habitat temperature daily coefficient of variation. t-crit- critical t value. Df = degrees of freedom.

	<i>t</i> -crit	<i>df</i>	<i>p</i>
Ht_{max}	-6.0675	5.1043	0.001633
Ht_{min}	0.92147	5.5285	0.3952
Ht_{mean}	-2.8813	3.789	0.04783
Ht_{cv}	-7.4267	6.8135	0.0001669

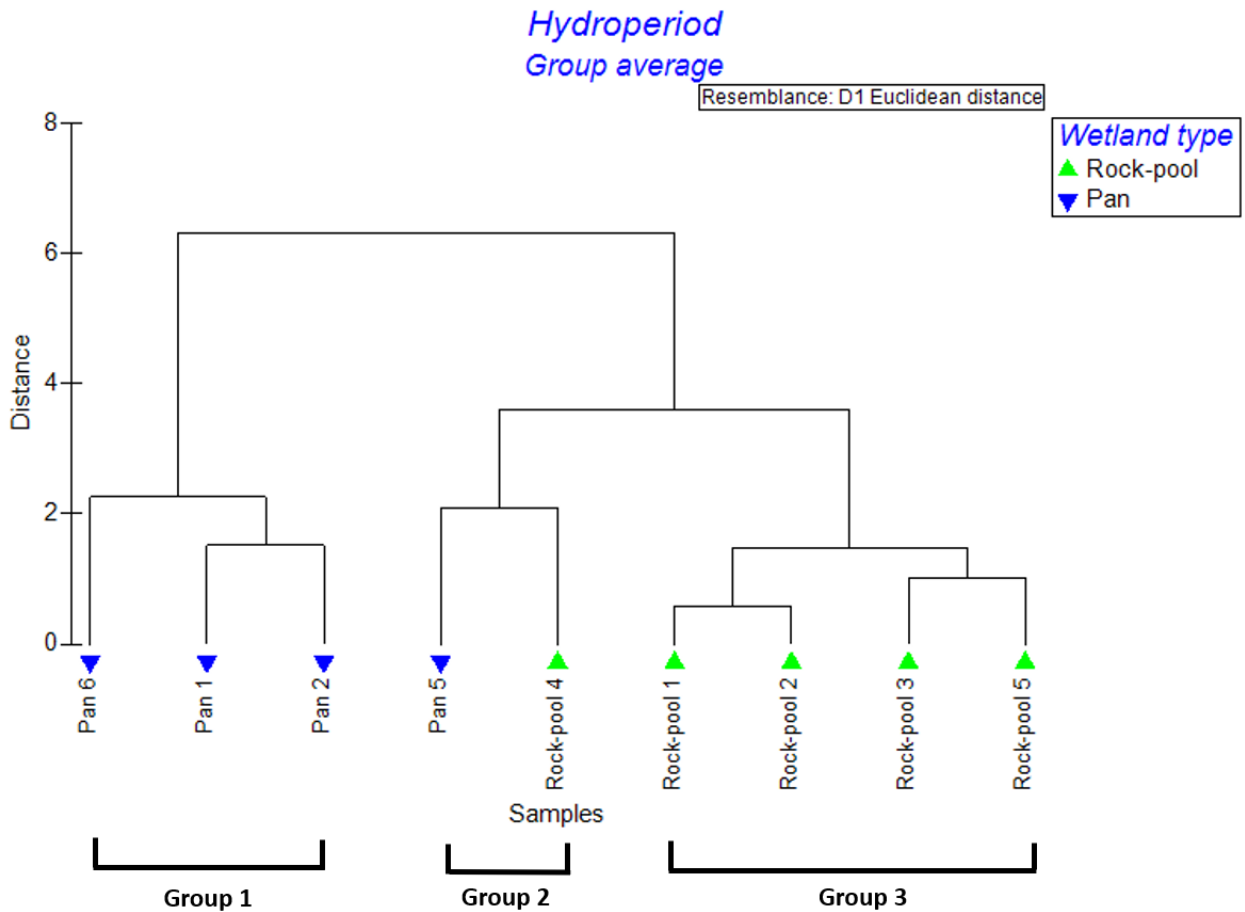


Fig 2. 11: Cluster dendrogram of wetland types based on thermal characteristics (H_{tmax} , H_{tmin} , H_{tmean} , H_{tcv}), highlighting the differences between rock-pools and pans. The Euclidean distance differentiated the ponds according to the temperature variables calculated for the synchronized *hydroperiod* over the period 2-7 November 2019. Groups were identified based on levels of similarity on the Y-axis (Distance).

2.3.2 Wetland physico-chemical characteristics

Pan wetlands had significantly higher electrical conductivity, turbidity and total dissolved solids compared to rock-pools ($p < 0.05$) (Table 2.3). Cluster analysis revealed two groups based on Euclidean distance similarity. Group 1 comprised

exclusively of pans while Group 2 comprised exclusively of rock-pools (Fig. 2.12). Rock-pool ecosystems were highly similar when compared to pans, with the former grouping at a similarity level of ~ 10, while the latter grouped at ~200. Within the more heterogenous pans, Pan 3 resembled Pan 4 while Pan 2 was more similar to Pan 5. Within the relatively homogenous rock-pools, rock-pools 1, 3 and 4 comprised a subgroup, while rockpools 2 and 5 comprised another.

Table 2. 4: Physico-chemical properties of Pans and Rock-pools. The physico-chemical data was obtained in hydroperiods in January and February 2020. t-crit =critical t value. Df = degrees of freedom.

	Pan	Rock-pool	<i>t</i> -crit	<i>df</i>	<i>p</i>
EC (μS/cm)	34.75 ± 25.5	5.4 ± 3.7	3.2254	7.2381	<0.05
Turb (NTU)	334.0 ± 149.07	0.17 ± 0.53	6.3339	7.001	<0.05
TDS (mg/L)	22.125 ± 16.71	3.1 ± 2.5	3.1913	7.254	<0.05

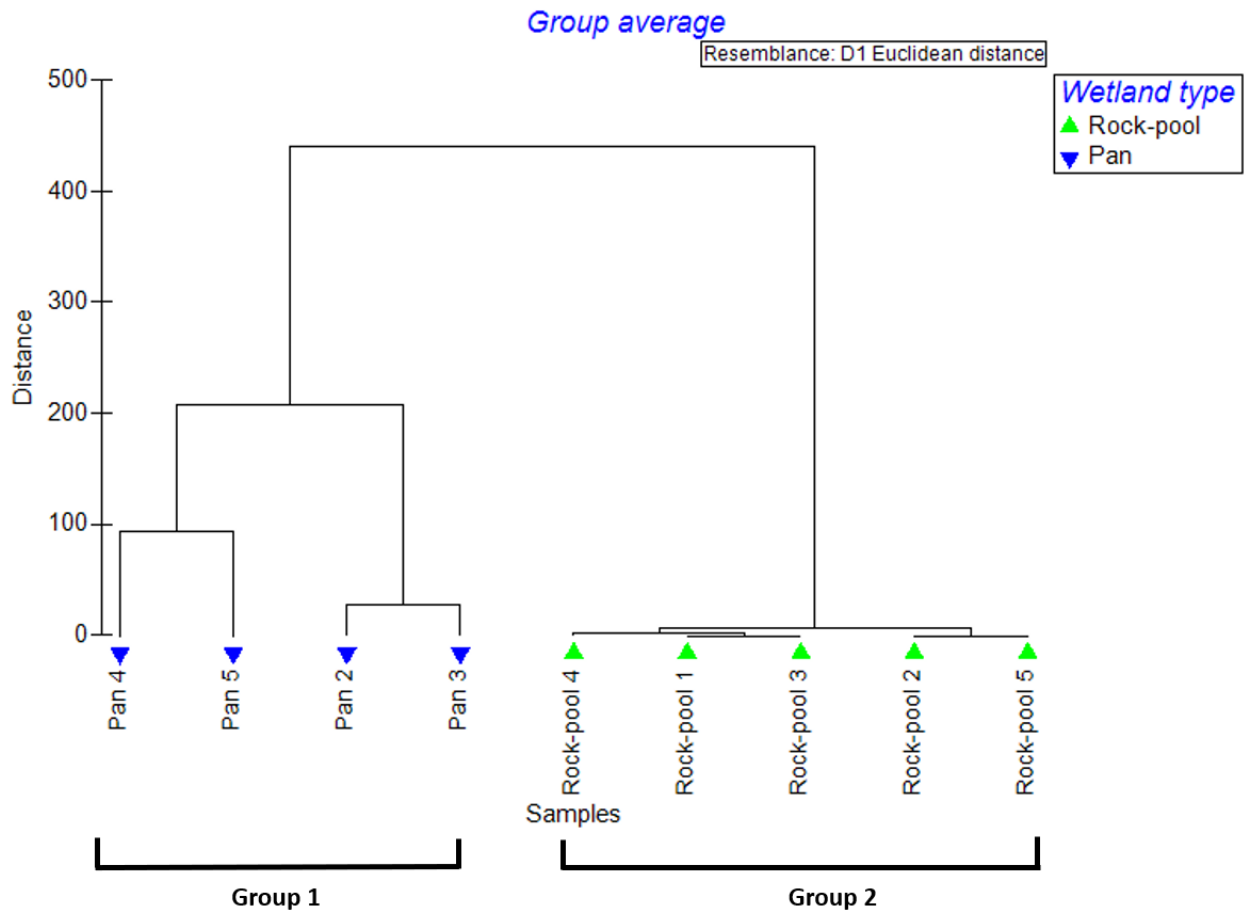


Fig 2. 12: Cluster dendrogram of wetland types based on physico-chemical characteristics (electrical conductivity ($\mu\text{S}/\text{cm}$), total dissolved solids (mg/L) and turbidity (NTU), highlighting the differences between rock-pools and pans. The Euclidean distance differentiated the ponds according to the physico-chemical variables measured from the ponds during a hydroperiod around six days after the wetlands filled. Groups were identified based on levels of similarity on the Y-axis (Distance).

2.4 Discussion

In contrast to the first hypothesis, that the mean daily temperatures would be similar between rock-pools and pan temporary wetland types, rock-pool mean temperatures were significantly higher than pan temperatures. Daily maximum temperatures were, however, higher in rock-pools than in pans, in line with the second hypothesis that

rock-pools would experience higher maximum daily temperatures. In contrast to the third hypothesis, that rock-pools would experience lower minimum daily conditions than pans given their smaller size and lower thermal inertia, rock-pools did not experience significantly lower daily minimum temperatures. But as such rock-pool ecosystems were indeed found to be more variable environments than pans, which was in line with the fourth hypothesis that more variable (higher CV) daily conditions will be observed in rock-pools than in pans. These patterns were evident in both the dry phase and the hydroperiods for the temporary wetlands. Of the two wetland types, rock-pools can, therefore, be considered as more variable environments and likely to experience more environmental stress extremes in the face of climate change.

The thermal dynamics of the temporary wetland types are, in part, aspects of the size of the depressions that collect water on their rocky outcrop landscapes (de la Fuente and Meruane, 2017). The rock-pools were much smaller and shallower than the pans. Given their small size and underlying geology, rock-pools inundate to capacity much faster than pans (Zacharias et al., 2007; Čížková et al., 2013). They also require much less rainfall to inundate to a point where dormant eggs can hatch. So even small rainfall events can result in the presence of a hydroperiod in rock-pools, whereby pans require large rainfall event for hydroperiod development. As such, hydroperiods occur more frequently in rock-pools than in pans. For example, during the period November 2019 and February 2020, Rock-pool 4 experienced 4 discrete hydroperiods (Fig. S3). These hydroperiods lasted between 7 to 14 days on average. During this same season, Pan

6, for example, only had a single hydroperiod, although this hydroperiod lasted over 30 days.

In addition to their size, the drivers of time-scale differences in hydroperiods between rock-pools and pans are also contributed to by their underlying geology (Reddy and DeLaune, 2008; Dodds and Whiles, 2010). The underlying rock precluding water loss through the sediment, since they are impermeable (Brendonck et al., 2010). Therefore, rock-pools only lose water through evaporation and direct water removal by animals and humans. However, the outcrops on which they are situated heat faster during the daily 24hour cycle than do the pans (see Fig 2.5). Rock-pools reached $H_{t_{max}}$ earlier during the day than the pans during the hydroperiods. This reduced time to reach $H_{t_{max}}$, along with the overall higher temperatures in rock-pools, increase evaporation levels in rock-pools, contributing to the reduction in their hydroperiod lengths.

The underlying geology also had implications for the physico-chemistry of the water in the rock-pools (Jocque et al., 2010). The rock-pools had very low electrical conductivity, total dissolved solids and turbidity values compared to pans. Given that the pans are clay-lined and much larger, there is conceivably considerably more allochthonous material in these temporary wetlands during the hydroperiod, than in rock-pools. These various allochthonous components, along with ions from the sediment, will breakdown or dissolve in the pan water, increasing electrical conductivity, total dissolved solids and turbidity in pan temporary wetland types (Reddy

and DeLaune, 2008). Indeed, the water in rock-pools supports this finding, and was noticeably clearer than the pans. Similarly, while primary productivity was not measured in the present study, the rock-pools likely have considerably lower primary productivity levels than the pans, although this has never been assessed to my knowledge. As such, in addition to the thermal dynamics, primary consumers such as the specialist branchiopods likely have limited resources e.g. food in the rock-pool relative to pan ecosystems (Brendonck et al., 2000).

The differences in volumes of water between wetland types may also have contributed to the extents of daily fluctuations during the hydroperiods (de la Fuente and Meruane, 2017). The daily fluctuations in rock-pools were considerably higher than in pans (see Fig. 2.5), and the statistically significant differences in $H_{t_{cv}}$ values between the temporary wetland types during the hydroperiods. The coefficient of variation is a measure of relative variability, and thus increased $H_{t_{cv}}$ could arise as a result of differences between pond types $H_{t_{max}}$, $H_{t_{min}}$ or a combination thereof. These differences in variability during the hydroperiods likely serve as drivers of thermal tolerances and performance for specialist species that are temporary wetland-type specific. However, in this instance, the increased $H_{t_{cv}}$ in rock-pools was driven primarily by elevated $H_{t_{max}}$ values, as $H_{t_{min}}$ values were not significantly different between pond types. This suggests that when contrasting rock-pool and pan specialist fauna with regards to their thermal biology, their Critical thermal maximum limits may differ more than their Critical thermal minimum limits. Ecologically, higher thermal limits to activity

may be more important for survival across the two systems than lower thermal limits to activity.

Differences in thermal dynamics between rock-pools and pans during the hydroperiod can serve as useful indicators of the thermal fitness of temporary wetland specialist crustaceans. However, the life cycles of these specialist organisms require the production of cysts (dormant eggs), which rest in the sediment during the often-prolonged dry phases (Brendonck et al., 1996). The dry phase temperature data outlines some of the conditions that these dormant eggs are exposed to, and similar to during the hydroperiod, significant differences in temperature dynamics were evident during the dry phase between temporary wetland types. Cysts in the rock-pool sediment are potentially exposed to much higher average ($H_{t_{mean}}$) and maximum ($H_{t_{max}}$) temperatures than those in pans. Similarly, the variability in temperature ($H_{t_{cv}}$) conditions between pan types differed significantly. Other differences between wetland types are also important to note, regarding conditions cysts are exposed to. It was observed that the sediment layer in rock-pools is very thin comprising more coarse-grained sediment when compared to that of pans. As such, cysts in the sediment in rock-pools are likely situated closer to the surface and exposed to more light than those in pans. Interacting environmental variables such as temperature and light likely have implications for cyst hatching success.

Within the context of shifting climates, rock-pools and their fauna may be more vulnerable to the increases in temperature projected for the region. The rock-pools are characterised by more extreme average and maximum temperatures, which may be

closer to threshold limits of specialist, and even generalist aquatic fauna. In addition, their small size makes them more vulnerable to increased temperatures hence increased evaporation rates during the hydroperiods (de la Fuente and Meruane, 2017). This also has significant implications for habitat quality through e.g. hydroperiod length reduction. Since a sufficiently long enough inundation period (wet phase) is required for hatching cysts to attain sexual maturity and produce their own cysts (Brendonck, 1996), reductions in hydroperiod may result in localised extinctions of these specialist taxa. However, temperature increases may have physiological implications for branchiopod specialists in both rock-pool and pan environments (see Chapter 3). These implications need to be considered within the context of their unique life cycles. As such, physiological studies focusing on the thermal biology of temporary wetland specialist crustaceans need to consider both the adult aquatic stage and the cyst stage, which persists under non-aquatic conditions.

Chapter 3

Thermal limits and preferences of branchiopods from temporary wetland arid zone systems



Plate 3: An oasis in the desert- searching for large branchiopods in a recently inundated rock-pool (Photo: Murphy Tladi).

Abstract

Large branchiopods are specialist crustaceans adapted for life in temporary, thermally dynamic wetland ecosystems. Certain 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 limits (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 better predictor of thermal preference and critical thermal limits than phylogenetic relatedness. 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, phylogenetic 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 phylogenetic relatedness were, however, 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). Similarly, temperature preferences differed significantly among species, 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* preferred 21-23 °C. Constrained anostracans basal thermal traits reported here suggest that anostracans might be more susceptible to projected climatic warming than spinicaudatans, irrespective of habitat type, albeit these taxa may also compensate through phenotypic plasticity.

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

3.1 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; Thuiller, 2007; Parmesan, 2006; Deutsch et al., 2018). Invertebrate lower and upper thermal limits 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). However, most empirical studies have focused on terrestrial (Addo-Bediako et al., 2000; Deutsch et al., 2018; Dillon et al., 2010; Hoffman et al., 2013;) and marine (Stillman, 2003; Stillman and Somero, 2000; Gunderson et al., 2016) organisms. To our knowledge, very little information is available on the thermal physiology of temporary wetland specialist fauna (see Lagerspetz et al., 2006).

Determining the thermal activity limits of organisms is a fundamental of biological and autecological investigations, providing insight on fitness and habitat suitability (Huey and Stevenson, 1979; Chown and Nicolson, 2004; Stillman, 2003; Stillman and Somero 2000; 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). 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). Basal 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). These have often been investigated using static and more ecologically relevant dynamic protocols (Chown and Nicolson, 2004; Terblanche et al., 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). These measures can also be linked (Huey and Kingsolver, 1993), where temperatures at which populations can perform at optimum are positively related 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). 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, however, 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 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., 2020a). These environments both 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 (see e.g. Leroi et al., 1994; 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 branchiopods. Climate models project that if mitigation measures would fail, Southern Africa is expected to be drier in future (Engelbrecht et al., 2015). 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 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 branchiopods would have higher thermal activity limits and wider temperature preference than pan specialist branchiopods, as smaller water bodies (rock-pools) both heat and cool more variably and rapidly than larger water bodies (pans). Indeed, the climate variability hypothesis predicts similar relationships, whereby a positive relationship exists between thermal tolerance traits 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 genetic relatedness was a better predictor of thermal fitness. The information from this study will help bridge the knowledge gap on large branchiopods thermal biology. 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.

3.2 Materials and methods

3.2.1 Species selection and collection

Preliminary surveillance showed anostracan *Branchiopodopsis tridens* and spinicaudatan *Leptestheria brevirostris* were found in rock-pools. In turn, the anostracan *Streptocephalus cafer* and spinicaudatan *Gondwanalimnadia* sp. were consistently found in pans between Palapye and Sherwood. Samples for this component of the thesis were collected during the austral summer rainy season in 2019 and 2020. From rock-pools and pans (Fig. 3.1), adult branchiopods were collected by gently pulling sweep nets through the water. A square sweep net (20cm × 10 cm; 500µm mesh) was used to sample rock-pools, while a round sweep net (diameter: 30 cm; 1mm mesh) was used to sample the wetlands. Captured animals were gently transferred from the sweep nets into 5L containers, filled with source water, following protocols by Martin et al. (2016). *Streptocephalus cafer* were collected from a single pan (22°52'16.0"S, 27°47'42.7"E) and a presently unidentified *Gondwanalimnadia* sp. (Tladi et al., 2020a) were collected from two pans in close proximity to one another as individual numbers were relatively low in both ponds (22°52'16.0"S, 27°47'42.7"E; 22°49'45.7"S, 27°37'17.9"E). *Branchiopodopsis tridens*

were collected from one rock-pool site (22°35'46.1"S, 27°07'16.5"E), while *L. brevirostris* were collected from another rock-pool site (22°35'55.6"S, 27°07'51.6"E) (Fig. 3.1). In the laboratory, samples were maintained in shallow containers (100 cm × 70 cm) using water (strained through a 20 µm filter) collected from their respective source wetlands, in climate chambers (HPP 260, Memmert GmbH + Co.KG, Germany) set at 25 °C (± 1 °C) under a 12:12 light:dark photcycle. These were kept at low but optimal densities 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.

3.2.2 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) (as in Machekano et al., 2020) (Fig. 3.2). 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. 3.2). 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). The samples were then examined every 0.5 minutes for cessation of motion and righteous response to mild mechanical stimulus.

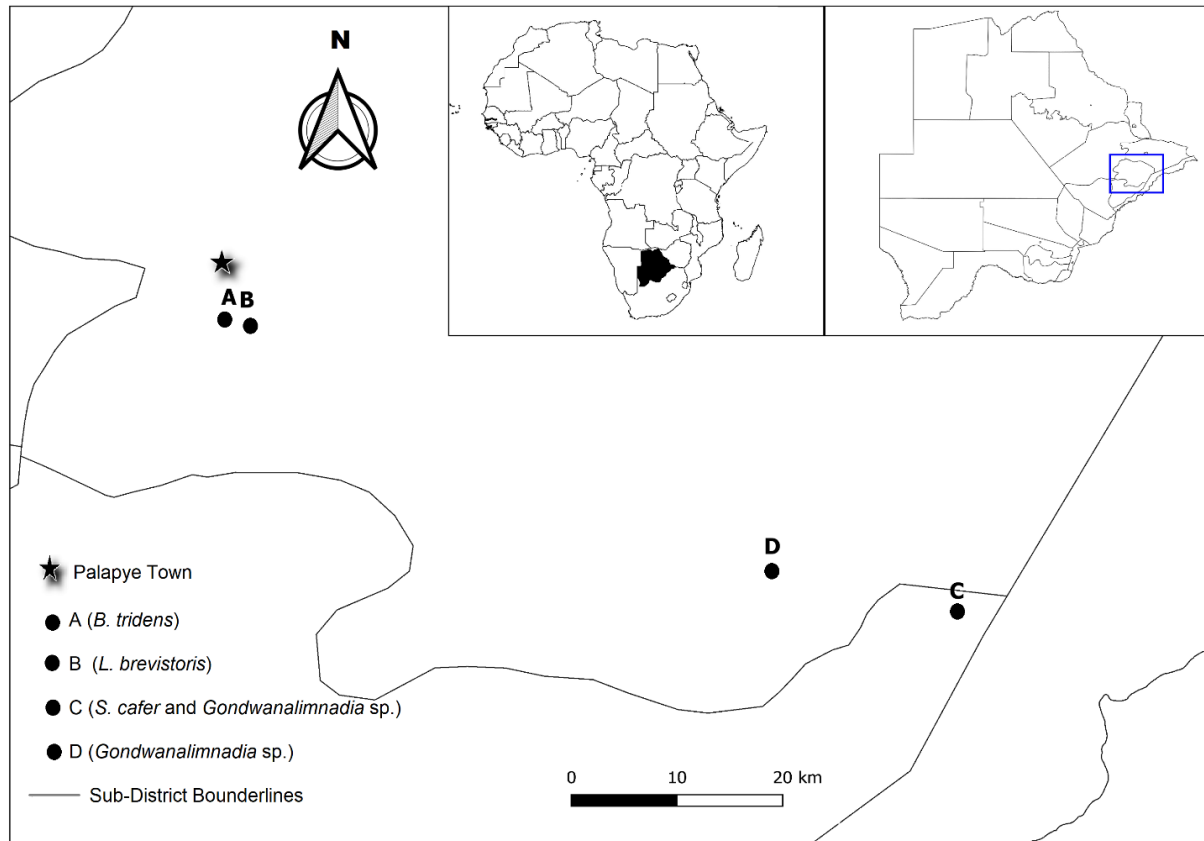


Fig 3. 1: Locations of the rock-pools for the collection of *Branchiopodopsis tridens* (A) and *Leptestheria brevirostris* (B), and pans for the collection of *Streptocephalus cafer* (C) and *Gondwanalimnadia* sp. (C and D) in central district, Botswana.

Critical thermal limits were thus 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* ($CT_{\min} n = 46$, $CT_{\max} n = 54$).

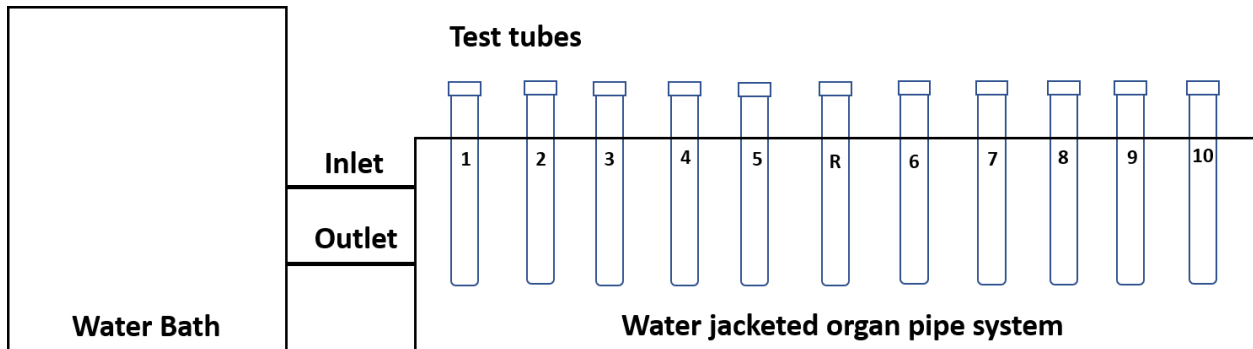


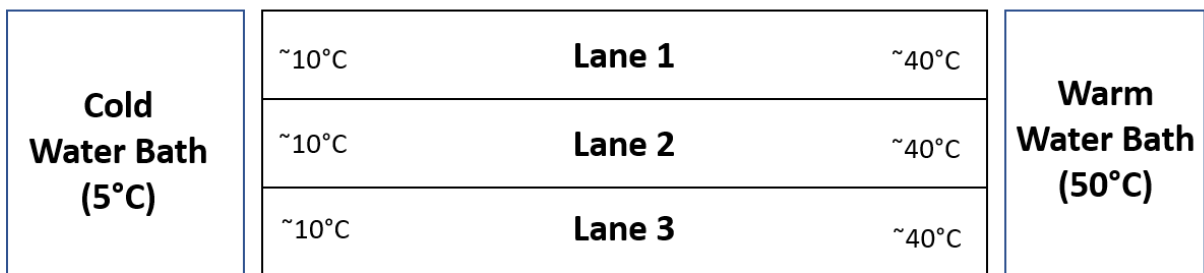
Fig 3. 2: Organ pipe design for the critical thermal limit experiment. The programmed water bath regulates the heat in the test tubes through circulation of temperature controlled 1:1 water:propylene glycol through the jacket system. Ten replicate test tubes (1-10) house the test animals, while the reference test tube (R) is used to monitor temperature within the test tubes.

3.2.3 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. 3.3). 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 (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 replicates 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.

a)



b)

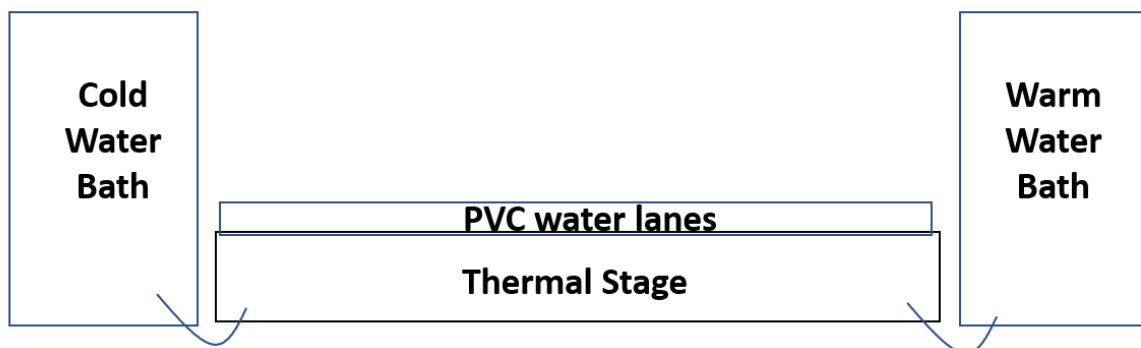


Fig 3. 3: Schematic representation of the thermal stage set-up showing a) aerial view of the 3-laned PVC pipes, each holding water, and b) lateral view of the PVC lanes on top of the metal thermal stage, fed by cold water on the left and warm water on the right from respective water baths.

3.2.4 Data analysis

Critical thermal limits

Differences in C_{tmax} and C_{tmin} 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.

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. Tukey tests were used post-hoc for pairwise comparisons of significant effects. All statistical analyses were performed in R v.4.0.2 (R Core Team, 2020).

3.3 Results

3.3.1 Critical Thermal Limits

High temperature to activity (CT_{max}) differed significantly across species ($\chi^2 = 144.16$, $df = 3$, $p < 0.001$) (Fig. 3.4a). Generally, this heat tolerance CT_{max} was highest in *L. brevirostris* > *Gondwanalimnadia* sp. > *S. cafer* > *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* ($p < 0.001$). Generally, cold tolerance (CT_{min}) was highest in *B. tridens* > *Gondwanalimnadia* sp. > *L. brevirostris* > *S. cafer*.

Low temperature to activity (CT_{min}) also differed significantly among species ($\chi^2 = 64.31$, $df = 3$, $p < 0.001$) (Fig. 3.4b). *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 *Gondwanalimnadia* sp. (median = 2.1 °C) ($p > 0.05$). In turn, *Gondwanalimnadia* sp. was significantly more cold tolerant than *L. brevirostris* and *S. cafer* (both $p < 0.001$), with those two species more similar statistically ($p > 0.05$).

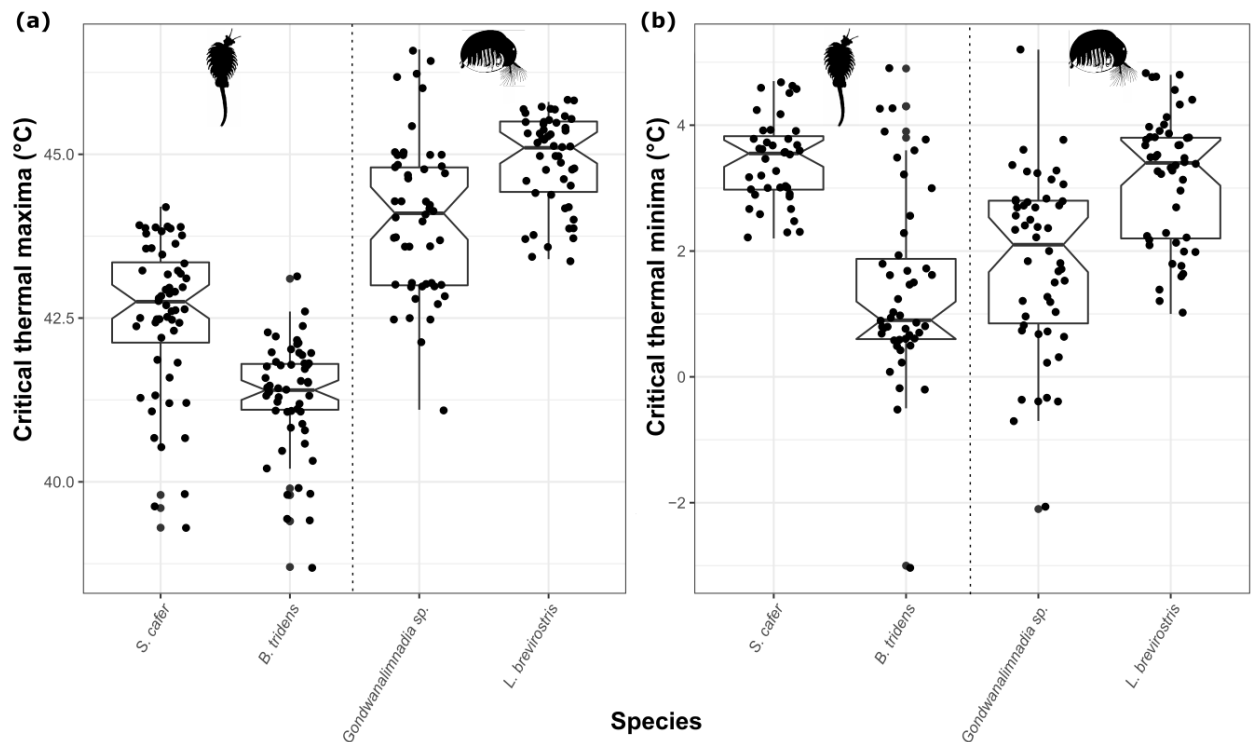


Fig 3. 4: Box plots showing (a) critical thermal maxima (°C) and (b) critical thermal minima (°C) of *Streptocephalus cafer*, *Branchiopodopsis tridens*, *Gondwanalimnadia* sp. and *Leptestheria brevirostris*. Jittered points are raw data. In the boxplots, the horizontal bar displays the median, the box gives the interquartile ranges and the whiskers show the largest and smallest values up to $1.5 \times$ interquartile range.

3.3.2 Thermal preference

Thermal occurrences among species differed significantly owing to a significant two-way 'temperature $\times$ 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. 3.5). *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. 3.5).

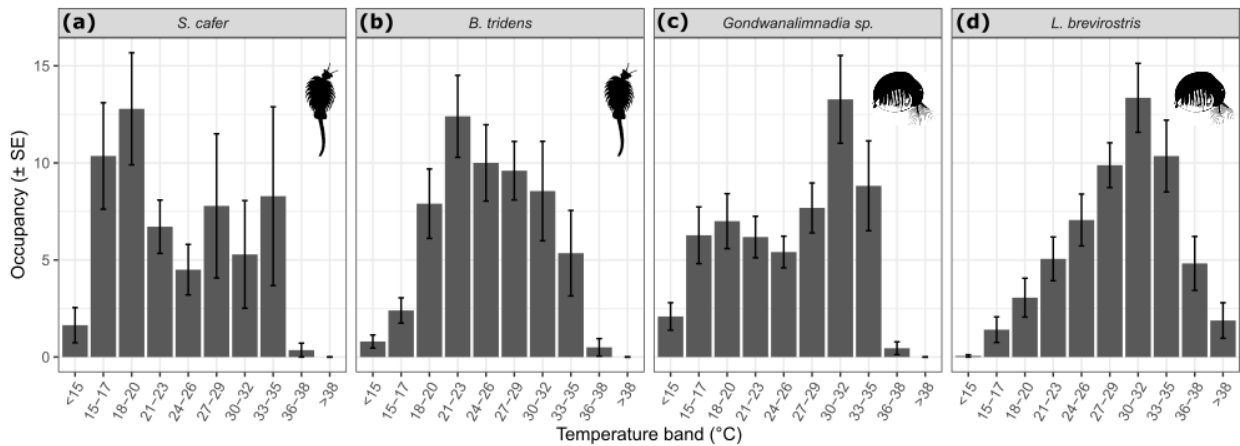


Fig 3. 5: Thermal preference occurrences of *Streptocephalus cafer*, *Branchiopodopsis tridens*, *Gondwanalimnadia* sp. and *Leptestheria brevirostris* across temperature bands. Medians are shown alongside standard errors (SE).

3.4 Discussion

The present study showed that, in contrast to our hypothesis, environment of origin was not a consistent determinant of thermal fitness. 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. 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 conserved 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 emergent. The rock-pool anostracan *B. tridens* exhibited significantly lower CT_{max} compared to the pan anostracan *S. cafer*. The spinicaudatans similarly showed significant differences between their respective heat tolerances, with the CT_{max} for the rock-pool species *L. brevirostris* higher than that of the pan taxon *Gondwanalimadia* sp. Whilst peak occurrences were similar between spinicaudatans, *L. brevirostris* exhibited the significantly greatest occurrences at highest temperatures, and was the only taxon to occur above 38 °C, and which seldom occurred below 15 °C. The findings of the present study contribute novel insights into thermal tolerances and preferences of understudied temporary wetland groups, and thus identifies species which may be most vulnerable to localised pressures as temperatures shift. Overall, all species here have a working thermal range of approximately ~15–40 °C. However, surface temperatures are expected to increase beyond 40 °C in future, especially when mitigation measures against climate change fail (Engelbrecht et al., 2015). This thus represent a significant threat to the fitness of these aquatic organisms in the face of climate change. However, organisms often cope to stressful temperatures through shifting their phenotypes (Sgrò et al., 2016) or through behavioural microhabitat selection (Pincebourde and Woods, 2020). The role of these mechanisms in

compensating for branchiopods' fitness under stressful environments is unknown and warrant future investigation.

Several factors might have an influence in the observed thermal fitness traits among taxa. Critical thermal limits in aquatic species are known to be limited by several factors including phenotypic plasticity, which is constrained by the genome (Chown and Nicolson, 2004; Sgrò et al., 2016). This thus limits capacities for intraspecific tolerance differences among populations of the same species. Furthermore, insect thermal fitness is highly subtle and constrained by 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 (Terblanche et al., 2007). Terrestrial organisms are thought to have higher critical thermal limits to activity than aquatic organisms perhaps owing to the constrained oxygen in water environments and the role of oxygen in thermal tolerance (Forster et al., 2012; Verberk et al., 2016). Overall, these thermal tolerance results suggest that anostracans are likely to be more markedly affected by predicted temperature increases and variability associated with climate change than spinicaudatans across both rock-pool and pan habitats. 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 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 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 branchiopods among discrete wetland habitats, as well as impacts of thermal regime on resting egg hatchability. 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, our results demonstrate that, even where taxa have adapted to different wetland types, their thermal fitness appears to be relatively similar within their taxonomic grouping.

Our results show that for temporary aquatic habitats, resilience to higher temperatures appears to be grounded extensively in taxonomic grouping, with spinicaudatans having significantly higher heat tolerance and preference, than anostracans. However, low temperature tolerance was not readily distinguishable between taxonomic groups, given species-specificity in responses. Overall, these results suggest that both pans and rock-pools are threatened by climate change with respect to their branchiopod assemblages. Further work should 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 branchiopod thermal fitness and ecology.

Chapter 4

Emergent effects of light and temperature on hatching success of *Streptocephalus cafer* (Branchiopoda: Anostraca) resting eggs



Plate 4: Life in the seemingly barren- sediment from a dry rock-pool, containing cysts of the crustacean *Branchiopodopsis tridens*. (Photo: Murphy Tladi).

Abstract

Resting egg production is considered the most common form of dormancy in aquatic invertebrates. Given that many taxa at least partially terminate resting egg state using environmental cues, knowledge on environmental drivers of hatching success is important, particularly within the context of climate change and environmental degradation. Fairy shrimp (anostracans) are temporary wetland specialists that are reliant on resting egg production for population persistence. Temporary wetlands are common in many arid regions projected to experience increases in temperature, and in areas often compromised by human-mediated activities. In this study, we assessed the combined effects of light and temperature on the hatching success of *Streptocephalus cafer* (Anostraca) dormant eggs from temporary wetlands in an arid environment. Both temperature and light altered hatching success, with emergent effects evident. Light caused a significant three-fold increase in hatching success overall, while temperature effects were non-linear, with hatching optimised at 27 °C, and especially under light conditions. These results are discussed within the context of shifting climates and disturbances to temporary wetland ecosystems.

Keywords: Botswana; Climate change; Environmental drivers; Fairy shrimp; Temporary wetlands.

4.1 Introduction

The most common form of dormancy in aquatic invertebrates is the production of resting eggs (Hansen, 2019). While widespread in the invertebrates, this is particularly

relevant for obligate temporary wetlands inhabitant crustaceans, whose population persistence is wholly dependent on resting egg (cyst) production (Hairston and Cáceres, 1996). These crustaceans produce cysts that can survive both wet and dry phases in temporary wetland habitats (Hairston and Cáceres, 1996; Jocque et al., 2010). Fairy shrimps are branchiopod crustaceans in the order Anostraca that live exclusively in ephemeral or temporary wetlands (Mioduchowska et al., 2018). During periods when these habitats are inundated with water (hydroperiod), nauplii rapidly hatch from the cysts, attain sexual maturity and, in turn, produce dormant resting eggs (Pinceel et al., 2013). These cysts usually remain in the diapause stage, also called the resting stage, until they go through the dry phase when the pond completely dries up (Brendonck, 1996). This dry phase is thus thought to be necessary to terminate dormancy of cysts of many fairy shrimp (Rogers, 2015). Generally, the first few days of inundation are characterised by the highest peak for hatching of cysts (Brendonck, 1996). However, not all cysts hatch in the first inundation after the dry phase, even if all hatching conditions are optimal (Pinceel et al., 2013). Such delayed hatching is useful in temporary wetlands in order to prevent complete population collapse, in events where hydroperiods are too short for hatched eggs to attain sexual maturity (Brendonck, 1996). These bet-hedging strategies have also been postulated to allow progenies from different timelines to hatch in the same hydroperiod, thus stabilising mutations that may have led to changes in the population (Pinceel et al., 2013). It has also been suggested that such bet-hedging evolutionary strategies facilitate fitness e.g. through hatching of eggs produced at a particular time, over multiple hydroperiods,

thereby guarding against stochastic threats that may be present during any particular hydroperiod (Pinceel et al., 2013).

While there is still paucity about drivers of crustacean cyst hatching success, mechanisms for cyst hatching have been explored. Light and temperature are considered among the primary drivers of cysts activation for many zooplankton species (Gyllstrom and Hansson, 2004; Hansen, 2019). Light exposure has been identified as an important cue for all major branchiopod crustacean groups, including fairy shrimps (Pinceel et al., 2013). Murugan and Dumont (1995), Mitchell (1990) and Pinceel et al. (2013) all identified light as an important cue in initiating hatching in different fairy shrimp species. Photoreceptors in the shell have been thought to be responsible for this phenomenon (Pinceel et al., 2013). Furthermore, different species have different pigmentations in the cysts which suggests that species of fairy shrimp hatch differently depending on light intensity and quality (Timms and Lindsay, 2011). Pinceel et al. (2013) also found that the difference in pigmentation occurs within populations and between individual cysts of the same batch. This may also be an adaptative strategy that helps control the bet-hedging and spacing of larval stages to minimise intraspecific competition. Temperature has also been identified as an important cue for fairy shrimps and other branchiopods, with optimal temperatures varying depending on the species (Brendonck et al., 1996; Atashbar et al., 2014; Chaves and Couto, 2014; Rogers, 2015).

In arid regions of Southern Africa, temporary wetland ecosystems are often degraded due to agricultural and other human-mediated activities, with implications for their fauna (Dalu et al., 2017a; Buxton et al., 2020a). In addition, many tropical regions of Southern Africa are projected to experience temperature increases and shifts in rainfall and soil-moisture availability (Engelbrecht et al., 2015). For temporary wetlands, this will likely mean fewer inundation events, shorter hydroperiods, warmer averages and more variable water temperatures. Given the reliance of many specialist temporary wetland fauna on successful egg hatching for population persistence, understanding egg-hatching dynamics within the context of shifting environments is key. Shifts in water quality, clarity and temperature will likely all have implications for dormant egg hatching success, and these processes could interact non-additively, with ramifications for populations and communities.

In this study, we determined the combined effect of light and temperature on the hatching success of *Streptocephalus cafer* (Lovén, 1847) (Branchiopoda: Anostraca) cysts. *Streptocephalus cafer* is a widespread species in Southern Africa, distributed across a range of climatic regions (Tladi et al., 2020b). We hypothesised that temperature would have a synergist effect on the hatching of cysts under light regimes.

4.2 Materials and methods

4.2.1 Sample collection

Eggs were collected from a dry pond 12 km North-West of Sherwood village, Central District, Botswana (27°47'43.07"E, 22°52'17.01"S). When completely inundated, the

pond is around 80 m long, 60 m wide and 1.5 m deep. Forming part of a small cluster of semi-connected ponds, the seasonal rainfed wetland has been observed holding water for up to six months of the year. Land-use in the area is characterized by free-ranging cattle and small-scale crop agriculture. During previous surveys of the pond, *S. cafer* had been sampled and identified (Tladi et al., 2020b), along with a *Gondwanalimnadia* species, *Triops granarias* and *Lovenula falcifera*. Numerous 5 cm deep dry sediment samples were collected from the centre to the northern edge of the pond, each placed in ziplock bags and transferred to the laboratory where samples were stored at ~21° C under dry conditions for seven months.

4.2.2 Laboratory procedure

Upon commencement of the experiment, sediment from ziplock bags were transferred into a twenty-liter plastic bucket and homogenized by shaking. The mixed sediment was then transferred into a five- liter beaker and filled with matured tap water. The mix was gently stirred with a plastic spatula, softening the sediment and allowing eggs to float. Floating cysts were then collected with a pasture pipette and transferred to a petri dish, filled with matured tap water. The petri dish with cysts was then placed under a dissecting microscope to distinguish *S. cafer* resting eggs from other crustacean eggs. Three Nunc 96 well-plates were used for the experiment. Four replicate rows (A, B, C and D) of eight wells each were filled with distilled water per well-plate (Fig. 4.1). Five *S. cafer* cysts were collected using sharp pointed soft forceps and transferred into each well. The well-plates were then placed into their respective treatment conditions within an incubator as shown in Table 4.1. This was repeated three times using a fresh batch

of sediment on each occasion, ensuring that eggs for each treatment were exposed to the water for the same duration before incubation. Temperature treatments spanned observed *in situ* early hydroperiod temperatures (18°C - 30°C) and higher temperature (32°C and 37°C) as the region is projected to warm in future (Engelbrecht et al., 2015). A fluorescent white 22 W LED aquarium lamp (2 x 11, 1W LED system) was used for lighting in the incubators with measured visible light produced ranging 700 to 1000 lux (Environmental Quality Logger Model 850071, Sper Scientific Ltd) depending on the location of the well plate under the light lamp. The starting time for each 96 well plate incubation was recorded immediately following placement in the incubator.

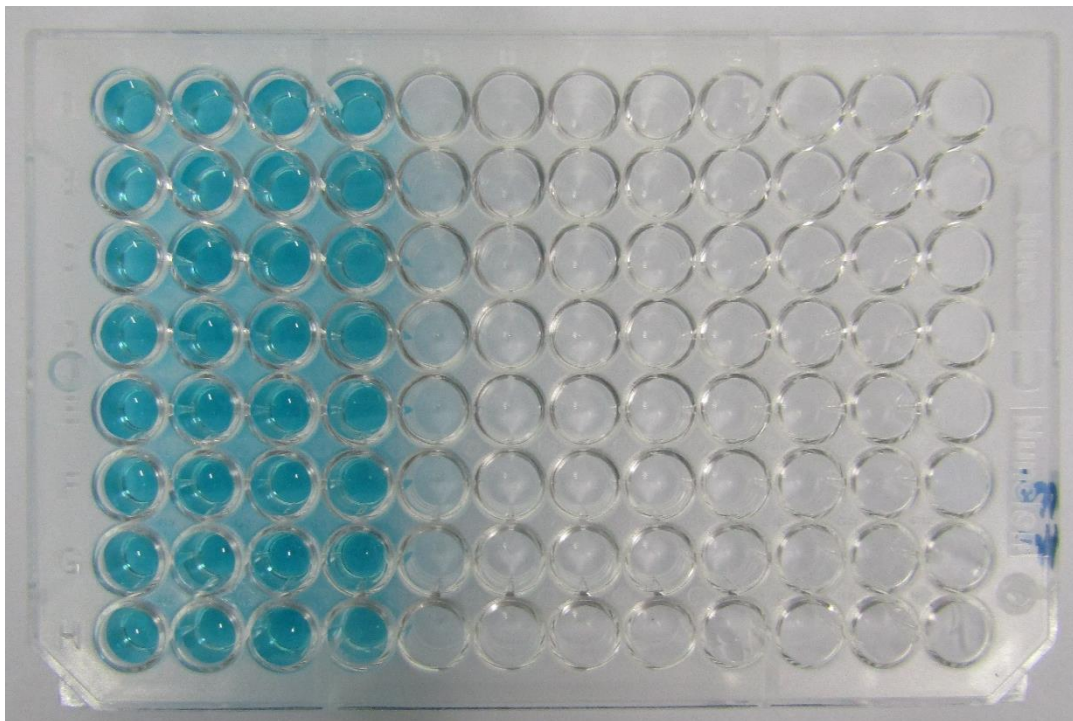


Fig 4. 1: Nunc 96 well-plates were used for the experiment, whereby four replicate rows (A, B, C and D) of eight wells each were filled with distilled water per well-plate, holding five *Streptocephalus cafer* cysts each. The blue dye water was used for illustration purposes for this image.

Table 4. 1: Temperature and light treatments used for testing the hatching success of *S. cafer* resting eggs in 96 well plates, placed within an incubator. H = hours. Lighting in the incubators during the 12 H light phase produced a measured visible light ranging 700 to 1000 lux.

Temperature (°C)	Light regime	
18 ($\pm$ 0.5)	12 H Dark: 12 H Light	24 H Dark
22 ($\pm$ 0.5)	12 H Dark: 12 H Light	24 H Dark
27 ($\pm$ 0.5)	12 H Dark: 12 H Light	24 H Dark
32 ($\pm$ 0.5)	12 H Dark: 12 H Light	24 H Dark
37 ($\pm$ 0.5)	12 H Dark: 12 H Light	24 H Dark

Data collection and analysis

The numbers of hatched cysts were counted by observing each well using a dissecting microscope (Stemi DV4, Carl Zeiss). The numbers of nauplii observed for each respective well were recorded individually. These numbers were then converted to percentage hatches (0, 20, 40, 60, 80 or 100%) and transferred to a grow-out chamber for species verification. To cater for light effects, data from the three trials were pooled, with each of the four rows (A, B, C, and D as above) from the well plate used as a replicate. The mean percentage hatching data across the 24 wells was therefore used as each replicate datum point ($n=4$) per treatment. The relationships between fairy shrimp hatchability and experimental treatments were tested using linear models, where light regime (fixed factor with two levels) and temperature (fixed factor with five levels), and their two-way interaction, were included as predictors of hatch rates.

Further, linear models including temperature regime continuously as linear and quadratic terms were compared using second-order derivations of Akaike's information criterion (lower values indicate a better fit). Only significant terms were included to obtain the most parsimonious model. Diagnostic plots were used to confirm normal distribution and residual homogeneity. Statistical analyses were performed in R v3.5.1 using the base *stats* package (R Development Core Team, 2018).

4.3 Results

The final model included both light regime and temperature as significant predictors of hatchability, as well as their interaction (Table 4.2). Overall, significantly greater (~3 times) hatchability was exhibited under the Dark:Light as compared to the Dark regime, irrespective of temperature. When separately comparing linear and polynomial (first- and second-order) temperature terms, inclusion of a quadratic term minimised information loss ($\Delta AICc = 45.91$; first-order $t = 8.14$, $p < 0.001$; second-order $t = -8.20$, $p < 0.001$), indicting non-linear temperature effects which, in turn, interacted with light regime (Fig. 4.2). In particular, hatchability differences between light regimes were significantly most pronounced at intermediate temperatures, with Dark group mean hatching rates ranging from 1 - 12 %, and Dark:Light groups ranging from 9 - 32 % across temperatures (Fig. 4.2).

Table 4. 2: Linear model coefficients considering fairy shrimp hatchability as a function of light regime (2 levels), temperature (5 levels), and their interaction. Significant effects are emboldened.

Term	df	F-value	p-value
Light	1	213.29	< 0.001
Temperature	4	49.20	< 0.001
Light:Temperature	4	5.75	< 0.01

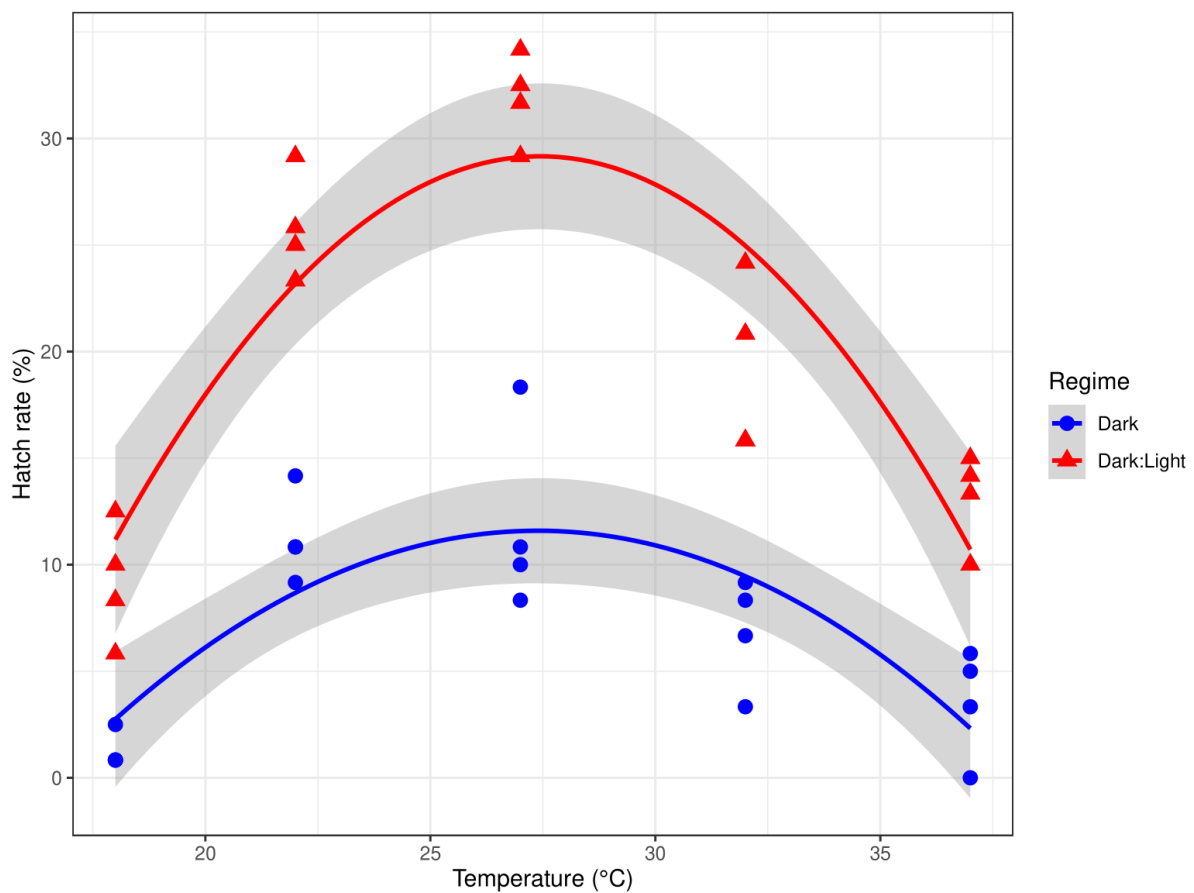


Fig 4. 2: Effects of temperature on fairy shrimp hatchability between two light regimes. A quadratic temperature term was included owing to non-linearities of the temperature effect. Point are raw data and shading represents 95 % confidence intervals.

4.4 Discussion

Both light and temperature independently influenced hatching of *S. cafer* cysts. The optimum temperature treatment was 27 °C while the most sub-optimal were 18 °C and 37 °C, irrespective of light conditions. Similarly, the presence of light (12h:12h, Dark:Light) had implications for hatching success irrespective of temperature, with light presence always promoting hatching. Nevertheless, significantly positive emergent effects of light were evident at intermediate temperatures under Dark:Light conditions. Although the percentage hatching success for the treatments varied, there was no 100% hatchability for any treatment, consistent with results in related studies (Saengphan et al., 2005). Since we did not account for non-viable or diapause eggs, (Brendonck, 1996), the potential presence of such eggs in the experiment were considered random effects equally distributed across the balanced treatment design.

These results suggest that in addition to potential bet-hedging strategies associated with different types of dormant eggs, microhabitat conditions within and across temporary wetlands likely have implications for *S. cafer* hatching success. Specifically, the effects of light on percentage hatching success of cysts has implications for resting eggs buried in the sediments of ponds. This suggests that the deeper the cysts are buried the less likely they will hatch. In arid Botswana, these environments are often heavily utilised by livestock for drinking, facilitating eutrophication (Buxton et al., 2020a). Movement of large numbers of livestock around the edges and through temporary wetlands may force cysts deeper into the sediment with implications for light exposure and ultimately hatchability. In addition, livestock activity and livestock-induced eutrophication likely elevate turbidity levels, with implications for light levels

reaching the cysts. Similarly, the region is projected to experience increases in temperature (Engelbrecht et al., 2015), with temperature effects shown in our study to be mediated by light regime. Considered in addition to increased temperature effects on hydroperiod lengths and pressures on these systems as water sources for livestock, such effects are likely to reduce resting eggs hatching success under projected climate change.

Small temporary wetlands are particularly vulnerable to disturbance given their small size and variable hydroperiods, and are often overlooked in conservation for the same reasons (Dalu et al., 2017a). Anostracans and other resting-egg producing branchiopods are characteristic fauna for temporary wetlands (Brendonck, 1996), and reduced hatching success of these are likely to affect wider ecosystem processes, undermining ecosystem functioning (Mabidi et al., 2018) and services offered by these environments. For example, Buxton et al. (2020b,c) identified temporary wetlands as potential mosquito breeding sites, suggesting that loss of temporary wetland specialists may indirectly facilitate larval mosquito numbers through loss of predation and competition pressure. A better understanding of threats compromising hatching success of temporary wetland specialist fauna is required for the prediction of future environmental scenario outcomes in these ecosystems (Mabidi et al., 2018).

Chapter 5

General discussion



Plate 5: Digging for a different type of Botswana diamond- searching to no avail in a drying pan, for a lost temperature data-logger holding valuable data (Photo: Ryan Wasserman).

5.1 Synthesis

This study was the first of its kind in the region, and one of the few globally that has investigated aspects of the thermal biology of large branchiopods within the context of specialist wetland types. In addition, the study added insight into the role of environmental cues in facilitating large branchiopod cyst hatching success. The Central District in Botswana offered an ideal opportunity to conduct this work, given the prevalence of rock-pool and pan ecosystems across the arid landscapes that characterise the region. The presence of a restricted, and largely predictable rainy season also facilitated the possibility of comparing wetland environments and their specialist fauna at the same time.

The present study highlighted the differences in thermal dynamics between rock-pools and pans. As expected, the shallow and small rock-pools were generally warmer than the pan wetlands (Chapter 2). To our knowledge this is the first time anyone has thoroughly contrasted the thermal dynamics between these two wetland types. These findings highlight the potential role of environment of origin as an important determinant of thermal physiology for temporary wetland specialists. But since more than one large branchiopod taxa have representatives that are specialist in either rock-pool or pan temporary wetland ecosystems, a contrast between the importance of environment of origin and phylogenetic relatedness as determinants of thermal performance of large branchiopods of the region could be assessed. As such, the thermal physiology of specialist anostracans and spinicaudatans from rock-pools and pans were contrasted (Chapter 3). Overall, pond type specialists exhibited differences in physiological

thermal fitness, measured as critical thermal limits and thermal preference. Spinicaudatans had a higher thermal tolerance than anostracans, suggesting that phylogenetic relatedness was a more important determinant of thermal performance than environment of origin. In the spinicaudatans, *L. brevirostris* (rock-pool specialist) had a higher thermal tolerance than *Gondwanalimnadia* sp. (pan specialist). A different pattern was observed in anostracans where a pan specialist (*S. cafer*) was tolerant to higher temperatures than a rock-pool specialist (*B. tridens*). Thermal preference results also grouped the species according to taxa with anostracans preferring lower temperatures than spinicaudatans. However, thermal preference results suggest that *S. cafer* prefers lower temperatures than *B. tridens*. In spinicaudatans, a similar trend as in critical thermal limits was observed with *L. brevirostris* preferring higher temperatures than *Gondwanalimnadia* sp.

Phylogenetic relatedness was crudely inferred by the known taxonomy of the studied species, rather than a formal phylogenetic study. However, no in-depth molecular work has been done on large branchiopods in Southern Africa which hinders that type of study, if it was ever repeated at a finer resolution using closely related species. To contribute towards the addressing of this knowledge gap, the complete mitochondrial DNA of the four studied species were determined. For two of these species (*S. cafer* and *Gondwanalimnadia* sp.), the descriptions have been published (files S1 and 2), with the third (*L. brevirostris*) under review (file S3) and the fourth in preparation. The hope is that moving forward, researchers studying large branchiopods in the region will follow suite and consider describing the complete mitochondrial DNA of their study

species. This type of description is quite simple and cost-effective and can be readily achieved if ecologists work with a taxonomist and a molecular specialist. A considerable contribution could be made within the pool of researchers working on large branchiopods in Southern Africa, if they decided to adopt this component to their research.

While Chapter 3 dealt with the physiology of adult branchiopods, Chapter 4 dealt with the role of temperature in determining cyst hatching success. While only one species was assessed, I strongly feel this is representative of what may be largely happening with the other species and forms a significant baseline for studies on other species.. Chapter 4 outlined that light interacts with temperature with emergent effects on cyst hatching success. These results confirm findings on other crustacean taxa highlighting the role of both light on hatching of dormant cysts (Van der Linden et al., 1986; Pancella and Stross, 1963; Kashiyaama et al., 2010).

5.2 Conclusions

Temporary wetlands are distinct with environmental conditions varying according to pond types. The differences in the characteristics of these environments are important for the biota that live in these environments, particularly for the specialist taxa. Irrespective of environment of origin, this study highlights that from a thermal limits' perspective, anostracans are likely to be more vulnerable to warming temperatures than spinicauatans, albeit organisms can shift their thermal phenotypes. Anostracans

had lower CT_{max} values than spinicaudatans. However, when considering the temperature dynamics in the environments, rock-pools are likely to be more vulnerable than pans with implications for their specialist taxa. The rock-pools were all much smaller and shallow, and had shorter hydroperiods than pans. It is likely that increased temperatures will reduce hydroperiod inundation times, and this could preclude the attainment of sexual maturity for rock-pool specialist branchipods, ultimately leading to localised extinctions. Furthermore, anostracans having lower CT_{max} values also mean they are more vulnerable than spinicaudatans. In anostracans, the threat is greater for *B. tridens* since they occur in rock-pools which experience higher temperatures than pans. The risk, therefore, is less for *S. cafer* as pans experience virtually lower temperatures and less fluctuations. In spinicaudatans, the threat of increasing temperature also appears to be greater for *L. brevirostris* than *Gondwanalimnadia* sp just because *L. brevirostris* is found in rock-pools. This species, however, requires to be protected in Botswana since this study is the first record of its siting in the country and its distribution is still not yet fully understood. Furthermore, the environmental characteristics seem to have implications on the hatching success of the large branchiopods as shown by the representative *Streptocephalus cafer*. Which implies that the hatching success of these cysts depend on factors that could result in the cysts being buried deep in the sediment such as livestock walking on sediment and pushing cysts deeper underground.

5.3 Future Research

More work is required on temporary wetland fauna in Botswana, incorporating other areas not covered by the current work. In addition, work should also extend to the region, including e.g. very basic distribution records, which to date are not updated. For example, *L. brevirostris* in this study represents the first record of this species outside Namibia. The only other record of this species is its type locality on the Waterberg Plateau, east of Otjiwarongo in north-western Namibia, in 1920 (Barnard, 1924). The type locality is approximately 1,300 km to the west of our new record. The original 1920 collection included two specimens; a single ovigerous female and a juvenile (Barnard 1924), and there are no data regarding the form or ecology of the type locality. However, an examination of the regions east of Otjiwarongo using Google Earth Pro reveal a landscape rich in rock outcroppings that is similar to our study area, with potential to hold rock-pools. Other rock outcrop habitats between the two areas should be examined for additional populations of this species.

Further investigations on the thermal biology of other large branchiopods is also warranted. There is also need for mapping and characterising temporary wetlands in the region to understand the distribution and vulnerability to anthropogenic behaviour. This could be coupled with detailed analysis of physico-chemical characteristics and the influences of altitude and vegetation on these parameters. Therefore, analysis on physico-chemical drivers of specialisation of large branchiopods to pond types could be assessed. For example, although, the pan specialists such as *S. cafer* might be absent in rock-pools due to the short hydroperiods of rock-pools, rock-pool specialists

such as *B. tridens* have not been recorded in pans. Understanding why rock-pool specialists cannot persist in non-rock-pool temporary wetlands is a major gap.

The hatching success of different large branchiopods species can be determined and compared. The effect of environmental cues might differ according to species depending on the temporary wetland they specialise in since rock-pools usually have a thinner layer of sediment than pans. There is also need to understand the genetic relatedness on thermal physiologies of large branchiopods by looking at closely related taxa to increase resolution on this conclusion. Furthermore, the mechanism which results in minimal genetic change in large branchiopods can be investigated to determine the mutations that may occur and how they are corrected. This could be done by tracking mutations in different generations of progenies of the same brood. There is also scope for exploring the usefulness of these large branchiopods in aquaculture live feed. Given their contributions to secondary productivity dynamics are unparalleled among crustaceans, given their rapid growth and attainment of sexual maturity. Rock-pool species are particularly interesting in this regard given that they have evolved to attain adulthood and sexual maturity within days of hatching. Indeed, *B. tridens* adults with egg-sacs (ie sexually mature) were observed in rock-pools by the third day of inundation.

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Supplementary material

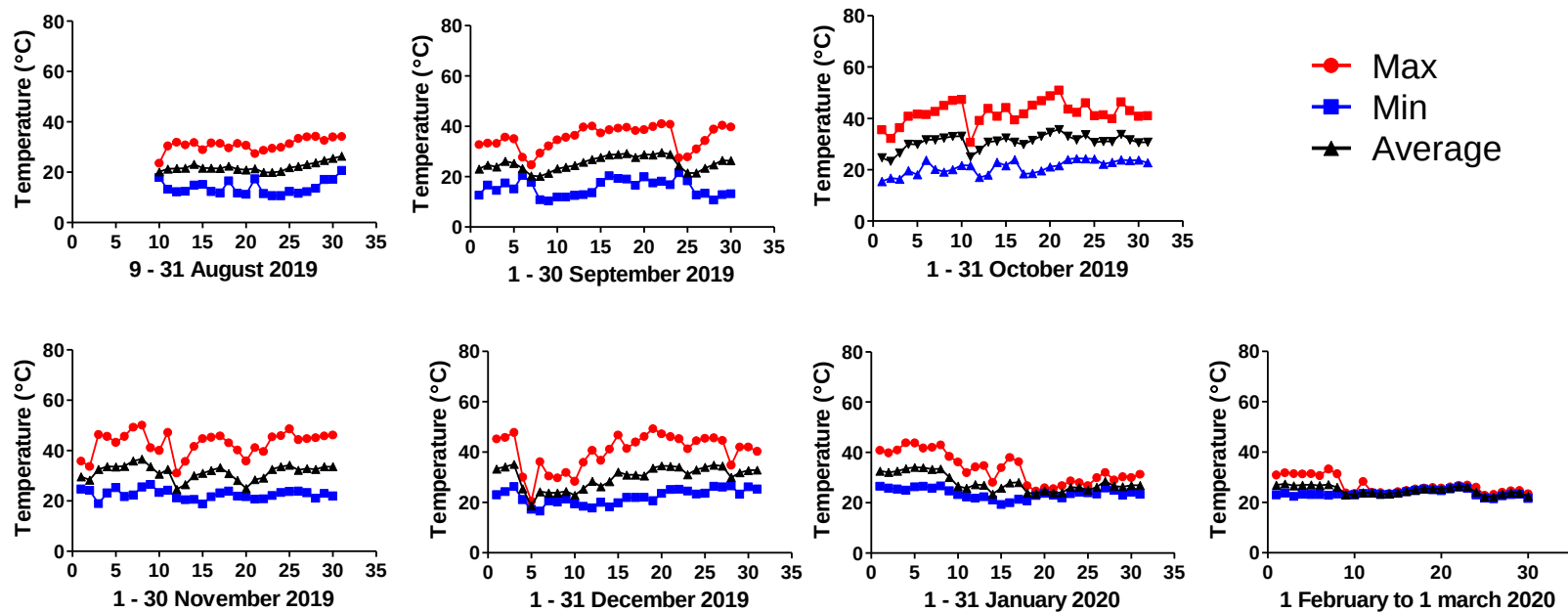


Fig S 1: Temperature records of a pan in Botswana International University of Science and Technology campus (22°37'26.4"S 27°07'35.3"E) (Pan 1). Programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) (0.5°C accuracy) were used for the monitoring of temperatures in the ponds at 1 hour time intervals throughout the sampling period. Loggers were placed in the deepest part of selected wetlands.

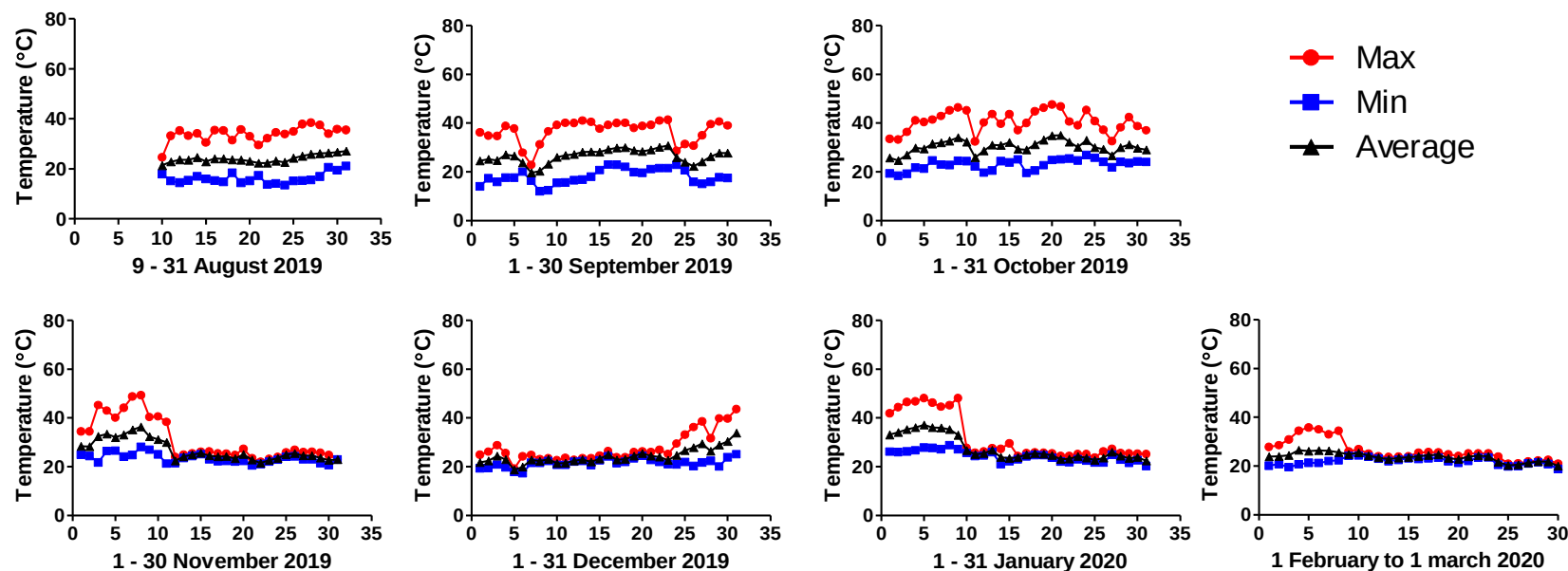


Fig S 2: Temperature records of a pan along Palapye to Martins Drift road (22°49'45.7"S 27°37'17.9"E) (Pan 2). Programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) (0.5°C accuracy) were used for the monitoring of temperatures in the ponds at 1 hour time intervals throughout the sampling period. Loggers were placed in the deepest part of selected wetlands.

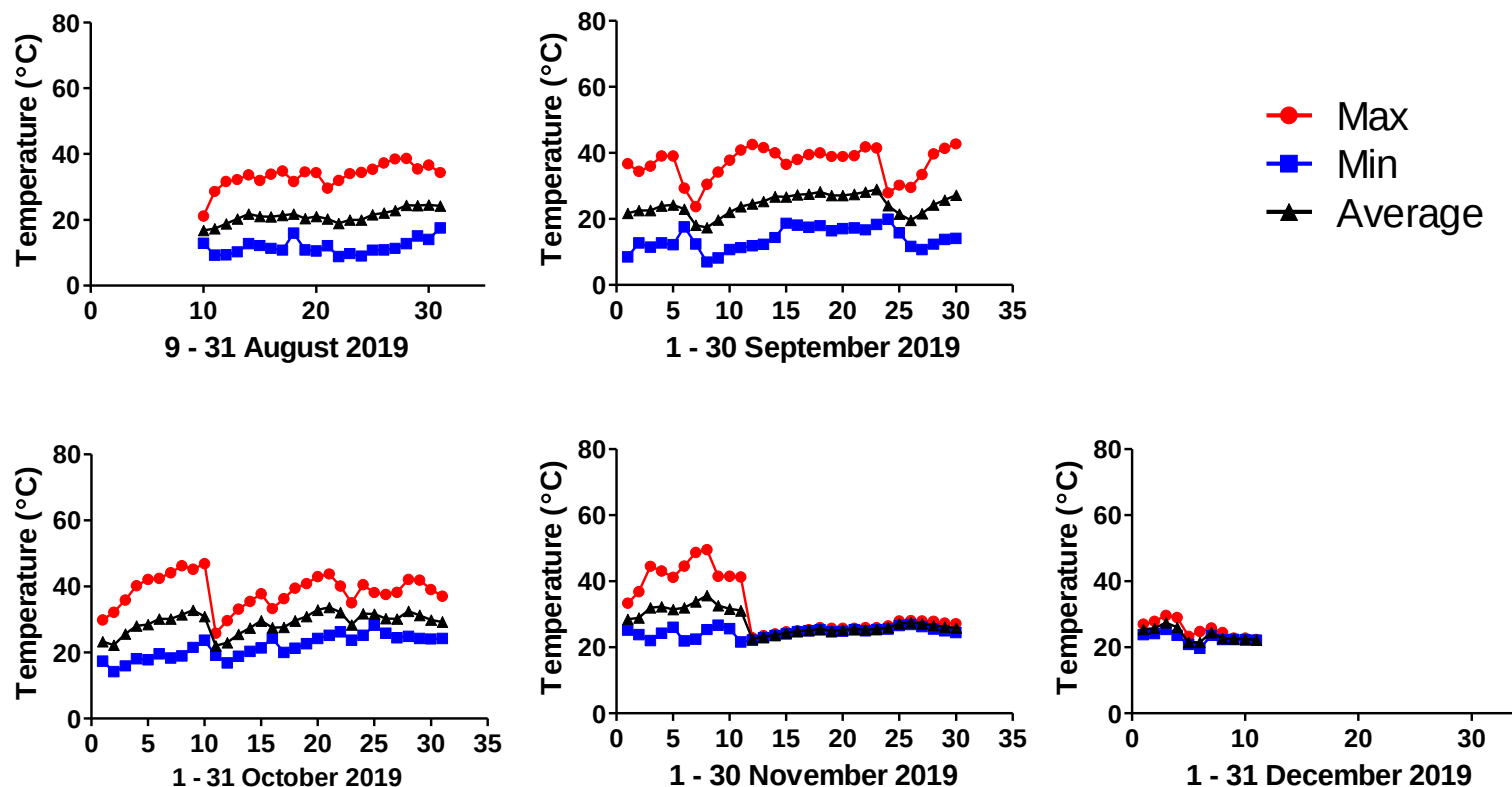


Fig S 3: Temperature records of a pan along Palapye to Martins Drift road (22°52'16.0"S 27°47'42.7"E) (Pan 3). Programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) (0.5°C accuracy) were used for the monitoring of temperatures in the ponds at 1 hour time intervals throughout the sampling period. Loggers were placed in the deepest part of selected wetlands.

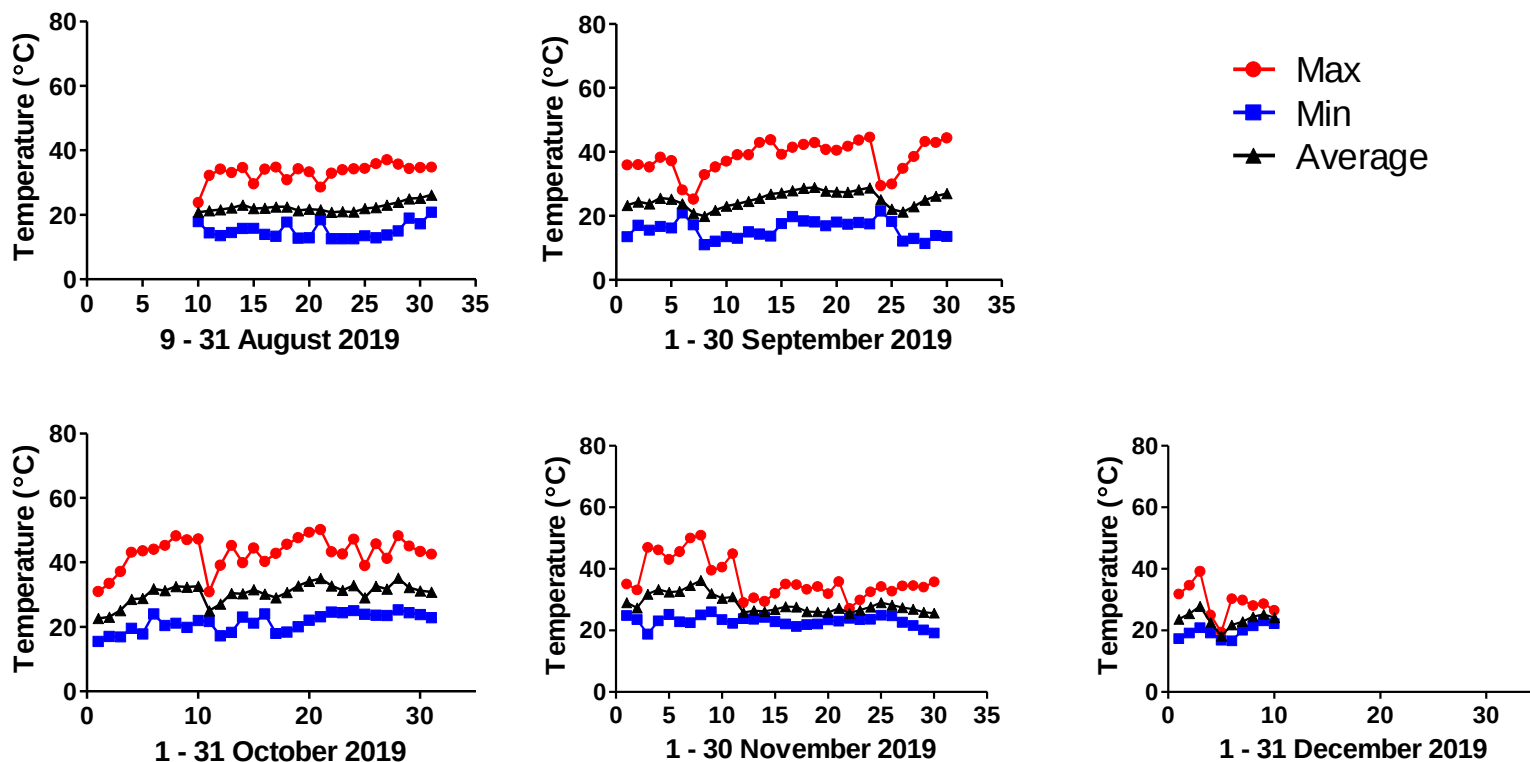


Fig S 4: Temperature records of a pan along Palapye to Martins Drift road (22°42'56.8"S 27°12'32.4"E) (Pan 4). Programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) (0.5°C accuracy) were used for the monitoring of temperatures in the ponds at 1 hour time intervals throughout the sampling period. Loggers were placed in the deepest part of selected wetlands.

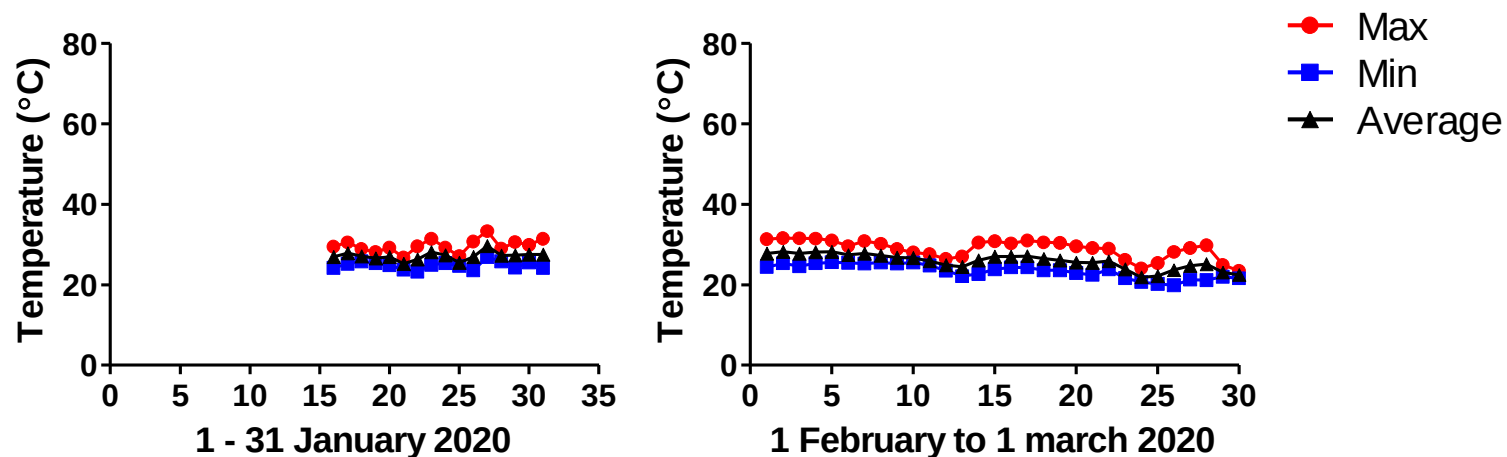


Fig S 5: Temperature records of a pan along Palapye to Martins Drift road (22°50'25.5"S 27°39'34.2"E) (Pan 5). Programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) (0.5°C accuracy) were used for the monitoring of temperatures in the ponds at 1 hour time intervals throughout the sampling period. Loggers were placed in the deepest part of selected wetlands.

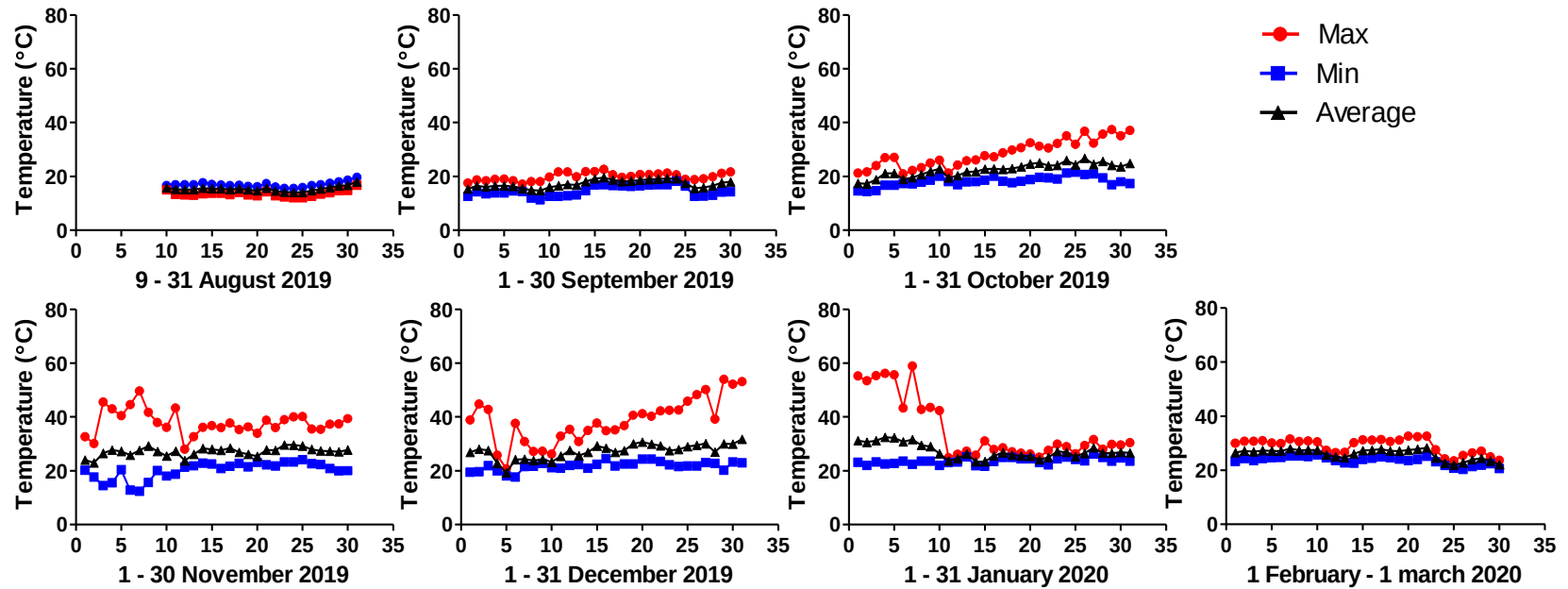


Fig S 6: Temperature records of a pan along Palapye to Martins Drift road (22°32'43.1"S 27°09'56.8"E) (Pan 6). Programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) (0.5°C accuracy) were used for the monitoring of temperatures in the ponds at 1 hour time intervals throughout the sampling period. Loggers were placed in the deepest part of selected wetlands.

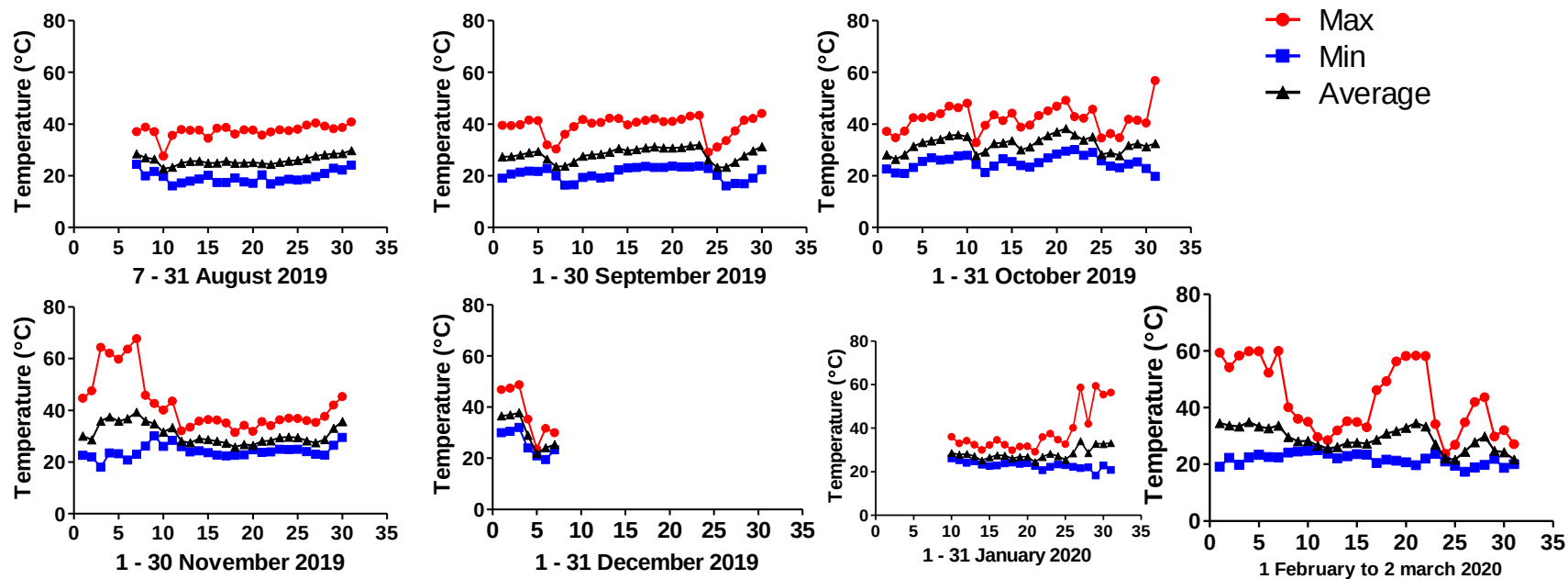


Fig S 7: Temperature records of a rock-pool in Botswana International University of Science and Technology campus (22°35'48.4"S 27°08'05.5"E) (Rock-pool 1). Programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) (0.5°C accuracy) were used for the monitoring of temperatures in the ponds at 1 hour time intervals throughout the sampling period. Loggers were placed in the deepest part of selected wetlands.

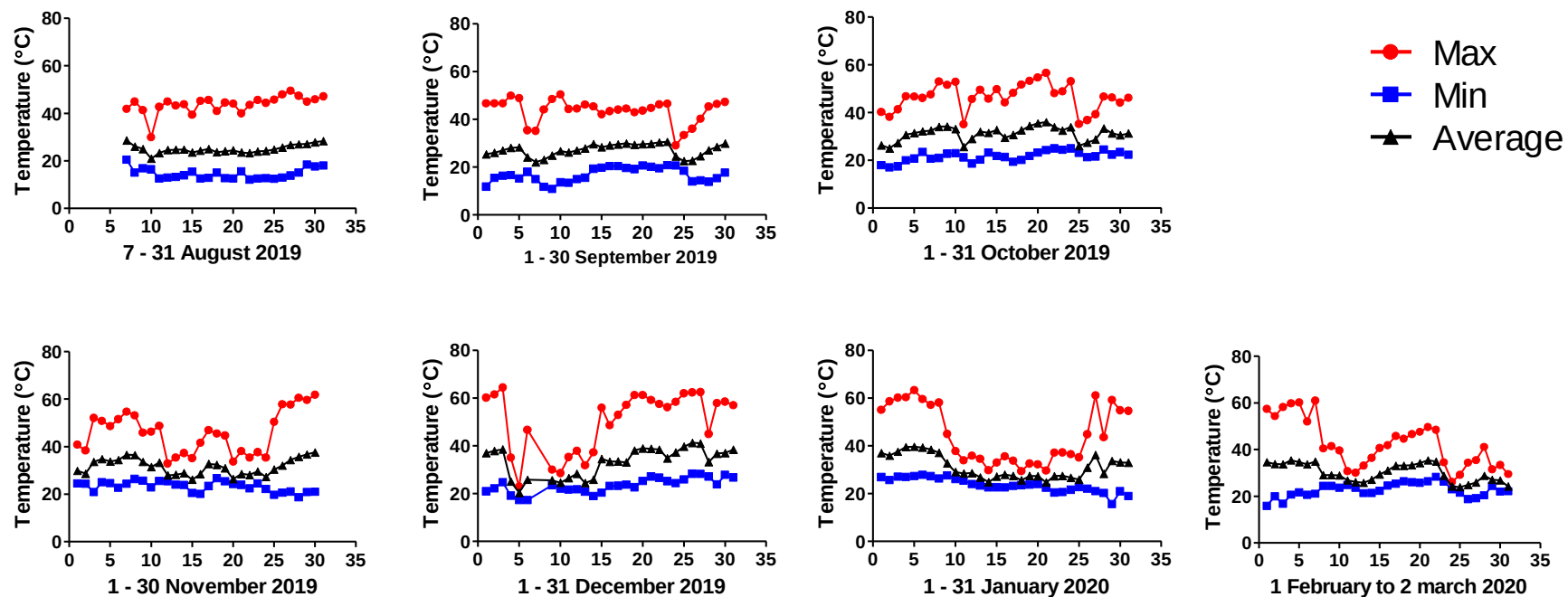


Fig S 8: Temperature records of a rock-pool in Botswana International University of Science and Technology campus (22°35'49.6"S 27°07'59.6"E) (Rock-Pool 2). Programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) (0.5°C accuracy) were used for the monitoring of temperatures in the ponds at 1 hour time intervals throughout the sampling period. Loggers were placed in the deepest part of selected wetlands.

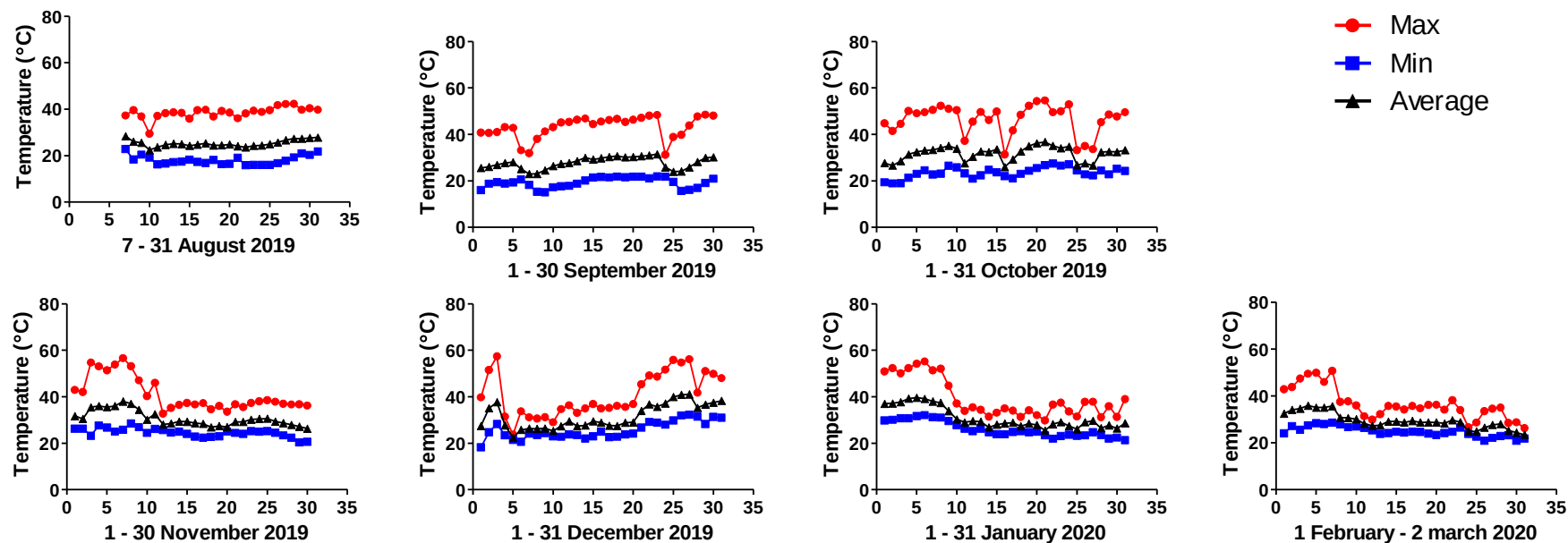


Fig S 9: Temperature records of rock-pool in Botswana International University of Science and Technology campus (22°35'45.8"S 27°07'15.8"E) (Rock-Pool 3). Programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) (0.5°C accuracy) were used for the monitoring of temperatures in the ponds at 1 hour time intervals throughout the sampling period. Loggers were placed in the deepest part of selected wetlands.

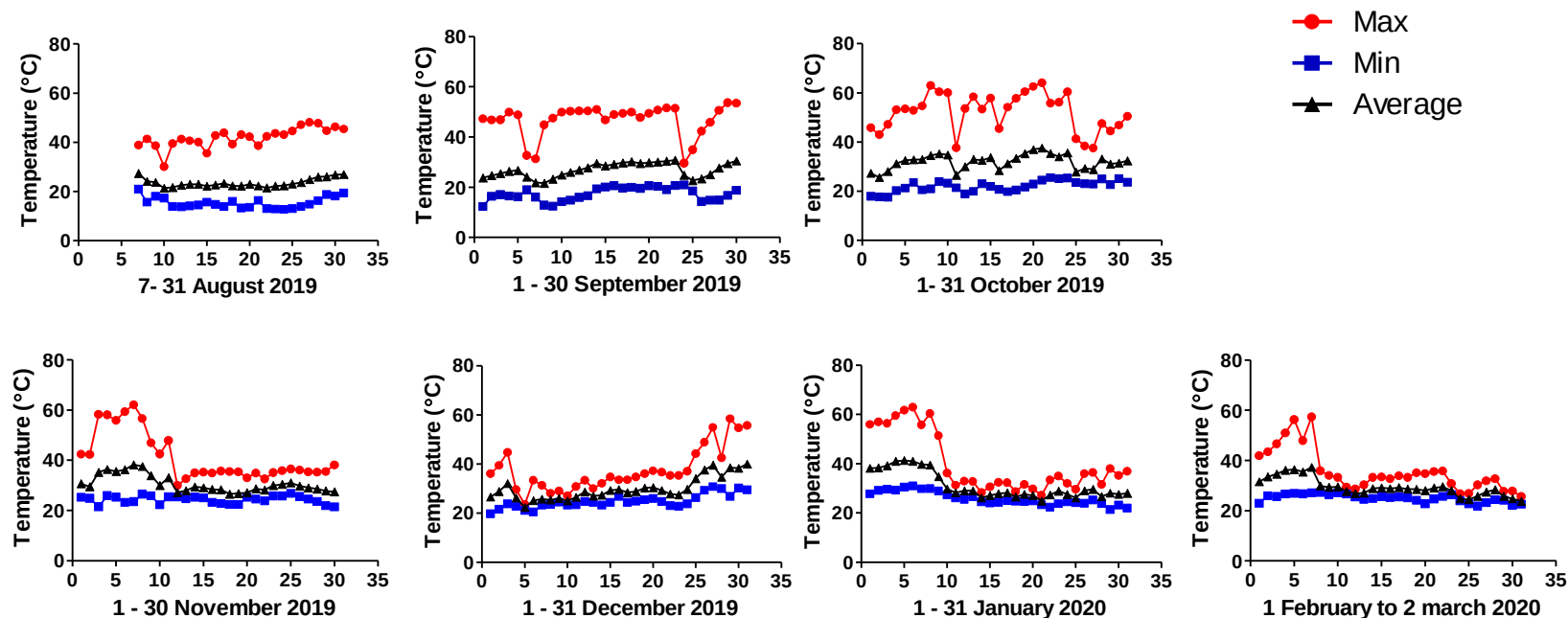


Fig S 10: Temperature records of a rock-pool in Botswana International University of Science and Technology campus (22°35'46.1"S 27°07'14.6"E) (Rock-Pool 4). Programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) (0.5°C accuracy) were used for the monitoring of temperatures in the ponds at 1 hour time intervals throughout the sampling period. Loggers were placed in the deepest part of selected wetlands.

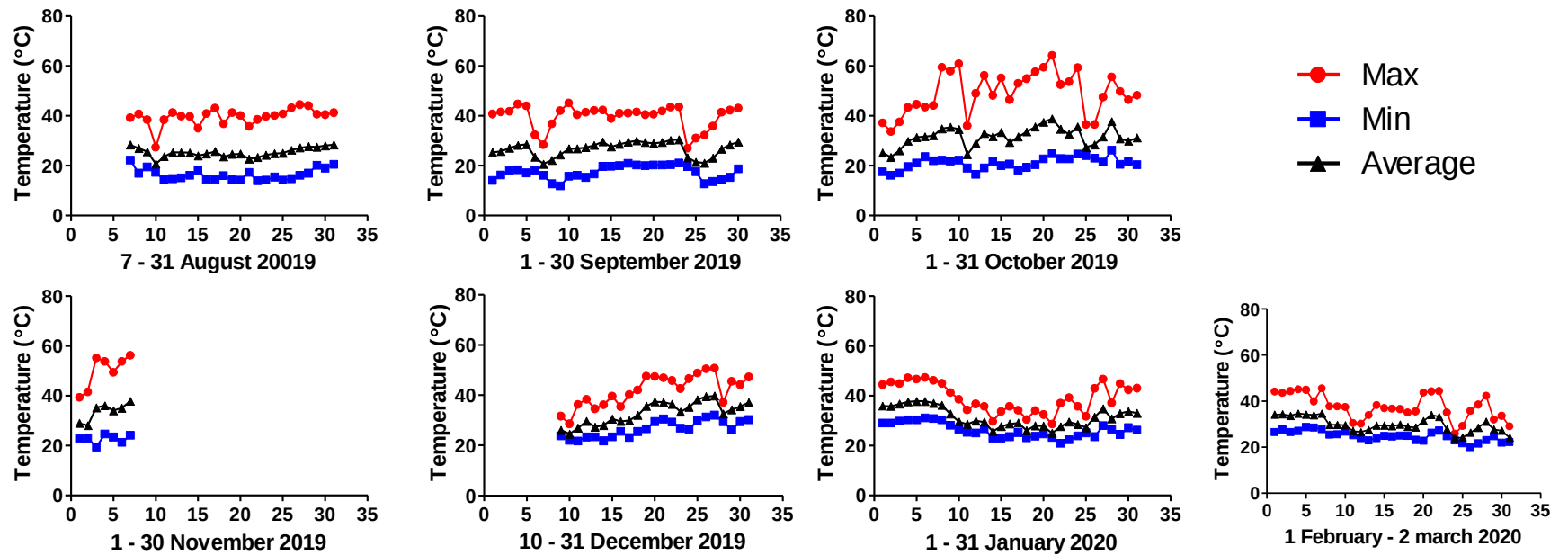


Fig S 11: Temperature records of a rock-pool in Botswana International University of Science and Technology campus (22°35'55.6"S 27°07'51.5"E) (Rock-Pool 5). Programmable data logger probes and software (HOBOWare Pro, version 3.7.16, Massachusetts, USA) (0.5°C accuracy) were used for the monitoring of temperatures in the ponds at 1 hour time intervals throughout the sampling period. Loggers were placed in the deepest part of selected wetlands.

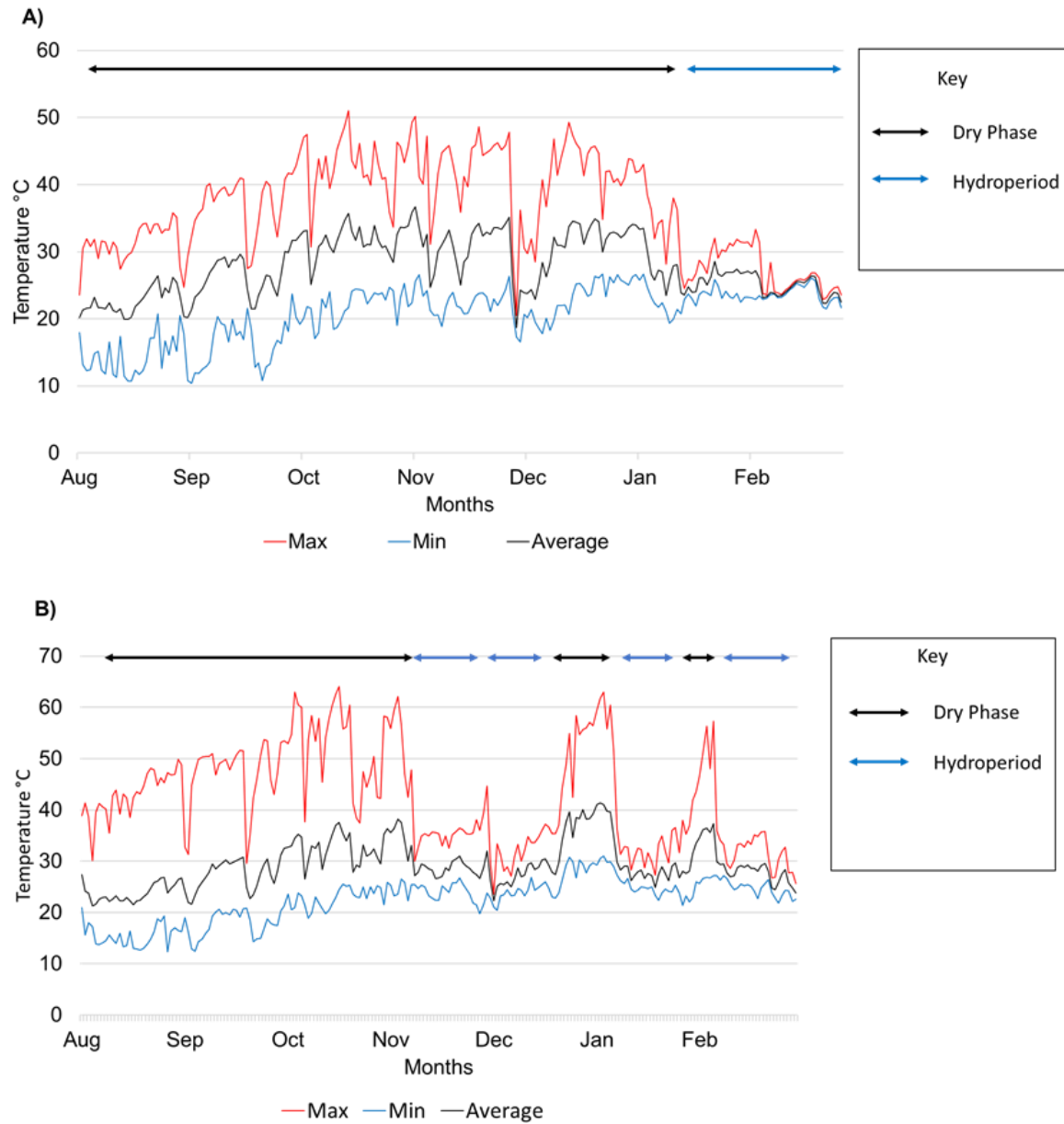


Fig S 12: An example of the number of hydroperiods experienced per pond type; A) a pan and B) a rock-pool. Note that rock-pools, as smaller aquatic environments underlined with bedrock experience more, but shorter, hydroperiod events that do pans.

The complete mitogenome of the fairy shrimp *Streptocephalus cafer* (Lovén, 1847) (Crustacea: Branchiopoda: Anostraca) from an ephemeral pond in Botswana, southern Africa

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ABSTRACT

Fairy shrimps (Anostraca) constitute an important component of seasonally aquatic habitats, but few complete mitochondrial genomes have been published for this group. Here, we report the mitogenome of a common southern African species, *Streptocephalus cafer*, from Botswana (accession number: MN720104). Low-coverage shotgun sequencing recovered two contigs 15653 bp and 1347 bp in length that are separated by a repetitive region of unknown length within the non-coding control region. The mitogenome's GC content is 31.80%. Phylogenetic analysis using protein-coding genes confirms the sister taxon relationship of *S. cafer* with the only other congener whose mitogenome has been reconstructed to date, the Asian *S. siindhornae*.

ARTICLE HISTORY

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African anostracans;
Illumina; mitogenome;
phylogenetics;
Streptocephalidae

Fairy shrimps (Anostraca) are freshwater crustaceans that occur exclusively in seasonally astatic aquatic habitats (Brendonck et al. 2008; Rogers 2009). Similar to other branchiopod members of the order, fairy shrimps produce dormant eggs which allow the survival of the next generation through dry periods (Brendonck et al. 2008; Rogers 2009) and demonstrate rapid body growth rates to facilitate attainment of sexual maturity within limited hydropereod windows (Rogers 2009). The Anostraca originated in the Cambrian (Harvey et al. 2012), with the monobasic Streptocephalidae probably appearing during the early Cretaceous, approximately 105 million years ago (Daniels et al. 2004). The genus *Streptocephalus* has about 66 species worldwide, with numerous species occurring in southern Africa (Rogers 2013; Rogers and Padhye 2014; Shu et al. 2018). Despite their ecological significance, only a few complete mitogenomes of Anostracans have been published (Fan et al. 2015; Liu et al. 2016). Here, we describe the first complete genome of a widespread southern African fairy shrimp, *Streptocephalus cafer* (Lovén, 1847) (sensu Hamer et al. 1994).

Specimens of *S. cafer* were collected from a temporary pool on the outskirts of Palapye, Central District, Botswana (27.16616 E, 22.54507 S) and stored separately in 1.5 mL tubes containing 80% ethanol. Voucher specimens from the locality were lodged at the Kansas Biological Survey (DCR-1136). Total genomic DNA was extracted using the CTAB method

(Doyle & Doyle 1987). One µg of DNA was used to prepare a genomic DNA library using the NEBNext DNA Library Preparation Kit (Massachusetts, USA). The library was then sequenced on an Illumina Hi-Seq platform using 2 × 150 chemistry with an average insert length of 350 bp.

The sequencing run yielded 59,848,500 paired-end sequences. The complete mitogenome was assembled using NOVOPlasty v3.5 (Dierckx et al. 2017) and annotated in MitoZ v2.4. The mitogenome assembly resulted in two contigs (15653 bp and 1347 bp, respectively) separated by a repetitive region of unknown length within the non-coding control region. MITOS annotation on the longest contig identified all 13 protein-coding genes, 22 tRNAs and 2 rRNAs, typical of crustaceans. The GC content of the total assembly was estimated at 31.8%. MitoZ annotation reported several instances of non-canonical start codons and truncated stop codons, consistent with other studies on arthropods (Monsanto et al. 2019).

Protein-coding sequences from *S. cafer* and the complete mitogenomes of nine related species were aligned in MAFFT v7.429 (Katoh et al. 2009). A Bayesian phylogenetic tree was reconstructed with BEAST2 (Bouckaert et al. 2014) using default parameters, except that the substitution model was changed to HKY (Hasegawa et al. 1985) with four gamma categories. BEAST2 was run for 50,000,000 iterations with 30% burn-in. The convergence of the chain and Effective Sample

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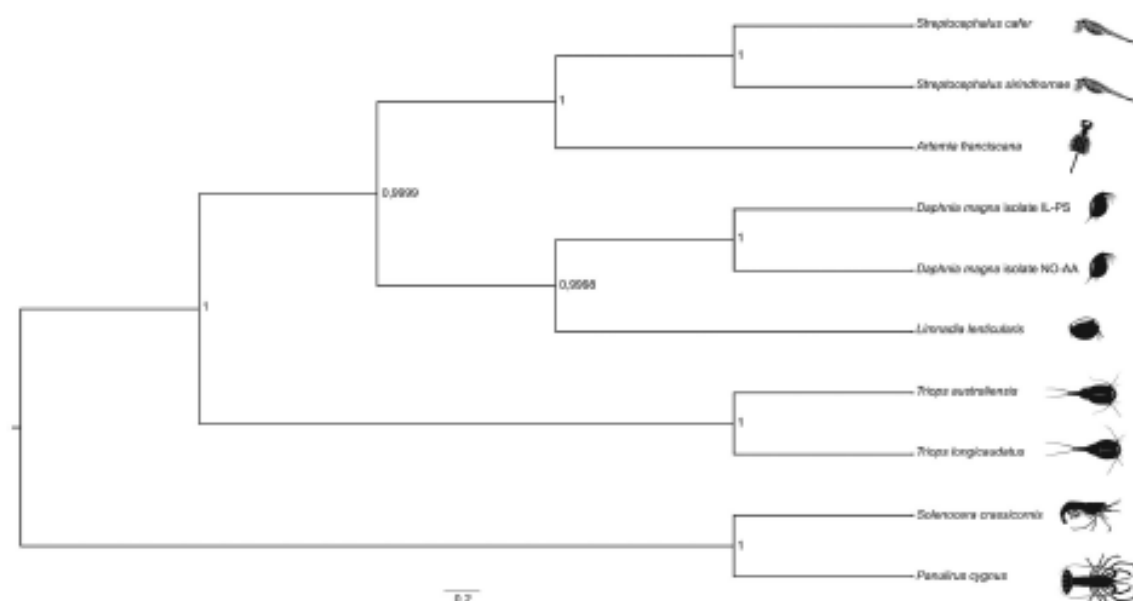


Figure 1. A Bayesian phylogenetic tree constructed in BEAST2 using mitogenome sequences of *Streptocephalus cafer* (NCBI accession number MN720104) and nine other crustacean species *Streptocephalus sirindhornae* (NC_026704.1), *Daphnia magna* isolate L-PS (MH683649.1), *Daphnia magna* isolate NO-AA (MH683655.1), *Artemia franciscana* (X69067.1), *Limnadia lenticularis* (NC_039894.1), *Penaeus cygnus* (KT696486.1), *Solenocera crassicornis* (KU899137.1), *Triops australiensis* (LK91946.1) and *Triops longicaudatus* (AY69934.1). The numbers next to each node represent posterior probability and the scale bar shows the scaled substitution rate.

Size (ESS) was assessed in Tracer v1.7 (Rambaut et al. 2018), and the resulting phylogenetic tree was visualized in FigTree v1.4 (Rambaut and Drummond 2012) (Figure 1). It confirms the sister taxon relationship of *S. cafer* with the only other *Streptocephalus* species whose mitogenome has been reconstructed to date, the Asian *S. sirindhornae*.

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Disclosure statement

No existing competition and/or financial interest has been declared by the authors. The authors declare that they own the opinions, findings, conclusions or recommendations expressed in this material.

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The complete mitogenome of an undescribed clam shrimp of the genus *Gondwanalimnadia* (Branchiopoda: Spinicaudata), from a temporary wetland in Central District, Botswana

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ABSTRACT

Clam shrimps (Spinicaudata) are a widespread and diverse crustacean group that frequent temporary aquatic habitats, but few complete mitochondrial genomes have been published for this group. Here, we report the mitogenome of an undescribed *Gondwanalimnadia* species from Botswana. Raw sequences were assembled into a single circular genome with a total length of 15,663 bp. Thirteen protein-coding genes, 22 tRNAs, and 2 rRNAs were identified using the MITOS pipeline. The mitogenome's GC content is 33.52%. Phylogenetic analysis using protein-coding genes confirmed that *Gondwanalimnadia* sp. is closely related to another member of the Limnadiidae, *Limnadia lenticularis*.

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KEYWORDS

African Spinicaudata;
Illumina; mitochondrial
DNA; phylogenetics;
Crustacea

Spinicaudatans are an order of freshwater crustaceans commonly known as spiny clam shrimps. These large branchiopod crustaceans occur exclusively in seasonally astatic aquatic habitats (Brendonck et al. 2008). Similar to other branchiopods, clam shrimp rapidly attain sexual maturity and within short hydroperiod windows, produce dormant eggs that can withstand dry periods (Brendonck et al. 2008; Rogers 2009). They are thought to be omnivores with diets consisting of detritus, plankton and algae (Hethke et al. 2019). *Gondwanalimnadia* spp. belong to the Limnadiidae (Weeks et al. 2012; Rogers et al. 2016), a family in the order Spinicaudata with a global distribution, except for Antarctica (Bellec and Rabet 2016). As with many spinicaudatan families, little is known about their genomic make-up, and their correct placement within the branchiopod phylogeny is not fully resolved (Bellec and Rabet 2016). Here, we describe the complete mitogenome of an undescribed *Gondwanalimnadia* species obtained from Botswana, and investigate its phylogenetic placement among several other branchiopods.

Specimens of the undescribed *Gondwanalimnadia* species were collected from a temporary pond on the outskirts of Palapye, Central district, Botswana (GPS 27.16616 E, 22.545075) and preserved in 80% ethanol. Voucher specimens from the locality were deposited at the Kansas Biological Survey (DCR-1138). Genomic DNA of high molecular weight was extracted using the CTAB method (Doyle and

Doyle 1987). An indexed DNA library was constructed using NEBNext DNA Library Preparation Kit (Massachusetts, USA), and 1 µg of genomic DNA as template. The DNA library was sequenced on the Illumina Hi-Seq platform (California, USA) using 2 × 150 chemistry with an average insert size of 350 bp.

The Illumina sequencing run yielded a total of 23,096,464 raw sequences. The complete mitogenome was assembled using NOVOPlasty v3.5 (Dierckx et al. 2016) and annotated using MITOS Web Server (Bernt et al. 2013). The assembly resulted in a single circular genome with a total length of 15,663 bp. The MITOS pipeline identified 13 protein-coding genes, 22 tRNAs, and 2 rRNAs, typical of all crustaceans. The GC content of the assembly was estimated at 33.52%. Several instances of non-canonical start codon and incomplete stop codons were observed, as for other arthropods (Monsanto et al. 2019; Jagatap et al. 2019). Protein coding sequences from the studied species, eight closely related species with complete mitogenomes in the NCBI database, and an out-group (*Dysdercus evanescens*) were aligned in MAFFT v7.429 (Katoh et al. 2009). A Bayesian phylogenetic tree was reconstructed using package BEAST2 (Bouckaert et al. 2014). The program's default settings were used, except that the substitution model was set to HKY (Hasegawa et al. 1985) with four gamma categories. BEAST2 was run for 50,000,000 iterations with 30% burn-in. Markov chain convergence and

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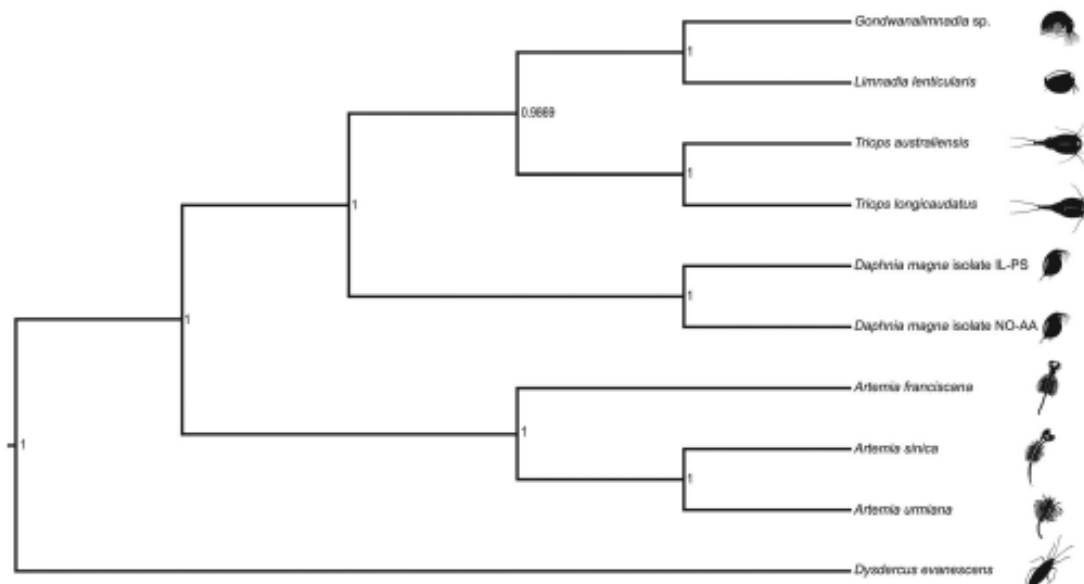


Figure 1. A Bayesian phylogenetic tree constructed in BEAST2 using mitogenome sequences of an undescribed *Gondwanalimnadia* sp. (NCBI accession number MN625703) and nine related species. *Daphnia magna* isolate IL-PS: MH683649.1, *Daphnia magna* isolate NO-AA: MH683655.1, *Limnadia lenticularis*: NC_039394.1, *Triops australiensis*: LK391946.1, *Triops longicaudatus*: AY639934.1, *Dydacus evanescens*: NC_042437.1, *Artemia urmiana*: NC_021382.1, *Artemia sinica*: NC_042147.1, *Artemia franciscana*: X69067.1. The numbers on the tree indicate the posterior probability estimated for each node.

Effective Sample Size (ESS) were visually inspected in Tracer v1.7 (Rambaut et al. 2018). The phylogenetic tree was visualized in FigTree v1.4 (Rambaut and Drummond 2012), placing the uncharacterized *Gondwanalimnadia* sp., along with another limnadiid (*Limnadia lenticularis*), in a monophyletic clade with cladoceran and notostracan representatives (Figure 1).

Disclosure statement

The authors declare that there is no existing competition and/or financial interest. Therefore, opinions, findings, conclusions or recommendations expressed in this material are owned by the authors.

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File S3

The complete mitogenome of *Leptestheria brevirostris* Barnard, 1924, a rock-pool clam shrimp (Branchiopoda: Spinicaudata) from Central District, Botswana

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Keywords: Africa, Illumina next-generation sequencing, mitochondrial genome, spinicaudatan

Abstract

Clam shrimps (Spinicaudata) are a widespread and diverse group of branchiopod crustaceans, yet few mitochondrial genomes have been published for this taxonomic group. Here, we present the first mitogenome of *Leptestheria brevirostris* from Botswana, a clam shrimp previously thought to be endemic to Namibia. Massively parallel sequencing of a single specimen facilitated the reconstruction of the species' 15,579 bp circularized mitogenome. The reconstructed phylogenetic tree confirms that *L. brevirostris* forms a monophyletic group with other diplostracan branchiopods, and that these are the sister taxon to Notostraca. Our record presents a significant range extension from the type locality in Namibia, and the mitogenome reconstructed here is the first to be reported from a leptestheriid clam shrimp.

Spinicaudatan clam shrimps are a group of branchiopod crustaceans found in seasonally astatic aquatic habitats on all continents except Antarctica (Brendonck et al., 2008; Rogers 2009). These shrimps produce resting eggs (cysts) capable of withstanding prolonged dry conditions between hydroperiods (Brendonck, 1999). As spinicaudatan clam shrimps tend to occupy and feed in the benthic portions of pools, they are often overlooked (Brendonck et al., 2008; Rogers, 2009). Here, we describe the complete mitogenome of *Leptestheria brevirostris* Barnard, 1924, a leptestheriid clam shrimp that was collected from a rock-pool temporary wetland in Central District, Botswana. This species has not been reported since it was initially described (Barnard, 1924), and our collection represents a significant range extension from its type locality in north-western Namibia. This study forms the basis for more comprehensive phylogenetic studies to better understand the evolution of branchiopod crustaceans and their relatives.

Whole specimens of *L. brevirostris* were collected from a temporary rock-pool (22°35'55.50" S; 27°7'51.78" E), preserved in 80% ethanol and identified using the relevant literature (Barnard, 1924, 1929; Brendonck, 1999). Voucher specimens from the same locality were deposited at the Kansas Biological Survey (DCR-1140). Genomic DNA of high molecular weight was extracted from a single specimen using the CTAB method (Doyle & Doyle, 1987). A genomic DNA library was constructed from 1 µg of genomic DNA as template using a NEBNext Library Preparation Kit (Massachusetts, USA) and sequenced on an Illumina HiSeq 4000 platform using 2 × 150 bp chemistry following the manufacturer's instructions.

The sequencing run produced 22,933,816 paired-end sequences. The assembly of raw sequences using MitoZ v2.4_alpha pipeline (Meng et al., 2019) resulted in a single circular genome with a total length of 15,579 bp. Annotation of the reconstructed genome in MITOS webserver (Bernt et al., 2013) identified 13 protein-coding genes, 22 tRNAs and 2 rRNAs, as is typical of all crustaceans. Instances of non-canonical start codons and truncated stop codons were observed (Monsanto et al., 2019; Jagatap et al., 2019). The protein-coding DNA sequences of the study species were aligned with those of eight closely related crustaceans in MAFFT v7.429 (Katoh et al., 2013). A consensus Bayesian phylogenetic tree was reconstructed for the study species using BEAST2 v2.6.2 (Bouckaert et al., 2014). The program's default settings were used, except that the substitution model was set to HKY (Hasegawa et al., 1985) with four Gamma categories. Ten independent runs, each comprising 500 million iterations with 150 million initial burn-in steps were executed in parallel. Final log and tree files were combined in BEAST2 LogCombiner (Rambaut and Drummond 2014), and convergence of the independent runs and Effective Sample Size (ESS) were checked in Tracer v1.7 (Rambaut et al., 2018). The Bayesian phylogenetic tree was rooted using the anostracans *Artemia sinica* and *Streptocephalus cafer*, and visualized in FigTree v1.4 (Rambaut and Drummond, 2016) (Fig 1).

Our results grouped *L. brevirostris* with other spinicaudatans, and confirmed the monophyly of cladocera and spinicaudatans and are consistent with previous studies on crustaceans mitogenomics (Luchetti et al., 2019) (Fig. 1).

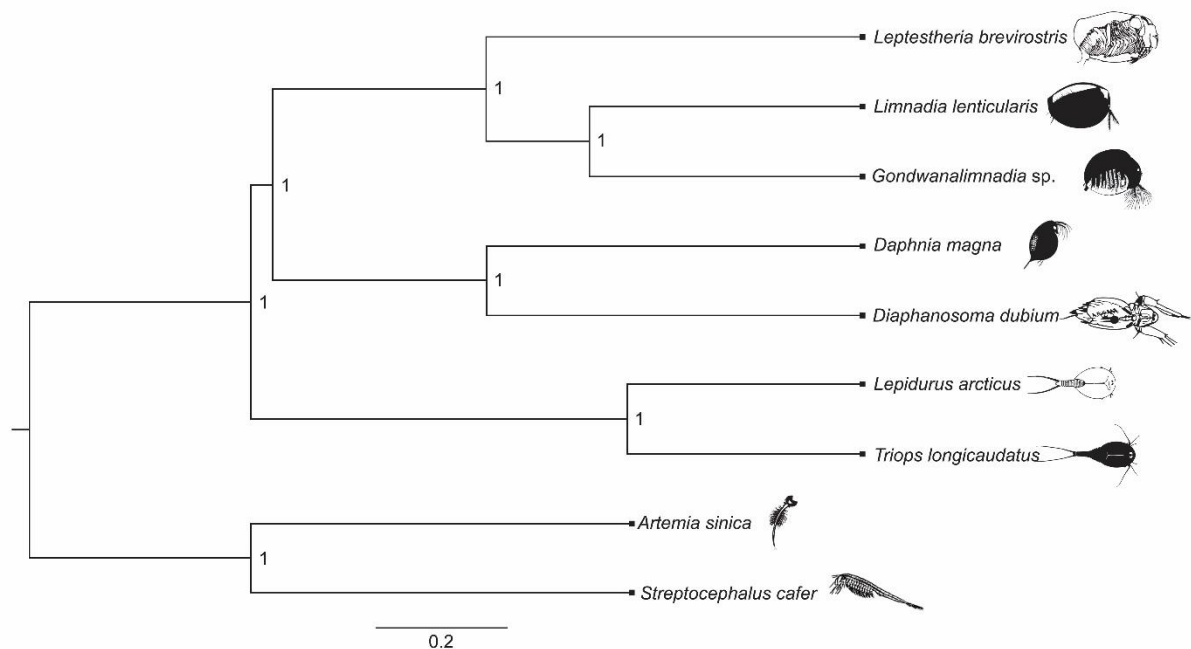


Fig. 1. A Bayesian phylogenetic tree constructed in BEAST2 using mitogenome sequences of *Leptestheria brevirostris* (NCBI accession number MN548772) and nine crustacean species: *Limnadia lenticularis* (NC_039394.1), *Gondwanalimnadia* sp. (MN625703.1), *Daphnia magna* (MK370029.1), *Diaphanosoma dubium* (NC_037488.1), *Lepidurus arcticus* (MK579380.1), *Triops longicaudatus* (KM516710.1), *Artemia sinica* (MK069595.1), and *Streptocephalus cafer* (MN720104.1). The numbers on the tree indicate the posterior probability of each node. The scale beneath the tree is expressed in number of substitutions per time unit.

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Disclosure statement

The authors declare that there is no existing competition and/or financial interest. Therefore, opinions, findings, conclusions or recommendations expressed in this material are owned by the authors.

Data Availability

Raw sequence data generated for this study are available at NCBI Short Reads Archive (SRA) under BioProject ID:PRJNA649895. The assembled and annotated mitogenome of the study species was submitted to NCBI database under accession number MN548772.

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