Environmental characteristics of ephemeral rock pools explain local abundances of the clam shrimp, *Paralimnadia badia* (Branchiopoda: Spinicaudata: Limnadiidae)

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ABSTRACT

The conditions of ephemeral freshwater pools are highly variable through time, and their inhabitants must be able to tolerate these changing conditions to survive. Although much research has focused on large branchiopod hatching requirements, there is comparatively little information available on the environmental conditions endured by adult clam shrimp populations. A suite of physical and chemical characteristics, especially pool depth, influence the presence or absence of clam shrimp populations in rock pools on granite outcrops in the Wheatbelt region of Western Australia. Here we examine multiple environmental variables of temporary rock pools and how they may affect adult populations of the clam shrimp, *Paralimnadia badia*.

KEYWORDS: habitat, physiochemical, temporary pool, pool morphology, hydroperiod, environmental variability

INTRODUCTION

Clam shrimp are branchiopod crustaceans (Branchiopoda: Spinicaudata) that are obligate dwellers of temporary waters (Dumont & Negrea 2002). Populations survive dry periods in the form of resting eggs produced by mature females or hermaphrodites. Their eggs lie dormant until appropriate conditions are met for hatching (Bretonck 1996, Brendonck & Riddoch 2000, Brendonck & De Meester 2003). Upon inundation, a fraction of resting eggs hatch, leaving behind a bank of dormant eggs in the sediment (Brendonck 1996, Brendonck & De Meester 2003). Occasionally, eggs will continue to hatch even after the initial inundation, resulting in multiple generations of shrimp in a single pool (Benvenuto et al. 2009). Often, rain will fill the pool and eggs will hatch but not reach sexual maturity before the basin dries. In these instances, the egg bank is vital to ensure long-term population persistence (Bretonck & De Meester 2003). In this way shrimp ‘hedge their bets’ against the unpredictability of their environment which has led to speculation as to which factors might promote this pattern of hatching (Mossin 1986, Bretonck et al. 1996, Kuller & Gasith 1996, Simovich & Hathaway 1997, Beladjal et al. 2007).

Many physical environmental parameters, including dissolved oxygen, pH, osmotic pressure, light, and temperature, have been individually implicated in regulating hatching in various large branchiopod species (Bishop 1967, Belk 1977, Scott & Grigarick 1979, Mitchell 1990, Schönbrunner & Eder 2006). However, initial hatching conditions are not sole predictors of clam shrimp presence, as temporary pool environmental conditions change throughout the duration of their inundation and drying regimes (Jocqué et al. 2007a). Here we aim to determine environmental factors that are associated with maintenance of adult populations of clam shrimp.

For a hatched population to be sustained, the conditions of the pool must meet survival requirements after initial hatching. Long term maintenance of populations of several species of *Daphnia* have been assessed in relation to habitat characteristics and each species appeared to thrive under different environmental conditions, occupying different pools as a result (Pajunen & Pajunen 2007). Both the size and permanence (length of hydroperiod) of temporary pools have been found to play important roles in predicting species richness (Kiflawi et al. 2003). Additionally, since larger pools will resist evaporation for longer periods of time, pool size has been shown as an important factor structuring local communities (Jocqué et al. 2007b).

Our study sought to answer how temporary pool environments relate to adult clam shrimp populations. Here we determine which parameters best explain abundances of adult populations of the clam shrimp *Paralimnadia badia* (Wolf 1911; the Australian species *Limnadia* have recently been moved to a new endemic genus *Paralimnadia*, Rogers et al. 2012) in a variety of temporary rock pools on granite outcrops in the semi-arid, Wheatbelt region of Western Australia.

⁴Deceased March 2013

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METHODS

Study Sites

Although the Wheatbelt region of southwestern, Western Australia is generally flat, occasional large granite outcrops (or ‘inselbergs’ sensu stricto, Withers 2000) can be found in the area (York Main 1997, Withers 2000). The rainy season in this part of Australia lasts approximately from May to October and is the milder time of the year, with mean maximum air temperatures ranging from 20.3°C to 23.7°C (Commonwealth of Australia, Bureau of Meteorology, 26 Sept 2011).

Three outcrops received adequate rainfall for sustained hydration: Holland Rock (Shire of Kent, 33°21.299’S; 118°44.639’E; 13 pools sampled), Dingo Rock (Shire of Lake Grace, 33°0.558’S; 118°36.321’E; 8 pools sampled), and Rockhole Rock (Shire of Bruce Rock, 31°55.970’S; 117°45.209’E; 11 pools sampled). Data from Bruce Rock were collected at a later date than Holland and Dingo (May 2009) and were only used to construct Fig. 3.

Field Sampling

Study pools were selected for a range of sizes and depths, and were sampled for physiochemical parameters and quantities of clam shrimp and other macroinvertebrates in late April to late May 2007. Water quality [temperature, pH, dissolved oxygen (DO), and electrical conductivity (EC)] of each of the three sites was monitored for five consecutive days at four times per day (12:00, 14:00, 16:00, and 18:00) to assess diurnal change, however for most analysis only the average of the mid-afternoon readings (14:00 and 16:00) were used as a result of incomplete sampling across the other two time periods. Pools on Holland and Dingo Rocks were sampled with a YSI 556 multi-probe (YSI, USA). Pools on Rockhole Rock were sampled with a Hydrolab Quanta multi-parameter meter (Hydrolab, USA). Pool dimensions measured included maximum length (l), maximum width (w) and mean depth (d) (each averaged from three measurements). Pool volumes were estimated from these dimensions assuming the shape to be half of an ellipsoid (Formula: 4/3πlwd, Baron et al. 1998).

Each pool was sampled across the entire volume for three, 3-min periods with sweep nets of mesh sizes 2.0 mm and then 0.5 mm. Paralimnadia badia abundance approximately halved each sampling event over the three successive rounds of sampling. Consequently, after three rounds in each pool approximately 90% of clam shrimp had been captured. Paralimnadia badia were sorted from other macroinvertebrates in white plastic collecting trays, species were identified, counted, and then returned to the pool alive.

Analysis of habitat variables and species distributions

Volume and EC values were log_{10} transformed to achieve normality. All other variables had normally distributed residuals (using Shapiro-Wilk test for normality). One-way ANOVAs compared the means of environmental variables between pools with and without *P. badia*.

All parametric analyses were made with JMP Pro 10 (SAS Institute Inc. 2012). Ordinations of environmental data were produced by Principal Components Analysis (PCA) in PRIMER multivariate software (PRIMER-E 2006). Although not suitable for analysis of biotic community structure, the implicit underlying Euclidean Distance matrix of this method makes it suitable for environmental data, with an advantage over nMDS ordination in that ordination axes are interpretable (Clarke & Warwick 2001). Correlations between pairs of environmental variables were then examined with draftsmen plots. Combinations of highly correlated variables (Spearman rank higher than 95%) were reduced to a single, representative variable.

The PRIMER BEST procedure (Clarke & Ainsworth 1993) identified the combination of mean water quality and habitat variables for each pool, which best rank-correlated with clam shrimp abundances. These best correlating environmental variables are likely to be those that are most important in defining clam shrimp abundance. Prior to BEST analysis, environmental variables were normalized to the maximum value encountered (Olsgard et al. 1997, Clarke & Warwick 2001) and draftsmen plots were created to determine which variables were highly correlated with each other (i.e., 95% or greater, Bob Clarke, Plymouth Marine Laboratory UK pers. comm.). Data were then log_{10} transformed to enhance a linear relationship between variables and finally standardized to account for different variable scales (Clarke & Warwick 2001).

A canonical discriminate analysis (CDA) was then used (JMP Pro 10, SAS Institute Inc.) to determine which environmental variables best described the variation between groups (presence / absence of *P. badia*; see below). Bivariate regressions were then made to describe relationships between *P. badia* abundance, estimated fitness and habitat pH. Fitness was estimated from the size of the carapace, which is directly related to the number of resting eggs per clutch in female clam shrimp (Weeks et al. 1997).

RESULTS

Of the 21 pools studied on Holland and Dingo Rocks, 13 contained clam shrimp populations. Pools with and without *P. badia* had different abiotic conditions. Pool depth was related to pool volume and surface area (shown as factor loadings clustering on the loading plot; Fig. 1), which is expected because of the common formula used to derive pool volume. Examined individually, pH was the only abiotic parameter that differed significantly between the two groups (Table 1). Pools containing *P. badia* typically had lower pH than those without clam shrimp. Pools containing *P. badia* had overlapping ranges of all variables with pools lacking *P. badia*. The water temperature of the pools without *P. badia* was also higher, but not significantly so (p=0.06). Pool volume, surface area, conductivity, and TDS were higher in the pools that had *P. badia* populations, albeit not significantly.

Mean depth was the most important sole environmental variable explaining clam shrimp abundance according to the BEST analysis (Spearman Rank correlation: rho = 0.375). When pH was added to this model, this water quality variable further helped to explain clam shrimp distribution at rho = 0.413.
Table 1. Habitat characteristics of 21 total pools with *P. badia* present (n=13) and absent (n=8). Bold type indicates statistically significant difference (p<0.05) between pool environmental variable with and without *P. badia*.

<table>
<thead>
<tr>
<th></th>
<th>Pools without <em>P. badia</em></th>
<th>Pools with <em>P. badia</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>21.2</td>
<td>0.41</td>
</tr>
<tr>
<td>Conductivity (mS/cm)</td>
<td>0.24</td>
<td>0.03</td>
</tr>
<tr>
<td>Dissolved oxygen (%)</td>
<td>113</td>
<td>2.75</td>
</tr>
<tr>
<td>pH</td>
<td>7.09</td>
<td>0.17</td>
</tr>
<tr>
<td>Depth (mm)</td>
<td>30.7</td>
<td>5.36</td>
</tr>
<tr>
<td>Volume (L)</td>
<td>136</td>
<td>41.3</td>
</tr>
<tr>
<td>Surface area (m²)</td>
<td>7.52</td>
<td>1.41</td>
</tr>
</tbody>
</table>

Figure 1. PCA of pool environmental variables. Length of vector indicates strength of correlation between variable and component axes. Asterisks indicate significant correlation of the component with the original variable (*p<0.05*). Pools with *P. badia* present are filled circles. Pools without *P. badia* are empty circles.
Figure 2. Results of a canonical discriminate analysis (CDA) on all seven dependent variables using data from Holland and Dingo outcrops only. Pools without *P. badia* present are empty circles; pools with *P. badia* present are filled circles. Canonical correlation 1 explains ~100% of the differences between present/absent pools. Ellipses show the means of the centroids of *P. badia*-present pools and *P. badia*-absent pools. Asterisks indicate significant correlations of the canonical scores with each of the original variables (*p*<0.05).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Canonical 1</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>-0.48</td>
<td>0.006*</td>
</tr>
<tr>
<td>Dissolved Oxygen</td>
<td>-0.18</td>
<td>0.096</td>
</tr>
<tr>
<td>pH</td>
<td>3.08</td>
<td>0.004*</td>
</tr>
<tr>
<td>Depth</td>
<td>0.05</td>
<td>0.027*</td>
</tr>
<tr>
<td>Surface Area</td>
<td>-0.03</td>
<td>0.022*</td>
</tr>
<tr>
<td>Conductivity</td>
<td>-0.94</td>
<td>0.721</td>
</tr>
<tr>
<td>Volume</td>
<td>-0.91</td>
<td>0.020*</td>
</tr>
</tbody>
</table>

Figure 3. Regression of CC1 on the quantity of *P. badia* present in all pools. Canonical 1 (consisting largely of volume, depth, and surface area) is negatively correlated with the quantity of *P. badia* (p=0.0052).
The CDA correctly classified 17 of 21 pools in its assignment of pools into groups that either had *P. badia* or not, or 81% of the total pools in this comparison (Fig. 2). The first canonical correlation (CC1) explained ~100% of the important variation that differed between pools with and without *P. badia*. Five original dependent variables were correlated strongly with CC1: temperature, depth, and surface area were negatively correlated with CC1, while volume and pH were significantly positively correlated with CC1 (Fig. 2). Volume, depth, and surface area are all measures describing pool size. Temperature and pH are variable that are closely associated and influenced by pool size, which suggests that CC1 is mainly describing the size of the pools. This ‘pool size’ variable is negatively correlated with the quantity of *P. badia* (Fig. 3). Figure 4 displays the diurnal variability of pH in pools on Dingo and Holland rocks.

**DISCUSSION**

Our study confirms that temporary rock pools are highly variable environments (Bayly 1982, Williams 2001). To survive this environment, pool inhabitants must cope with extended periods of drought between wet phases as well as the widely variable conditions throughout the inundation phase.

There is a wealth of literature available that quantifies the habitat requirements for large branchiopods to break dormancy (Moore 1963, Belk 1977, Brendonck 1996, Schönbrunner & Eder 2006). The information available regarding the maintenance of populations of clam shrimp mainly suggests that the size of the pools is of key importance (Kiflawi et al. 2003 & Jocqué et al. 2007b). Frequently, clam shrimp nauplii will hatch from resting eggs, but then the population will crash before they have reached maturity (Jocqué et al. 2007b). Much of the available information regarding hatching or sustaining populations is focused on the range of a single parameter (i.e., only temperature or pH; Belk 1977, Scott & Grigarick 1979, Mitchell 1990, Schönbrunner & Eder 2006). However, by combining all parameters into a multivariate BEST analysis, we showed that pool depth combined with pH best explained *P. badia* abundance in temporary outcrop pool habitats. A canonical discriminate analysis confirmed pool depth and pH as the most vital explanatory variables, while additionally implicating volume, surface area, and temperature as important habitat variables. Temperature was negatively correlated with volume, depth, and surface area, as might be expected: as pool sizes increase, maximum pool water temperatures decrease because larger pools can be more resistant to temperature change. The sampling times used in this analysis were from the warmest parts of the diurnal cycle, and thus the larger volume pools should have been more resistant to warming, explaining the negative correlation.

The environmental variables that described pool morphology were all positively correlated with one another and with CC1. Canonical correlation 1 correctly predicted which pools contained *P. badia* 81% of the time, illustrating that pool morphology was a key habitat characteristic for *P. badia* abundance. This may well be because pools that are on the more ephemeral end of the continuum evaporate before clam shrimp populations have reached sexual maturity.

The apparent association between pH and the presence of *P. badia* (Table 1) is likely an artifact of the sampling regime (i.e., pH values used in this analysis were only from the middle of the day and thus do not take into account the significant diurnal change observed; Fig. 4). It is possible that the variability of pH, like temperature is associated with the size of the pool and this is why it appears significant here. Otherwise it is not obvious why such a subtle difference in mean pH between the two groups (0.45) would have an impact on *P. badia* populations. More information is necessary to determine the significance of this observation.

Clam shrimp species previously reported from these pools were *Eulimnadia dahli* Sars, 1896 and *Paralimnadia badia* (Timms 2006, Weeks et al. 2006). Whether these
genera never coexist within the same pool or whether one succeeds the other in the same pools is still unknown. Preliminary evidence suggests that *P. badia* may predominate in pools in the winter (rainy) season, while *E. dahli* may be more common in the summer (Timms 2006).

These data represent the results of an observational and not a manipulative study and so can only suggest a potential relationship between the above variables, not necessarily a direct causal relationship. There may also be other factors beyond pool morphology that could affect the distribution of this species. It is possible that clam shrimp had not yet dispersed to the non-*P. badia* pools, although this scenario is unlikely considering the proximity of the pools to each other on each outcrop. Overflow and wind-mediated propagule dispersal have been shown to be extremely effective in temporary pool metacommunities (Brendonck & Riddoch 1999, Vanschoenwinkel et al. 2008a, Vanschoenwinkel et al. 2008b). Another possibility is that the presence of a predator excluded *P. badia* from some pools and not others, although this seems unlikely. Some clam shrimp were consumed by predacious water beetle larvae (Dytiscidae) during sampling, but this was a rare occurrence in our observations. Furthermore, in every pool in which these predacious larvae were present, *P. badia* was also present. Additionally, 81% of pools that harboured anuran larvae also had *P. badia*. Our sampling was not exhaustive enough to prove that no predator had an effect on *P. badia* populations, but simply suggests that we did not observe such a phenomenon during our sampling period.

Generally, there is thought to be a continuum of factors structuring communities in habitats ranging from permanent freshwater bodies to temporary freshwater environments (Wellborn et al. 1996). Some work has suggested that predation is the dominant structuring force in the most permanent communities while competition is more powerful in structuring pools that are less permanent (Wellborn et al. 1996, Wilbur 1987). Indeed, priority effects may play a role in early colonizers monopolizing particular pools (De Meester et al. 2002, Jocqué et al. 2010).

Another factor that may affect the habitat quality of *P. badia* is dryland salinity. Extensive land clearing in the region has resulted in increased salinities in many aquatic habitats (Anon 1996, National Land and Water Resources Audit 2001). Dust and saline solutes from salt flats around the rock outcrops are likely to be both more alkaline and also more buffered. Some outcrop pools are also showing higher alkalinity through increased bicarbonate concentrations than would be expected from rainfall interaction with acidic granite rock (Pinder et al. 2005).

Pool morphology appears to influence the presence of *P. badia* in temporary rock pools. Temperature may also be an influential factor, especially as it is correlated with the size of the pools. Many of these outcrops are already functioning as islands in an ecologival desert that is the mono-agricultural Wheatbelt landscape. Consequently, loss of these island habitats to clam shrimp at a local scale may lead to a significant threat to sustained existence for clam shrimp at a more regional scale.

ACKNOWLEDGEMENTS

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