

# Sexual reproduction and diapause of *Hexarthra* sp. (Rotifera) in short-lived ponds in the Chihuahuan Desert

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## SUMMARY

1. In the life cycle of monogonont rotifers it is generally assumed that diapausing eggs invariably hatch into amictic stem females which produce female offspring parthenogenetically. Diapausing eggs are only produced by later generations after sexual reproduction has been induced by environmental cues.
2. We show that populations of an undescribed *Hexarthra* species inhabiting small temporary ponds in the Chihuahuan Desert deviate from this life cycle pattern. These ponds may dry within days and up to 85% of females were mictic. Females producing male offspring and diapausing eggs were observed 1 or 2 days, respectively, after ponds had filled with water.
3. Under laboratory conditions, 7–46% of females hatching from re-hydrated sediments were sexual. Male offspring of these females can fertilise other mictic stem females leading to diapausing egg formation. In laboratory experiments, females produced fully developed diapausing eggs within 1.9 days at 20 °C and 1.2 days at 30 °C.
4. In addition, embryonic development time (1.1–0.3 days at temperatures between 12 and 30 °C) and juvenile period (2.1–0.5 days for the same temperature range) are shorter than those of other rotifer species. In short-lived habitats, the potential for rapid population development and production of new diapausing eggs may be crucial in the long-term survival of populations.

*Keywords:* bet-hedging, cyclic parthenogenesis, mixis, reproductive mode, temporary habitats

## Introduction

Organisms occurring in temporary aquatic environments are confronted with rapidly drying habitats that require them to disperse or persist under dry conditions. While some organisms undergo periods of quiescence, others depend on diapause for survival. Some organisms such as bdelloid rotifers, tardigrades, certain nematodes and chironomids respond directly to stressful conditions by forming resistant quiescent stages (Sømme, 1996; Ricci, 2001; Watanabe *et al.*, 2004). In many of these organisms dormancy is not restricted to a specific stage of the life cycle (Ricci,

1987; Ricci, Vaghi & Manzini, 1987; Ricci & Pagani, 1997; Nelson, 2002). In other organisms dormancy is limited to a diapausing embryonic stage. The eggs of notostracan, conchostracan and anostracan crustaceans enter diapause and survive periods of drought in temporary ponds (Dodson & Frey, 2001). Embryonic diapause is also characteristic of monogonont rotifers but generally only sexually produced diapausing eggs are able to survive adverse environmental conditions, not parthenogenetic subitaneous eggs (Gilbert, 1974, 1992). The life cycle of monogonont rotifers is heterogonic, with prevailing parthenogenesis and periodic sexual reproduction. Diapausing eggs of monogonont rotifers must be produced ahead of or during deteriorating environmental conditions. In fact it has been demonstrated in several species that diapausing eggs are produced under favourable

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conditions, when food resources are abundant and support high population densities (cf. Gilbert, 1992, 1993; Schröder, 2005).

It is generally thought that a stem female hatching from a diapausing egg is always amictic, meaning that it reproduces parthenogenetically, and gives birth to female offspring (e.g. Gilbert, 1992; Wallace & Snell, 2001; Serra, Snell & King, 2004). Only in the second or a later generation can mictic females appear, whose oocytes undergo meiosis and develop into male offspring if unfertilised or diapausing eggs if fertilised. Often production of mictic females is induced at high population density (Gilbert, 1992, 2002, 2003a, 2004; Stelzer & Snell, 2003; Schröder & Gilbert, 2004). Exclusively parthenogenetic reproduction in the first generations and mictic female production at high density imply that a minimum time is required before sexual reproduction produces new diapausing eggs. In other words, a temporary habitat which is colonised by populations of monogonont rotifer species must persist sufficiently for amictic stem females to hatch, undergo at least one generation of parthenogenesis, then produce mictic daughters that, if fertilised, produce diapausing eggs.

This monogonont life cycle involves a trade-off between parthenogenetic and sexual reproduction. Allocating resources to producing male offspring or diapausing eggs restricts the intrinsic growth rate of a lineage and decreases its frequency in the population (Serra & King, 1999). As asexual reproduction facilitates rapid colonisation when the habitat is suitable, sex should occur shortly before or when the habitat becomes unsuitable (Serra *et al.*, 2004). Therefore it may be advantageous in longer-lasting more predictable habitats for females coming out of diapause to reproduce exclusively by parthenogenesis and for the sexual reproduction of new diapausing eggs to take place later when high densities are reached. In fact, in some species mictic reproduction is blocked in the first generations after stem females hatch from diapausing eggs (Gilbert, 2002, 2003b; Schröder & Gilbert, 2004). For unpredictable habitats, the analysis of Serra *et al.* (2004) indicates that sex should occur as soon as possible, even if population densities are low. In extremely temporary habitats which may or may not dry out soon after they have filled, delaying diapausing egg production would be fatal if the habitat dries while reproduction is still exclusively amictic.

We report here on the life cycle of an undescribed species of the genus *Hexarthra* Schmarida (Hexarthriidae, Monogononta) that exhibits a unique deviation from the commonly recognised life cycle of monogonont rotifer species. This species regularly colonises very short-lived temporary rock pools (huecos) in parts of the north-western Chihuahuan Desert which are filled with water by unpredictable rainfall. *Hexarthra* is the dominant monogonont rotifer in these rock pools. Mictic reproduction is often observed within 1 or 2 days after the pools fill with water. This would be an extremely short period for one or more amictic generations to precede the first mictic generation assuming the *Hexarthra* life cycle followed the generally accepted pattern. We therefore tested the hypothesis that this rotifer's life cycle deviates from the typical pattern and that stem females hatching from diapausing eggs may reproduce sexually. Such a variation in the life cycle would allow for the fastest possible production of new diapausing eggs, in agreement with the scenario of Serra *et al.* (2004) for unpredictable habitats. Assuming that longer-lasting huecos provide a more predictable growth period for *Hexarthra* populations than short-lasting ones, we tested the hypothesis that the percentage of mictic stem females is higher in huecos that exist for shorter time periods on average. In addition we conducted laboratory experiments to test the assumption that life-history traits, such as embryonic development time, juvenile period and maturation of diapausing eggs, of this *Hexarthra* species are extraordinarily short permitting rapid population development and production of diapausing stages in ephemeral habitats.

## Methods

### Field studies

Aquatic habitats surveyed consisted of *huecos*, small water-catching depressions in the syenite porphyritic rocks at Hueco Tanks State Historic Site (El Paso Co., TX; 31°55.108'N; 106°02.478'W). These aquatic habitats may exist only for very short periods. The rock pools are filled with water by rain and may be dry again within a few days. This is especially true in the summer months when air temperatures can exceed 30 °C and rock pools may be dry again by as little as 1 day after they filled. During winter, when air temperatures typically do not exceed 20 °C during

the day, the rock pools may be filled with water for periods of 4 or more weeks.

Water samples were taken from the huecos within 24 h of rainfall in August and December 1998. Physical data including pond dimensions, temperature and pH were then recorded daily while the ponds persisted. A grab sample of 50–200 mL (according to pond size) was collected from each hueco on a daily basis starting the day after a rainfall until it was dry. Samples were brought to the laboratory in coolers and examined under a dissecting microscope. All females found were counted and categorised as female-producing, male-producing or diapausing egg producing, depending on whether they carried small male eggs, larger female eggs or whether a single diapausing egg was developing in the female's oviduct. When population densities became high, subsamples of 50 mL were counted. Females not carrying eggs were placed into individual wells of tissue culture plates containing about 0.4–1 mL of filtered (Millipore, 45 µm) Ozarka® spring water (Nestlé Waters North America Inc., Greenwich, CT, U.S.A.) with *Chlamydomonas reinhardtii* Dangeard (UTEX strain 90) as food. Females were monitored until they produced offspring or died.

#### *Determination of juvenile period, egg development time and production of diapausing eggs*

As *Hexarthra* females could only be maintained on standardised medium and food for a few days and a long-term culture of the species under laboratory conditions was not possible, the following experiments were carried out with unfiltered water taken from the species' natural habitat and from which all rotifers were removed. Algal cell counts were not taken at the time of collection, although chlorophyll *a* content was recently determined using EPA method 446.0 (U.S. Environmental Protection Agency, 1997); values for huecos range between 2 and 33 µg L<sup>-1</sup> total chlorophyll *a* and between 2 and 18 µg L<sup>-1</sup> chlorophyll *a* in the size fraction <20 µm.

Ovigerous females (>100) were collected from each of two huecos (Heart and Luisa's). Their offspring were used for the experiments. Individual newborn females (1–1.5 h old) were placed in tissue culture plate wells filled with about 1.5 mL water taken from the hueco. The rotifers were transferred to fresh unfiltered hueco water daily. Two sets of experiments (both at 12:12 day length) were conducted, the first at

12 and 25 °C (21 and 28 females, respectively), and the second at 20 and 30 °C (52 and 65 females, respectively). Culture plates were checked every 2 h when egg extrusion and hatching of offspring were recorded. Juvenile period was calculated as the time from birth to the deposition of the first egg. Embryonic development time was calculated as the time from egg extrusion to hatching. Data for embryonic development were fitted to the Bělehrádek (1935) function by nonlinear curve fitting using the Levenberg Marquard algorithm implemented in the ORIGIN 5.0 software package (Origin Lab Co., Northampton, MA, U.S.A.).

When the second set of experiments was conducted, water samples contained a high number of males, as mixis had occurred in the population at the time of collection. As sufficient numbers of males were present, we timed the production of diapausing eggs during these experiments. Ovigerous amictic females were placed in dishes with unfiltered water containing *Hexarthra* males but no additional females. For this reason hatching mictic neonates were likely to be fertilised in these dishes. After 2 h, neonates were collected and placed individually into tissue culture plates wells filled with 1.5 mL pond water. We observed that fertilised mictic *Hexarthra* females produce a single diapausing egg which is released when the female dies. The growing oocyte becomes clearly visible as a bright orange colour under a dissecting microscope, as it is supplied with large amounts of yolk from the vitellarium. When the diapausing egg matures and the shell is secreted, the colour changes from orange to dark brown. We determined the time taken for mictic females from hatching and subsequent fertilisation to produce mature diapausing eggs, based on the time when the colour of the egg shell turned from orange to dark brown. This change in colour during the ontogeny of the egg has been consistently observed in these populations over a 12-year period.

#### *Determination of mictic and amictic stem females hatching from diapausing eggs in different huecos in laboratory experiments*

About 4–10 g of fine sediment from each of five huecos was flooded in large Petri dishes with about 150 mL filtered spring water. Petri dishes were kept at 20 °C and checked every 24 h for hatching stem

females. These females were individually transferred to tissue culture plate wells filled with 1.5 mL water taken from the flooded sediment samples. Animals were transferred to fresh culture water daily and cultured until they produced eggs and could be typed as amictic or mictic. The proportion of mictic and amictic females among hatching stem females was calculated for each hueco. Differences among the huecos in the ratio of hatching mictic and amictic stem females were analysed using a log-likelihood ratio test (JMP statistical software package, SAS Institute, 2001). The mictic ratio of hatching stem females was plotted against habitat existence, which we calculated for each hueco as the percentage of all sampling dates over a 6-year period when water was present in a given hueco.

## Results

### Field studies

Immediately following a rainfall event in the summer, *Hexarthra* populations were established in seven huecos (Fig. 1a). The huecos held water for a maximum of 7 days before drying. In these graphs, only females whose reproductive status was determined are included. In some cases, a substantial number of females (0–67%) could not be assigned a reproductive category because they died without producing an egg. In two of the huecos (Pia's and Luisa's) amictic *Hexarthra* females were found within 24 h of the rainfall, and in Luisa's approximately 47% of the females were unfertilised mictic females. The remaining huecos developed detectable *Hexarthra* populations after 3–5 days. Four of the huecos (Pia's, Clammation, Jeff's, Stacia's) maintained high proportions of amictic females ranging from 57% to 81% on the day of drying. All populations contained fertilised mictic females before the huecos dried, indicating that diapausing eggs were produced in sufficient time to contribute to egg banks in the pools.

Following the winter rainfall event (Fig. 1b), a *Hexarthra* population was established only in Stacia's hueco. Again within 24 h, amictic females were observed. One day later, unfertilised and fertilised mictic females comprised approximately 21% of the population. Their proportion in the population increased up to 52% during the 30 days the pond was filled.

### Determination of juvenile period, egg development time and production of diapausing eggs

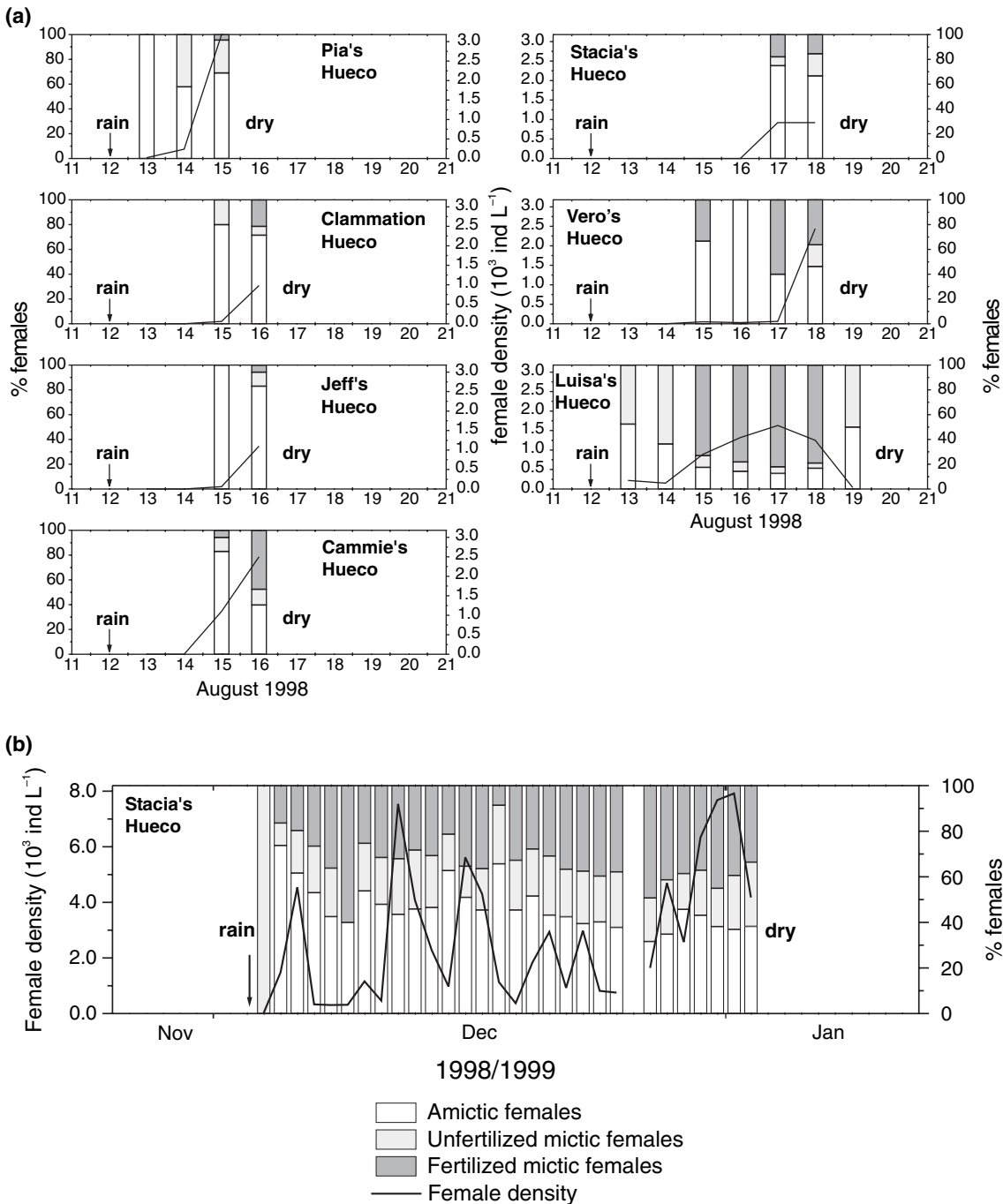
The juvenile period of *Hexarthra* females (i.e. the time that females require from hatching until they produce their first eggs) ranged from  $2.1 \pm 0.04$  days (mean and 95% confidence interval,  $n = 14$ ) at 12 °C to  $0.5 \pm 0.04$  days (mean and 95% confidence interval,  $n = 12$ ) at 30 °C (Fig. 2). Female subitaneous eggs developed and hatched within  $1.1 \pm 0.08$  days (mean  $\pm 1$  SE,  $n = 4$ ) at 12 °C and within  $0.3 \pm 0.01$  days (mean  $\pm 1$  SE,  $n = 15$ ) at 30 °C (Fig. 3). This resulted in generation times ranging between 3.2 days at 12 °C and 0.8 days at 30 °C. Mature amictic females carried  $1.8 \pm 0.06$  eggs (mean  $\pm 1$  SE,  $n = 84$ ). In contrast, unfertilised mictic females carried a large number of male eggs, ranging from 1 to 20. We found  $5.5 \pm 0.29$  mictic eggs per female (mean  $\pm 1$  SE,  $n = 159$ ). Mictic females that were fertilised after hatching produced a single diapausing egg which was released after death. Diapausing eggs required  $1.9 \pm 0.04$  days at 20 °C (mean  $\pm 1$  SE,  $n = 47$ ) and  $1.2 \pm 0.04$  days at 30 °C (mean  $\pm 1$  SE,  $n = 41$ ) to develop fully.

### Determination of mictic and amictic stem females hatching from diapausing eggs in different huecos

The percentage of mictic stem females hatching from diapausing eggs in laboratory experiments for five huecos is given in Table 1. The percentage of mictic stem females ranged from 6.7% to 45.5% of all stem females. The differences in the mictic ratios of hatching stem females among the huecos were significant (log-likelihood ratio test,  $\chi^2_{5,175} = 17.86$ ,  $P = 0.003$ ). A plot of the mictic ratio of hatching stem females against habitat existence showed no significant correlation (linear regression,  $R^2 = 0.488$ ,  $P = 0.19$ , Fig. 4). Stem females started to hatch from flooded sediment samples after <24 h at 20 °C. In a separate experiment at 12 °C, hatching did not start until 2.5 days after the sediment samples were flooded (T. Schröder and E.J. Walsh, unpublished data). At both temperatures hatching continued over the next 2–3 days.

## Discussion

This study is the first to report that mictic stem females of monogonont rotifers can be a natural and



**Fig. 1** (a) Reproduction of *Hexarthra* populations immediately following summer population establishment in seven huecos at Hueco Tanks State Historic Site, El Paso Co., TX. (b) Reproduction of the *Hexarthra* population immediately following winter population establishment in Stacia's Hueco. Bars indicate the proportion of amictic and mictic females (fertilised and unfertilised) and line graphs the density of all females in the huecos.

common part of the life cycle. The only other study with indirect evidence that stem females can produce male offspring was inconclusive (Bogoslovsky, 1958). He observed solitary larvae of the colonial species

*Sinantherina socialis* Linnaeus (1758) which were amphoteric (producing both female and male offspring) upon maturity. He concluded that these solitary larvae must have hatched from diapausing

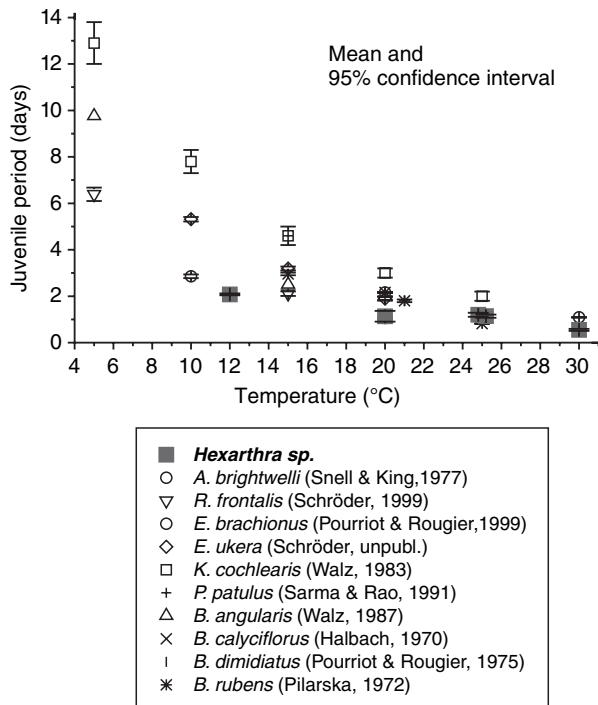


Fig. 2 Juvenile period of *Hexarthra* females at different temperatures in comparison with other rotifer species.

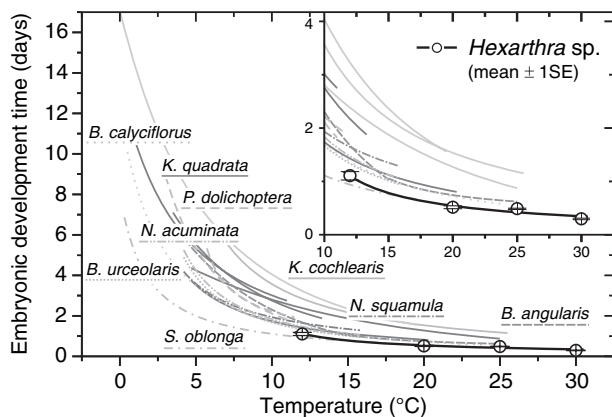


Fig. 3 Embryonic development times of *Hexarthra* at different temperatures in comparison with other rotifer species. Data for other species are taken from Halbach (1970), Herzig (1983), Rigler, MacCallum & Roff (1974), Schröder (1999), Walz (1983, 1987) and Zoufal (1989).

eggs, as he observed that subitaneous female offspring would form larval colonies before separating from their mother. However, R. Rico-Martinez & E.J. Walsh (unpublished data) followed the entire life cycle of *S. socialis* in laboratory cultures and found that larval offspring may leave their mothers either as

Table 1 Reproductive status of females hatching from sediments flooded in laboratory experiments. The sediments were collected from five huecos

Hueco	% mictic stem females	% amictic stem females	n
Luisa's	33.3	66.7	21
Stacia's	45.5	54.5	33
Vero's	30.8	69.2	39
Shelby's	6.7	93.3	30
Jamie's	9.5	90.5	21

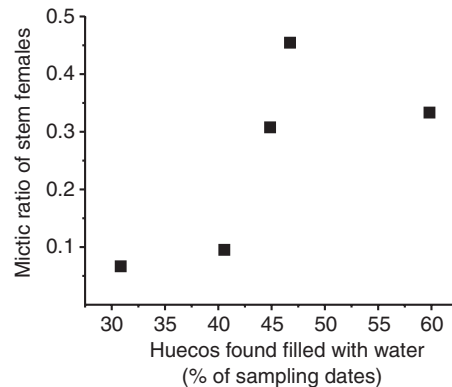


Fig. 4 The mictic ratio of hatching stem females plotted against habitat existence (measured as the percentage of sampling dates on which the respective huecos were filled with water).

single individuals or as larval colonies formed with siblings. Therefore the conclusion of Bogoslovsky (1958) that solitary larvae must have hatched from diapausing eggs is not necessarily valid.

To our knowledge, mictic females hatching from diapausing eggs have only been directly observed in a few instances in two laboratory-cultivated strains of *Brachionus calyciflorus* Pallas 1776 (Gilbert & Schröder, 2004). In each case the stem female produced only one or two male offspring and died thereafter. The net reproductive rate  $R_0$  of unfertilised mictic *B. calyciflorus* females usually ranges from 10 to 22 male offspring depending on food quantity and quality (Galindo & Guisande, 1993; Xi, Huang & Jin, 2001). Therefore, it seems likely that the appearance of mictic stem females in these *B. calyciflorus* strains is not a typical feature of the life cycle but rather a developmental anomaly. However, in the *Hexarthra* species inhabiting the Chihuahuan Desert rock pools, mictic stem females made up to 45.5% of the females hatching from diapausing eggs in the laboratory

experiments. Therefore, the hatching of mictic stem females from diapausing eggs must be considered a regular component of the life cycle.

Life-history traits of this *Hexarthra* species are adapted to short-lived habitats. As shown in Fig. 3, female embryonic development times are the shortest known among monogonont rotifers (see references in Fig. 3). Also, the juvenile period of females is shorter than almost any other known for monogonont rotifers (Fig. 2 and references therein). These comparisons have to be made with caution as data in the literature are for standardised food conditions, in contrast to ours. In our experiments, life-history parameters are probably more representative of natural conditions than data gained from standardised food conditions, as the source water contained a variety of food items. However, it may be that naturally available food is not the optimal diet for the species. If food conditions were sub-optimal, both life-history parameters we measured may be even shorter under optimal conditions, in particular the juvenile period. Rapid embryonic development combined with a short juvenile period contributes to rapid population growth through asexual reproduction. As population densities increase, mixis in response to crowding may also increase. In addition, continuous hatching of diapausing eggs could have also contributed to population growth in the huecos. It is not possible to separate diapausing egg hatching from population growth through parthenogenetic reproduction in field populations.

As shown in Fig. 1a, population densities in the huecos can reach 1000–3000 L<sup>-1</sup> in only 3–4 days. High population density is one of the factors known to induce sexual reproduction and, hence, the production of new diapausing eggs in monogonont rotifers as demonstrated for *B. calyciflorus* (Gilbert 2003a, 2004), *Epiphanes brachionus* Haring 1913 (Pourriot & Rougier, 1999) *B. plicatilis* O. F. Müller 1786 (Stelzer & Snell, 2003), *B. angularis* Gosse 1851, *E. senta* Müller, 1773, and *Rhinoglena frontalis* Ehrenberg, 1853 (Schröder & Gilbert, 2004). However, production of male offspring in the *Hexarthra* population could be observed as early as 1 day after the huecos were flooded by rain in the summer, and diapausing egg production took place as early as 2 days after the pools filled with water, as observed in winter 1998. Also, mictic females were present at very low population densities at both times (Fig. 1a,b).

It seems probable that the mictic females that produced male offspring and diapausing eggs in the huecos during the first days after flooding in the summer were in fact stem females. In our experiments we observed mictic stem females hatching from sediments which were not fertilised as males were not present in the dishes. However in nature, mictic stem females should theoretically be able to produce diapausing eggs if they were fertilised by males in the population. The winter field data (Fig. 1b) also support this assumption, as the first fertilised mictic females had already appeared in the population on the second day after the hueco was filled by rain. It seems unlikely that these fertilised females were of the second or subsequent generations, particularly as water temperature was 13–15 °C, for which our experimental data indicate a generation time of 1.5–2 days. The high number of male offspring produced by mictic females should lead to rapid fertilisation of mictic stem females and the production of diapausing eggs at an early stage. The mean egg ratio of unfertilised mictic *Hexarthra* females is about two to three times greater than that in other species. Maximum egg ratios of mictic females from other species range between three and ten eggs per female (T. Schröder, unpublished data), in contrast to *Hexarthra* where the maximum was 20 eggs per female. Thus, the male offspring of the first mictic stem females should very quickly reach high density and increase the chances that later hatching mictic stem females will be fertilised and produce diapausing eggs.

Under natural field conditions, *Hexarthra* are able to produce diapausing eggs almost immediately following a rainfall event (Fig. 1a,b), thus replenishing and/or restoring the egg bank for future population development. These observations are substantiated by our laboratory experiments. In these experiments, it took <1 day for stem females to start hatching from diapausing eggs, 0.5–1 day for females to reach maturity, 0.5–1 day for the first offspring to hatch and 1–2 days for diapausing eggs to develop fully. Such a rapid production of new diapausing eggs seems unlikely if the first generations are exclusively amictic. Serra *et al.* (2004) calculate that the period for *B. calyciflorus* from hatching of stem females to the formation of diapausing eggs is at least 2 weeks under optimal conditions at a temperature of 25 °C.

During winter, when lower temperatures prevail, the production of new diapausing eggs might take

longer, but the rock pools also persist for longer. It is unlikely that diapausing eggs in *Hexarthra* populations are entirely produced by mictic stem females, however. Particularly in the winter, but also in the summer when larger huecos are filled for longer periods, high proportions of mictic females were continuously observed over days to weeks in the populations at densities of more than  $10^3 \text{ L}^{-1}$ . The majority of these mictic females must be the offspring of amictic mothers, as it is improbable that such a high number of mictic females making up around 60% of the total population would continuously hatch from the sediments. It is unknown whether environmental signals induce the production of mictic offspring by amictic females in *Hexarthra*. It is possible that the proportion of a female's diapausing eggs hatching into mictic and amictic stem females is determined genetically, as well as the proportion of mictic and amictic offspring produced by parthenogenetic females.

Theory predicts that production of diapausing eggs in predictable environments should be delayed until, or just before, conditions deteriorate (King, 1980; Serra & King, 1999). In fact, the delay in mixis that has been observed in populations of several rotifer species (Gilbert, 2002; Schröder & Gilbert, 2004) prevents mixis in the first generations after stem female hatch, and has been shown to be an evolutionarily stable strategy (Serra, Snell & Gilbert, 2005). In the populations sampled here, *Hexarthra* uses a mixed strategy to produce new generations. A certain proportion of a clone's diapausing eggs hatches into stem females which are mictic and produce new diapausing eggs shortly after the rock pools fill. Other stem females reproduce asexually contributing to high population densities which may induce and facilitate sexual

production of diapausing eggs. This combination of strategies increases the probability of producing a viable egg bank for future population development.

Although the mictic ratio of hatching stem females in the sediment experiments was significantly different among huecos, we did not find a significant correlation of mictic ratio to the proportion of sampling dates that the rock pools were filled with water. This is not in agreement with the assumption that subpopulations in each of the huecos are adapted to different durations of habitat existence and that more investment in early production of new diapausing eggs could be observed in those huecos that tend to dry out sooner. The huecos studied are all within close proximity to each other (Table 2), and it seems possible that diapausing eggs are easily dispersed among the huecos by wind when the pools are dry. If gene flow among huecos prevents local adaptation, no significant differences in life-history traits would be expected among the huecos. The reasons for the observed differences remain unknown. It is possible that other factors might have effects that confound the influence of habitat existence. More importantly, variation in the frequency and amount of precipitation is great in the Chihuahuan Desert due to localised summer monsoon rains, so that habitat persistence is unpredictable. On some occasions even large huecos can be filled only partially by short rainfalls and then dry out very quickly, while they may last for several weeks when filled completely. Such unpredictability should favour higher percentages of diapausing eggs in the egg bank hatching into mictic stem females. This study demonstrates the flexibility of the rotiferan life cycle in response to environmental heterogeneity and, as more species from a greater variety of habitats are studied in greater detail, other alternative

**Table 2** Physical characterisation of selected huecos at Hueco Tanks State Historic Site (El Paso, Co., TX)

Hueco	Maximum dimensions (l × w × d) (m)	Season	Average water temperature (°C)	Distance to nearest neighbour (m)	Days with water	Days with rotifers
Stacia's	1.75 × 1.40 × 0.08	Aug.	27.8	2.98	6	2
Stacia's	2.05 × 1.60 × 0.11	Dec.	11.7	2.98	29	29
Luisa's	2.80 × 2.10 × 0.07	Aug.	25.2	3.63	7	7
Pia's	2.65 × 2.30 × 0.04	Aug.	28.5	18.35	3	3
Cammie's	1.55 × 1.00 × 0.06	Aug.	29.0	0.36	4	2
Clammation	1.53 × 1.00 × 0.05	Aug.	30.6	0.01	4	2
Vero's	3.00 × 1.90 × 0.08	Aug.	28.6	2.73	6	4
Jeff's	0.65 × 0.65 × 0.05	Aug.	28.8	0.55	4	2

strategies in the timing of mixis and diapause are likely to be found.

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