Rotifers from diapausing, fertilized eggs: Unique features and emergence

John J. Gilbert and Thomas Schröder
Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755

Abstract

Sexual reproduction in cyclically parthenogenetic rotifers results in the production of diapausing, fertilized (resting) eggs, which can survive for decades in sediment egg banks. Stem females hatching from these eggs can differ from genetically identical ones produced in subsequent parthenogenetic generations. Differences involve the inhibition of sexual reproduction, which may persist for many parthenogenetic generations; reduced morphology (apterous *Polyarthra,* shorter-spined *Brachionus*); and larger stores of lipid. These features of stem females may facilitate colonization by favoring population growth via female parthenogenesis and by decreasing food requirements for survival and reproduction. Fertilized eggs in dried sediments probably hatch soon after being flooded by rising water levels. Eggs in permanently submerged sediments may be in environments that inhibit hatching (e.g., darkness, low water temperature, low dissolved oxygen) and hatch only when brought to the sediment surface or resuspended into the water column by currents or bioturbation. Laboratory experiments show that the hatching of diapausing, fertilized eggs can require light and temperatures suitable for population development or specific changes in temperature consistent with a new growing season. The limited data that exist on their emergence from fertilized eggs in natural systems come from sediment traps, from the occurrence in the water column of apterous *Polyarthra* or empty egg shells, and from calculations of negative mortality rates of planktonic populations. Fertilized eggs often hatch at the beginning of the growth season but may continue to hatch intermittently thereafter. The extremely high genotypic diversity of some natural rotifer populations indicates extensive hatching of stem females from the sediment egg bank.

In cyclically parthenogenetic monogonont rotifers, diapausing eggs (often called resting eggs) are formed during periods of bisexual reproduction. Sexual periods occur when specific environmental stimuli induce amictic (female producing) females to produce some mictic-female offspring. Mictic females lay haploid eggs that develop parthenogenetically into males, if unfertilized, or into diapausing female embryos, if fertilized. Fertilized eggs are laden with dark-colored yolk and surrounded by a multilayered shell, and they can survive for decades in diapause before resuming development and hatching. This life cycle, including the environmental induction of mictic females, has been reviewed (Gilbert 1974, 1977, 1992, 1993; Pourriot and Snell 1983; Ricci 2001).

Environmental triggers for the initiation of sexual reproduction, and hence the production of diapausing eggs, have been extensively investigated in several species for many years but are not considered in this review. However, several general points are important. First, the type of signal that induces the production of mictic females can differ across genera. It can be high population density in *Brachionus* (Gilbert 2002, 2003*a,b*, unpubl. data; Stelzer and Snell 2003), *Epiphanes* (Pourriot and Rougier 1999; Schröder and Gilbert unpubl. data), and *Rhinoglena frontalis* (Schröder and Gilbert unpubl. data). The density cue is known to be a chemical in *Brachionus plicatilis* (Carmona et al. 1993; Stelzer and Snell 2003) and taxonomically specific in *Brachionus calyciflorus* (Gilbert 2003*b*). Other primary stimuli are long photoperiods in *Notommata* and *Trichocerca* (Pourriot 1963; Pourriot and Clément 1975) and dietary tocopherol (vitamin

E) in *Asplanchna* (Gilbert and Thompson 1968; Gilbert 1980*a,b*; Gilbert and Confer 1986). In most rotifers, the environmental signal that induces mictic females has not been identified.

A second general aspect of the life cycle is that there is a trade-off between rapid population growth via female parthenogenesis and the production of mictic females, males, and diapausing eggs during sexual reproduction (Snell 1987; Serra and Carmona 1993; Serra and King 1999; Gilbert 2002). In theoretical considerations that deal with the timing of sexuality in the rotifer life cycle, it is critical to focus on the environment at the time diapausing, fertilized eggs are produced rather than on the future environment in which these eggs hatch (Gilbert 1980*a*; Pourriot and Snell 1983). Models show that the optimal time and extent of sexual reproduction depend on the density dependence of population growth and on the predictability and carrying capacity of the environment (Hairston and Munns 1984; Snell 1987; Serra and Carmona 1993; Serra and King 1999; Ricci 2001; Spencer et al. 2001). These models indicate that production of mictic females sometimes should be delayed until the onset of deteriorating environmental conditions (Serra and King 1999). Alternatively, it has been suggested that sexual reproduction should occur during conditions favorable for population growth so that large numbers of young mictic females can be fertilized by males and produce many energy-rich diapausing eggs for a sediment egg bank (Gilbert 1974, 1980*a,* 1993; Snell and Garman 1986). In this strategy, the point is to produce many diapausing eggs whenever possible, rather than to wait and produce them at the end of the growing season when food availability and population size may have decreased.

There is no experimental evidence showing that mictic females are directly induced by unfavorable conditions. In fact, the tendency to produce mictic females in *B. plicatilis*

is higher when environmental conditions (e.g., food concentration, temperature, salinity) are good for population growth (Snell 1986) and when population-growth rates are high (Snell and Boyer 1988). Similarly, induction of mictic females by crowding in *Brachionus* spp., *Epiphanes senta,* and *R. frontalis* occurs without any decrease in fecundity (Gilbert 2002, 2003*b,* unpubl. data; Schröder and Gilbert unpubl. data). Clearly, the situation is different in *Daphnia,* where fertilized ephippial eggs are induced, at least in part, by low food availability within or across generations (Kleiven et al. 1992; Alekseev and Lampert 2001; LaMontagne and McCauley 2001). In rotifers, the only known case of poor environmental conditions directly inducing diapause involves parthenogenetic eggs of *Synchaeta pectinata* (*see below*).

A third general point about the rotifer life cycle is that sexual reproduction may be followed by a decline in population growth. The decline may result from the commitment to produce males and diapausing eggs. If so, it should be proportional to the probability of amictic females producing mictic daughters. This mictic rate is highly variable in both laboratory and natural populations but usually is <0.6 (Snell 1987; Carmona et al. 1995; Gilbert 2002, 2003*b*; Schröder and Gilbert unpubl. data). Pourriot (1965), however, noted that natural populations of *B. calyciflorus* and *Conochiloides natans* disappeared from the plankton after a sexual period in which all females were mictic. Alternatively, a population decline after a sexual period could reflect environmental factors that reduce the birth or survival rates of the amictic females in the population.

The fertilized egg is the only known diapause stage in the life cycle of most monogononts. Only two other diapause stages have been documented, each in only one species. For a population of *Keratella hiemalis,* eggs that had the appearance of being fertilized eggs (with dark-colored yolk and a multilayered shell) were produced parthenogenetically and called pseudosexual eggs (Ruttner-Kolisko 1946). In some strains of *S. pectinata,* starved or food-limited amictic females produce diapausing eggs in addition to typical, subitaneous eggs (Gilbert 1995, 1998; Gilbert and Schreiber 1998). These diapausing amictic eggs have a slightly thicker shell than the subitaneous eggs, but they differ from fertilized resting eggs in having a single shell layer, few (two to eight) nuclei, and no dark yolk (Gilbert 1995).

This review focuses on two largely ignored aspects of rotifers that hatch from fertilized eggs. First, these stem females, which initiate clonal lines via female parthenogenesis, can differ in several ways from genetically identical females produced in subsequent parthenogenetic generations. Differences involve morphological and physiological features that may facilitate colonization. Second, the time during the season when stem females hatch from fertilized eggs in the sediment is largely unknown. This information is critical for understanding seasonal patterns of abundance and genotypic variation in natural populations (King and Serra 1998). Factors that induce hatching in the laboratory are summarized, and some ideas about hatching in natural systems are presented and considered with the scarce data available.

Unique features of stem females hatching from diapausing, fertilized eggs

Introduction–Some old and new observations discussed in detail below demonstrate that females hatching from fertilized eggs can differ dramatically from their mothers and from genetically identical females produced in later parthenogenetic generations. In some rotifers, stem females are structurally reduced. In most rotifers, stem females are physiologically distinct in that they are exclusively amictic. In some rotifers, repression of the mictic-female phenotype extends into several or many subsequent generations of parthenogenetic reproduction. Finally, the relatively large amount of lipid reserves observed in the stem females of some rotifers may be a widespread phenomenon in monogonont rotifers. Although the ecological significance of the unique features of stem females is not always clear, some if not all of them certainly have the effect of maximizing reproduction via female parthenogenesis.

Morphology of Polyarthra—Nipkow (1952, 1961) was the first to demonstrate that the fertilized eggs of *Polyarthra* hatch into structurally reduced females, which produce daughters of the typical phenotype. In some species (e.g., *Polyarthra dolichoptera, Polyarthra remata*), the reduced form lacks only the paddlelike appendages, or fins, so characteristic of the genus (Fig. 1A). These specific forms, first described by Hood (1893), are called *aptera* (Fig. 1B). In other species (e.g., *Polyarthra euryptera, Polyarthra major, Polyarthra vulgaris*), the digestive system of stem females is also reduced. These specific forms are called *aptera reducta.*

Aptera reducta forms are similar in the different species (Nipkow 1952, 1961). The stem female produces just a single subitaneous egg, and this egg is produced before she hatches (Fig. 1C). Thus, much of the yolk from the fertilized egg has already been diverted to the next generation. When she hatches, her body cavity is almost completely filled by the egg inside the oviduct (Fig. 1D). The female lacks a functioning mastax, stomach, and gut, and her vitellarium is greatly reduced. The corona consists only of a simple circumapical ciliary wreath with a ventral gap, and the palps are missing. Shortly after the *aptera reducta* female hatches, the subitaneous egg is released from the oviduct and attached to the cloaca by a thread. This egg is almost as large as the mother (Fig. 1E). Within a few hours, the offspring, a *typica* form, hatches from the subitaneous egg. Thus, the period from the hatching of the stem female to the hatching of the second generation is shortened considerably. In comparison, at the same temperature, it takes 6 d from hatching for an *aptera* form of *P. dolichoptera* to produce its first specific *typica* form of offspring (Nipkow 1952).

The *aptera* phenotype may simply reflect a developmental constraint for the embryo of the fertilized egg, or it may have some unknown significance. The absence of paddles may allow more energy to be channeled into reproduction. Any costs for their development and maintenance would be avoided. Also, after hatching, females would not have to spend the considerable energy probably involved in spon-
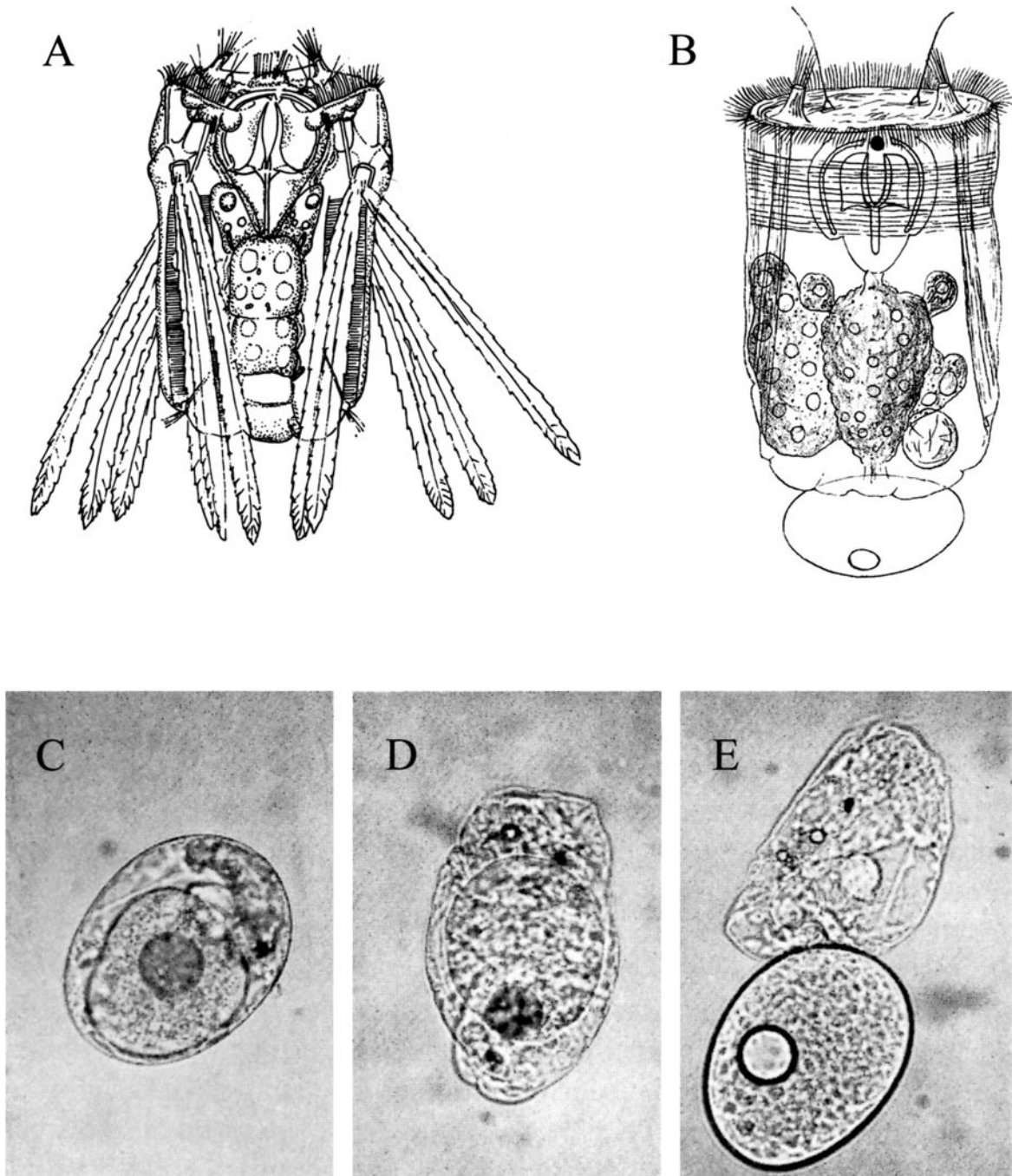
Fig. 1.   Typical and morphologically reduced forms of *Polyarthra.* (A) Typical form of *P. dolichoptera* (from Hollowday, in Koste 1978). (B) *Aptera* form of *Polyarthra* sp. (from Hood 1893). (C–E) *Aptera reducta* form of *P. vulgaris* (from Nipkow 1952); female still within shell of fertilized egg (C), recently hatched (D), and after ovipositing its single amictic egg (E). Female and egg (E) are 80–90 and 74 μm in length, respectively.

taneous or induced escape responses effected by paddle movements (Gilbert 1985; Kirk and Gilbert 1988). The trade-off, of course, is that *aptera* females would be more vulnerable to some invertebrate predators and competitors. For example, *Polyarthra*'s erratic and extremely rapid escape response is an effective defense against predation by *Asplanchna* (Gilbert 1985) and copepods (Williamson and Butler 1986; Williamson 1987; Williamson and Vanderploeg 1988) and against interference from *Daphnia* (Gilbert 1987, 1988). Experiments conducted by Magnien (1983) demonstrated that the *aptera* form of *P. dolichoptera* was very susceptible to predation by *Asplanchna girodi,* whereas the *typica* form was not.

It is also difficult to explain the evolution of the *aptera reducta* form, which produces one typical offspring shortly after hatching. If the *aptera* form reflects a developmental

constraint and if the energetic benefits of this form do not exceed the survival cost, then the *aptera reducta* form could be a device to decrease exposure of the susceptible stem female to predators. Alternatively, the *aptera* form may have originally evolved in the genus as a mechanism to conserve energy in an environment with low predation risk. If so, the *aptera reducta* form may have evolved in descendant species in environments with greater predation risk.

Spine development in Brachionus—A less dramatic example of body reduction in stem females occurs in *B. calyciflorus.* A kairomone from *Asplanchna* can induce the development and elongation of spines in this species, particularly the posterolateral spines (Gilbert 1999). In the absence of the *Asplanchna* kairomone, these spines may be completely lacking in some clones or long in other clones. Longer spines can be produced at low temperatures and food concentrations (Halbach 1970; Stemberger 1990). The length of the anterior and posteromedian spines increases with that of the posterolateral spines.

Some clones of a strain of *B. calyciflorus* from Piedmont Park Pond (Atlanta, Georgia) have long posterolateral spines when cultured in the absence of any predator and at a high temperature (20°C) and food concentration (2×10^4 cells ml^{-1} of *Cryptomonas erosa*) (Fig. 2A; Gilbert unpubl. data). However, stem females that hatch from fertilized eggs never have posterolateral spines, even when they prove to have the genotype for them by producing them in the next generation under the same conditions (Fig. 2B; Schröder and Gilbert unpubl. data).

Stem females of *B. calyciflorus* probably always lack posterolateral spines, even if fertilized eggs are produced under environmental conditions that promote their development, such as the presence of *Asplanchna* kairomones or low temperatures. Both Wesenberg-Lund (1930) and Nipkow (1958) noted that females hatching from fertilized eggs produced in nature always lacked posterolateral spines.

The ecological significance of reduced spine development in stem females of *B. calyciflorus* is unclear. The morphological reduction, like that in the *aptera* form of *Polyarthra,* may be either a developmental constraint or a cost-saving adaptation permitting greater reproduction. However, two studies were unable to detect any cost associated with the development and presence of long posterolateral spines (Gilbert 1980*c,* 1999; Stemberger 1990).

The absence of posterolateral spines observed in stem females of *B. calyciflorus* does not occur in some other, spinous congeners. For example, in an Australian strain of *Brachionus quadridentatus,* females hatching from some fertilized eggs had pronounced posterolateral spines (Gilbert 2001). Further research needs to be conducted on a variety of rotifers with body spines to determine if stem females lack spines or have shorter spines than those produced in the next generation(s) under the same conditions.
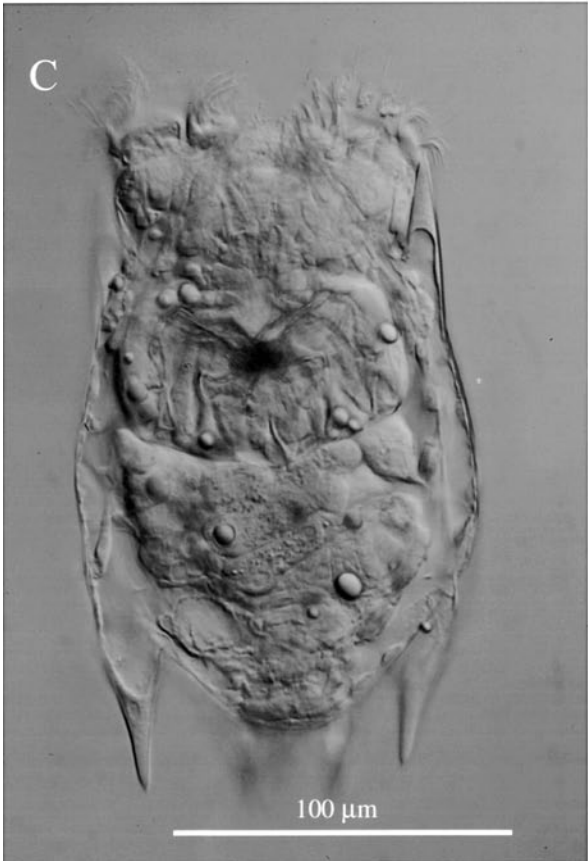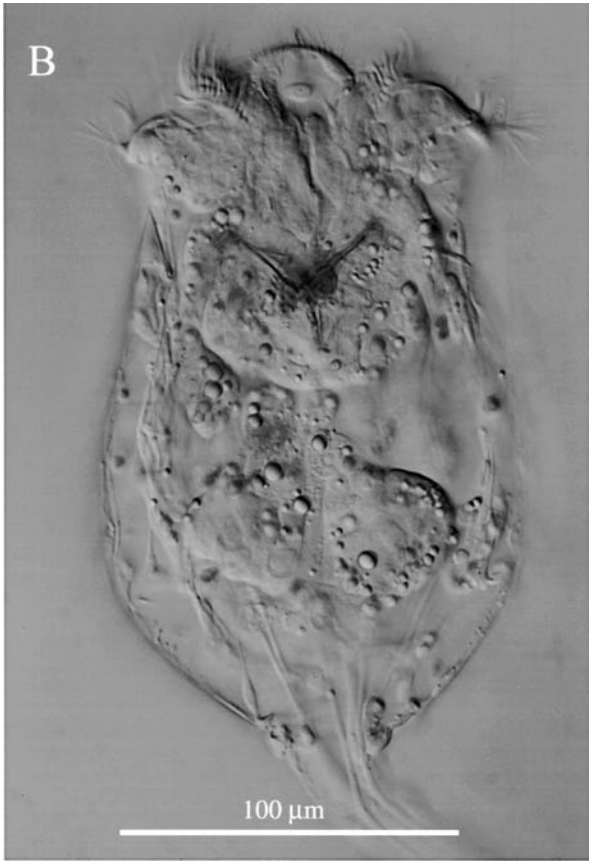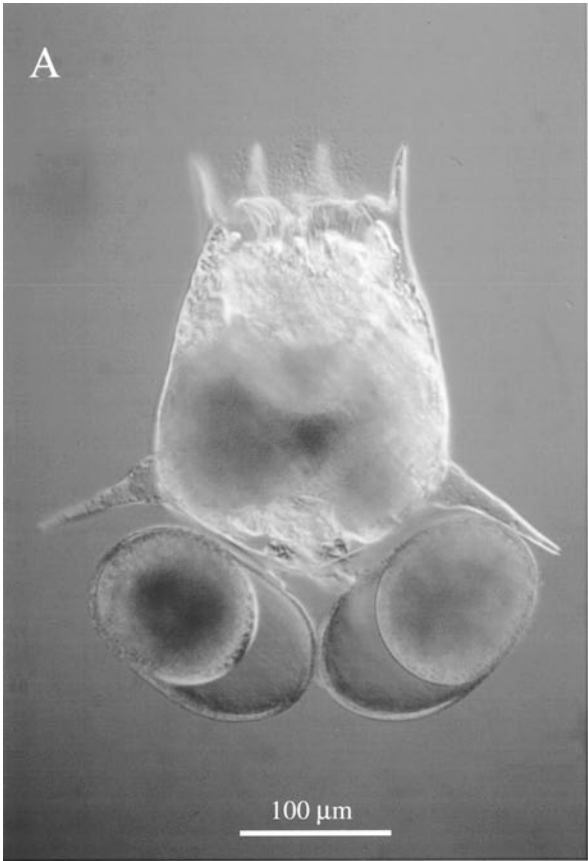
Female type—Stem females from fertilized eggs typically are amictic, even though their mictic mothers obviously developed under environmental conditions that induced the mictic-female phenotype. Indeed, it has been considered a rule of the rotifer life cycle that fertilized eggs hatch into amictic females (Wesenburg-Lund 1930; Ruttner-Kolisko 1974; Gilbert 1983). However, some exceptions have been noted recently. In each of two strains of *B. calyciflorus,* one from Piedmont Park Pond and another from a pond on the University of Florida campus in Gainesville, one or more fertilized eggs were observed to hatch into mictic females producing male offspring (Gilbert and Schröder unpubl. data). Hood (1893) reported another possible exception. He found some apterous *Polyarthra* carrying a single fertilized egg or a cluster of male eggs. Since apterous forms are known to hatch only from fertilized eggs (*see above*), these mictic females probably hatched from such eggs. Alternatively, it is possible that mictic females developing parthenogenetically might have been apterous; this, of course, would also be exceptional. Finally, the fertilized eggs of *Hexarthra* sp. from small, short-lived rock pools in the Hueco Tanks State Historic Site (El Paso, Texas) seem able to hatch into mictic females. In seven of these pools, males were observed within 1 d after the dried basins flooded (Walsh, pers. comm.). Such a rapid appearance of males can only be explained if the eggs hatched into mictic females.

The fact that female rotifers hatching from fertilized eggs in most habitats generally are amictic probably is an important life-cycle strategy. It enables them to establish clonal populations via female parthenogenesis, and thus to colonize the habitat for at least one generation, before committing to sexual reproduction. If stem females were mictic, they could produce female offspring only if they were fertilized by males from another clone, and these offspring would be diapausing embryos.

The mechanism that prevents fertilized eggs from developing into mictic females is not known. It has been suggested that the vitellarium of a fertilized mictic mother produces a factor in its yolk that is transferred to the oocytes and that represses the mictic-female phenotype (Gilbert 2002). In situations where mictic females do develop from fertilized eggs, the eggs may receive below-threshold amounts of the inhibitor.

Mixis response—Recent experiments with the Gainesville strain of *B. calyciflorus* demonstrated that amictic females hatching from fertilized eggs were less likely to produce mictic daughters in response to a standardized crowding stimulus than amictic clonemates produced in later parthe-

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Fig. 2.   *B. calyciflorus* (Atlanta, Georgia, strain). Live females photographed (J.J.G.) using Nomarski interference contrast optics and flash illumination. (A) Mictic female with long posterolateral spines and two fertilized eggs. (B) Female recently hatched from fertilized egg produced by long-spined mother, showing spine reduction (posterolateral spines absent; other spines reduced) and many lipid droplets in tissues. (C, D) Females recently hatched from parthenogenetic eggs produced by long-spined mothers, showing posterolateral spines and little lipid in tissues.
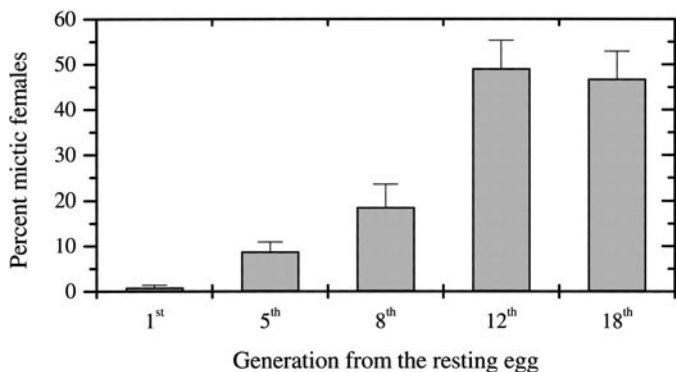
Fig. 3.   Effect of generation from the fertilized egg on the percentage of mictic females induced by a standardized crowding stimulus in *B. calyciflorus* (Gainesville, Florida, strain). Values are means (with 1 SE) of 10 clonal populations (from Gilbert 2002).

nogenetic generations (Gilbert 2002). The mixis response to crowding gradually increased but was significantly depressed for eight generations of amictic females (Fig. 3). A mechanism suggested for this multigenerational parental effect is an extension of the one just proposed to explain why stem females are amictic. An inhibitor preventing expression of the mictic-female phenotype may be transmitted through successive parthenogenetic generations at increasingly low concentrations (Gilbert 2002, 2003*a*). The effect of birth order within a generation was not tested but is a topic of interest. For example, if an inhibitor is involved, the amount of inhibitor passed on to an amictic female's daughters might decrease with the birth order of the daughter.

The reduced mixis response in early generations from the fertilized egg in *B. calyciflorus* may have evolved to ensure that sexual reproduction is delayed until clonal populations reach high densities and thus can produce large numbers of fertilized eggs (Gilbert 2002, 2003*a*). If fertilized eggs hatched from the sediment at about the same time of year, a delayed response to crowding would reinforce the effect of the crowding response in limiting sexual reproduction until a high density of the multiclonal population was attained. However, if fertilized eggs were to hatch for a longer period of time, then a delayed response to crowding could increase the reproductive fitness of later-hatching clones by preventing high densities of early-hatching clones from inducing them to commit to sexual reproduction at low population densities (Gilbert 2002). Thus, the ability of a late-hatching clone to maximize its contribution to the sediment bank of fertilized eggs would be increased if it continued female parthenogenesis in an inducing environment until it reached a higher population density. The ability of late-hatching clones to reproduce parthenogenetically while earlier-hatching ones are producing mictic females should increase the absolute number of fertilized eggs they can produce as well as the number of fertilized eggs they produce relative to those produced by the early-hatching clones.

A reduced tendency to produce mictic females in early generations from the fertilized egg occurs in other rotifers besides *B. calyciflorus.* A delayed response to a standardized mixis-inducing environment was first indicated in *B. plicatilis* (Hino and Hirano 1977). It has been demonstrated for the first one or several generations in *Asplanchna brightwelli* (Gilbert 1983), *Brachionus angularis,* and one strain of *R. frontalis* (Schröder and Gilbert unpubl. data), as well as for many generations in *E. senta* (Schröder and Gilbert unpubl. data). In contrast, the first generation from the fertilized egg was maximally sensitive to a mixis stimulus in the Piedmont Park Pond strain of *B. calyciflorus* and in another strain of *R. frontalis* (Schröder and Gilbert unpubl. data). Further research needs to be conducted to determine if a delayed mixis response is correlated with certain ecological conditions. The trait would seem more advantageous in permanent waterbodies, where there is time for many parthenogenetic generations, than in temporary ponds, where resting eggs should be produced before the habitat dries. The above-mentioned observation that fertilized eggs of *Hexarthra* sp. living in temporary rock pools often hatch into mictic females indicates the importance of early sexual reproduction in such ephemeral systems.

Lipid droplets—In many rotifers, the yolk produced by fertilized mictic females is especially dark in color and rich in nutrients because of high concentrations of shell-secreting granules, glycogen, and lipid droplets for the diapausing eggs (Wurdak et al. 1977, 1978). The investment in lipid probably explains the observation that stem females in *B. calyciflorus* appear to have considerably more macroscopic lipid droplets in their tissues than females hatching from parthenogenetic eggs produced under similar conditions (Fig. 2B–D; Gilbert unpubl. data). An exceptional amount of lipid droplets in stem females also was noted in this species by Bogoslowsky (1963) and in *P. dolichoptera* by Nipkow (1952, 1961). This pattern needs to be verified by further analysis with a variety of species, but it is consistent with the fact that in all species examined, fertilized mictic females produce many fewer fertilized eggs than amictic females produce subitaneous eggs (Gilbert 1993). For example, in a clone of *B. calyciflorus,* mean lifetime fecundity for these females was 3.3 and 29.8 eggs female^{-1}, respectively (Pourriot and Rieunier 1973).

A high-energy content of fertilized eggs may have evolved as a strategy to maximize the survival of stem females hatching in unpredictable or low food environments. If stem females had an especially high content of nutrient reserves compared to females from parthenogenetic eggs, they might be more resistant to starvation and below-threshold food concentrations and thus have an especially high potential to survive between hatching and encountering conditions suitable for growth and reproduction. This interval could be especially long for stem females. Since hatching cues may not be linked to good food conditions, these females may hatch when food availability is insufficient for growth and reproduction. Also, they may hatch from deep-water sediments located considerable distances away from epilimnetic food. In contrast, females hatching from parthenogenetic eggs are likely to immediately experience the same suitable food resources that were available to their mothers. In addition, the eggs from which they hatch probably are in places where food is available. Many rotifers carry their eggs until they have hatched, and many oviposit them in the plankton or on substrata where they live.

In *Daphnia,* the two fertilized eggs in the ephippium are also well provisioned with yolk (Zaffagnini 1987). Although there is detailed information on oogenesis in both parthenogenetic and fertilized eggs (Zaffagnini 1987), it is not clear whether the amount of yolk reserves in these two types of eggs would differ if they were produced under the same conditions. The life history of ex-ephippial and parthenogenetic females has been compared in *Daphnia magna* (Arbaciauskas 1998; Arbaciauskas and Lampert 2003). Ex-ephippial females were smaller at birth and had a higher metabolic rate, growth rate, and fecundity. The first two features led to a lower tolerance to starvation and thus to the authors' suggestion that stem females are adapted to predictable, high-food conditions. Thus, the strategy of nutrient provision in stem females may differ in *Daphnia* and rotifers.

General conclusions—In rotifers, females hatching from fertilized eggs appear to be particularly well suited for colonization. Perhaps most importantly, these stem females typically are amictic and may produce a number of parthenogenetic generations of amictic females less likely to produce mictic daughters in response to inducing environments than amictic females from later generations. Therefore, bisexual reproduction is delayed, allowing rapid population growth by female parthenogenesis to found large clonal populations that can then produce large numbers of diapausing, fertilized eggs for a sediment egg bank. Furthermore, stem females seem to have an especially large reserve of stored nutrients, which could be used to increase tolerance to food limitation, and hence survival, until food availability increases. Finally, morphological reduction in the stem females of some species may conserve energy that could be diverted to reproduction. Examples are apterous females in some species of *Polyarthra* and less spinous females in *B. calyciflorus.*

The morphological and physiological characteristics of stem females in rotifers may be analogous to some generation-dependent traits in aphids. Aphids, like rotifers, have a heterogonic life cycle. The fundatrix, or stem mother, hatches from a diapausing, fertilized egg and differs morphologically from females in the next parthenogenetic generations. Differences involve the antennae and legs as well as the number of ovarioles in the gonad (Hille Ris Lambers 1966; Wellings et al. 1980; Dixon 1998). For example, in four species of Callaphidinae, the fundatrix generation has two more ovarioles than the next one or two parthenogenetic generations (Wellings et al. 1980). The generational difference in ovariole number is not determined by diet, but rather, it is programmed by some unknown mechanism (Wellings et al. 1980).

The effect of generation from the fertilized egg on the mixis response in *B. calyciflorus* and other rotifers illustrates an unusually persistent parental effect, or transgenerational phenotypic plasticity. In strains of *B. calyciflorus* and *E. senta,* this effect suppressed or completely inhibited the response to crowding for at least 8 and 7 generations, respectively (Gilbert 2002; Schröder and Gilbert unpubl. data). Environmentally induced parental effects described in other organisms involve only a few generations (Mousseau and

Fox 1998; Agrawal et al. 1999; Alekseev and Lampert 2001).

Time of hatching from diapausing, fertilized eggs

Introduction—Most investigations of the hatching of fertilized eggs have been conducted in the laboratory with a very limited number of species, mostly of the genus *Brachionus* (Gilbert 1974, 1993, 2001; Pourriot and Snell 1983). Newly oviposited fertilized eggs kept in laboratory conditions suitable for population growth typically hatch only after a latent period or a minimum diapause period. This period lasts for a few days to several weeks in species of *Brachionus* (Pourriot and Snell 1983; Gilbert 2001) but can be much longer in other genera (Nipkow 1952; Pourriot and Snell 1983). In *B. plicatilis,* several days of nuclear divisions and development occur before the fertilized egg enters diapause or hatches (Hagiwara et al. 1995). Laboratory conditions that trigger hatching after the latent period are known for some rotifers. This knowledge may provide some insight into when hatching should occur in situ, but very few data are available on when, or to what extent, hatching actually occurs during the year in natural systems.

Hatching environments—Detailed laboratory investigations of environmental conditions that stimulate the hatching of fertilized eggs have been conducted with *B. angularis* and *Brachionus budapestinensis* (Pourriot et al. 1983), *B. calyciflorus* (Pourriot et al. 1982), *B. plicatilis* (Blanchot and Pourriot 1982*b;* Minkoff et al. 1983; Hagiwara and Hino 1989; Hagiwara et al. 1989, 1995), *Brachionus rubens* (Pourriot et al. 1980, 1981; Blanchot and Pourriot 1982*a*), and *E. senta* and *R. frontalis* (Schröder unpubl. data). Light may or may not be required, and there usually is a range of temperatures within which the eggs are most likely to hatch. Conditions that prevent fertilized eggs from hatching may vary greatly among species and include low or high temperatures, darkness, high salinity, and low dissolved oxygen concentrations (Gilbert 1974, 1993; Pourriot and Snell 1983). In some rotifers that can be readily cultured, fertilized eggs could not be induced to hatch (Gilbert unpubl. data).

In natural systems, the diapausing, fertilized eggs of rotifers become part of a sediment egg bank and may remain viable for tens of years (Nipkow 1961; Marcus et al. 1994; Viitasalo and Katajisto 1994) and even up to 100 yr (Y. Fu 1991, in Kotani et al. 2001). If the eggs become buried in the sediment, their ability to hatch probably depends on physical and biological processes that mix the sediments and bring them to the surface where they can experience hatching cues (Cáceres and Hairston 1998; Hairston and Kearns 2002). Since sediment mixing is greater in shallow water, buried diapausing eggs should have a greater chance of hatching there (Hairston and Kearns 2002).

A number of factors may determine if and when recent or old diapausing, fertilized eggs at the sediment surface hatch, but these factors are poorly understood. For example, can an environment that encourages hatching occur at the sediment surface, or must eggs be resuspended into the water column to receive a stimulus to hatch or to avoid a condition that inhibits hatching? If the latter is true, then processes that

facilitate resuspension may be critical for hatching (Nipkow 1961; Gilbert 2001). These processes likely are the same ones described for sediment mixing (Hairston and Kearns 2002) and thus should be more pronounced in shallow water.

In waterbodies that partially or completely dry out, the flooding of exposed, dried sediments provides an obvious hatching environment with well-oxygenated water and light (Gilbert 2001). For example, a great number and diversity of rotifers hatch from flooded dried sediments of floodplains and experimental and natural billabongs (Boulton and Lloyd 1992; Nielsen et al. 2000; Langley et al. 2001; Schröder 2001). Also, the transfer of dried sediment to culture medium in the laboratory usually leads to the emergence of hatched rotifers from fertilized eggs (Gilbert 2001, unpubl. data). For this reason, fertilized eggs in shallow-water sediments may be especially likely to experience the periodic cycles of drying and flooding that will induce hatching (Gilbert 2001). In some crustaceans, drying and rehydration of sediments are known to increase emergence from diapausing eggs (Arnott and Yan 2002).

The transportation of recently produced fertilized eggs of rotifers to the margins of a waterbody by wind and currents, as described by Wesenberg-Lund (1930), may distribute the eggs to shallow sediments and hence maximize future hatching. Furthermore, in some species, morphological characteristics of the shells of fertilized eggs (e.g., spines, gelatinous coats, vacuoles) may facilitate flotation, transport to the littoral zone, and retention in that zone (Gilbert 1974; Ruttner-Kolisko 1974).

Sediments submerged in the basins of permanent waterbodies contain large numbers of diapausing, fertilized eggs that can be stimulated to hatch in the laboratory (Nipkow 1961; May 1986, 1987; Mnatsakanova and Polishchuk 1996; Duggan et al. 2002). Densities of fertilized eggs of *B. plicatilis* in a small brackish-water pond in Florida were as high as 194 eggs $(cm^3)^{-1}$ at the surface of the sediment (Snell et al. 1983). However, most fertilized eggs, even ones recently produced, may be in sediment environments that inhibit hatching. Inhibiting conditions at and just below the sediment surface could be low temperature, low light, little dissolved oxygen, and high concentrations of dissolved carbon dioxide, sulfide, and organic compounds. In Lake Vechten (The Netherlands), the sediment depth at which the oxygen concentration declined to zero was 0.05 mm in November and 1.3 mm in the winter (Sweerts et al. 1991). Therefore, the hatching of diapausing eggs may depend on their suspension into the water column. The probability of resuspension by currents should decrease with the depth and thermal stratification of the water column and should increase with wind velocity.

The number or fraction of diapausing, fertilized eggs in sediment egg banks that hatches at a given time, or during a given year, probably is extremely variable and may be small or large. The large numbers of eggs in egg banks, as well as the ability of these eggs to remain viable in diapause for decades, suggest that fertilized eggs of considerable age occasionally hatch and introduce into the plankton genotypes that were produced long ago and under different environmental conditions. As discussed above, the hatching of old eggs buried in the sediment should depend on factors that bring these eggs to the sediment surface. The storage effect of egg banks should promote the maintenance of genetic and biotic diversity and also slow the rate of evolution (Hairston and De Stasio 1988; Ellner and Hairston 1994; Hairston 1996; Hairston et al. 1996; Cáceres 1997; Ricci 2001).

Relationship between hatching conditions and conditions for population development—Theoretically, diapausing eggs should hatch under conditions suitable for population growth (Pourriot and Snell 1983). This would increase the probability that stem females would survive to develop clonal populations through female parthenogenesis. There is some evidence for such a relationship with temperature. Pourriot et al. (1983) found that fertilized eggs of a cold-water strain of *B. angularis* hatched at high rates between 5°C and 16°C but rarely hatched at 22°C. Conversely, they found that fertilized eggs from a warm stenotherm, *B. budapestinensis,* had low hatching rates at 5°C but high ones at 18°C or 22°C.

The most extensive studies linking hatching temperatures with preferred temperatures for population growth were conducted by May for rotifers from Loch Leven. For some—but not all—species, there was a correlation between the temperatures at which fertilized eggs hatched from sediment in the laboratory (May 1987; May et al. 2001) and the temperatures at which populations occurred in the lake (May 1983; May et al. 2001). For *Keratella cochlearis* and *Synchaeta kitina,* eggs readily hatched at 5°C, 10°C, and 15°C, and natural populations developed throughout this temperature range (May 1983, 1987). For *Notholca squamula,* eggs readily hatched only at the colder temperatures (5°C and 10°C), and lake populations almost always developed below 10°C (May 1983, 1987). Similarly, for the cold stenotherm *P. dolichoptera,* eggs hatched only at 5°C and 10°C, and populations occurred primarily below 15°C (May 1983, 1987). In contrast, for *Trichocerca pusilla,* both egg hatching and population development generally were restricted to temperatures above 11–12°C (May et al. 2001). No eggs hatched at 5°C, some hatched at 10°C, and about seven times as many hatched at 15°C.

Recent evidence shows that critical temperature conditions for the hatching of diapausing, fertilized eggs can involve changes in water temperature that normally occur across seasons. *R. frontalis* is a cold stenotherm that occurs in the winter and spring in small ponds and temporary floodplain habitats when temperatures range from 1°C to 17°C (Wesenberg-Lund 1930; Pourriot 1965; Schröder 2001). In the floodplains of the Oder River, fertilized eggs are produced in April and May shortly before the waters dry up (Schröder 2001). Fertilized eggs produced in the laboratory by a floodplain clone hatched at 6°C, but only if they had been exposed previously to 20°C; virtually no hatching occurred if the eggs were kept continuously at 6°C or 20°C (Schröder unpubl. data). The requirement of a high-temperature signal ensures that diapausing eggs produced in the spring will not hatch before the ponds dry out.

For euryhaline rotifers, salinity may be another environmental factor that focuses the hatching of diapausing, fertilized eggs during conditions suitable for population development. In *B. plicatilis,* however, there is no strong evidence for or against such a pattern. In a strain of the species from

Israel, Minkoff et al. (1983) found that optimal hatching occurred at 16‰ and indicated that this salinity also was optimal for population growth. Hagiwara et al. (1989) found that both population growth and hatching of diapausing eggs in *Brachionus rotundiformis* occurred at high rates for a broad range of salinities (8–32‰).

Hatching of fertilized eggs in natural systems—Direct evidence for the hatching of fertilized eggs in nature is very scarce and difficult to obtain. The little information available is based on the recognition of apterous females in *Polyarthra*, the appearance of shells of recently hatched fertilized eggs in the plankton, and the emergence of females from the sediment into in situ traps. Some indirect evidence for the hatching of diapausing, fertilized eggs from the sediment has been provided from negative death rates of planktonic populations estimated from birth and reproductive rates (Bosselmann 1979; Arndt 1988; Mnatsakanova and Polishchuk 1996). Negative death rates involve errors in estimating reproductive and birth rates (Bosselmann 1979) and also could be caused by horizontal movements of the population (Mnatsakanova and Polishchuk 1996). Accordingly, they probably can provide convincing evidence that hatching of fertilized eggs has occurred only when large numbers of such eggs hatch.

It is clear that rotifer populations in flooded basins, previously completely dried, must initially develop from the hatching of fertilized eggs in the sediment. Similarly, it is likely that the flooding of dried sediment exposed at the margins of waterbodies soon leads to the hatching of fertilized eggs in those sediments. Thus, the hatching of fertilized eggs can be assumed to occur whenever dried sediment is flooded with water at an appropriate temperature for hatching. By contrast, little is known, or can be assumed, about when fertilized eggs hatch from permanently submerged sediments.

Two studies provide indirect evidence of emergence from a sediment egg bank by determining when negative death rates occurred for planktonic populations. For *K. cochlearis* in Lake Esrom (Denmark), diapausing eggs appeared to hatch in the early spring when the population began to develop (Bosselmann 1979). Similarly, for three species of *Brachionus* in Lake Chirtovo (Russia), negative death rates coincided with periods when populations began to develop (Mnatsakanova and Polishchuk 1996).

Direct evidence that diapausing, fertilized eggs have hatched comes from observations of the occurrence of the *aptera* form of *P. dolichoptera*. Carlin (1943) observed *P. dolichoptera* for 6 yr consecutively in the Motalaström at Fiskeby, Sweden. This rotifer occurred at very low abundances in the fall and winter, beginning in October or November, and then reached higher abundances from April to June. It was absent from July to September. Even though *P. dolichoptera* was present in the plankton continuously for 8–9 months of the year, apterous females seemed to be hatching only during brief periods (7–10 d) at temperatures below 10°C occurring months before peak development in April to June.

Similar patterns for the appearance of apterous stem females in populations of this species were found by Amrén
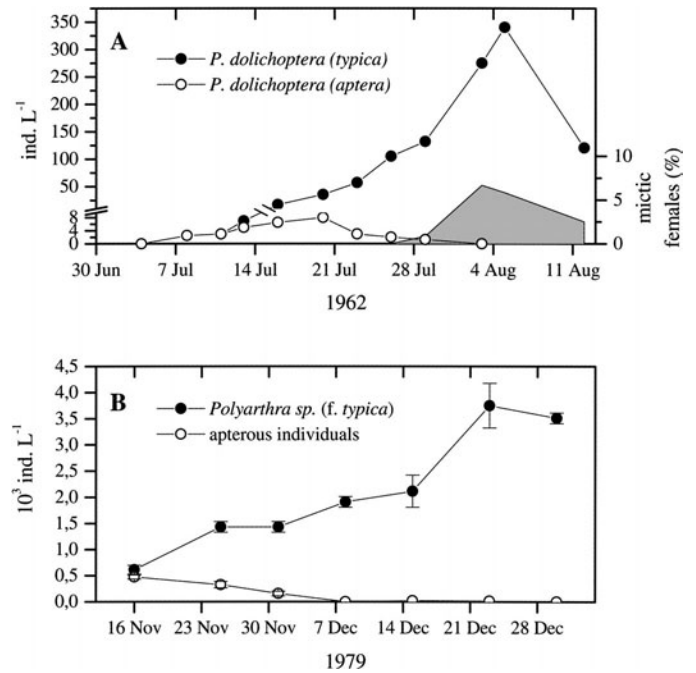

Fig. 4. Phenology of *typica* and *aptera* forms of *P. dolichoptera*. (A) Population in a shallow Spitsbergen pond in the summer of 1962 (from Amrén 1964). The proportion of mictic females in the population is indicated by the shaded area. Note the different scale after the axis break on the y-axis. (B) Population (~98% *P. dolichoptera*) in Star Lake (Vermont, U.S.A.) in the winter of 1979 (from Magnien 1983).

(1964) in a Spitsbergen pond and by Magnien (1983) in a small Vermont lake (Fig. 4). In the Spitsbergen population, the abundance of apterous females increased for about 2 weeks, indicating that continuous hatching from fertilized eggs had occurred during this period. Afterward, there was a period of pronounced population growth and the appearance of mictic females, but no more apterous females were found. The study of the Star Lake population missed the beginning of the hatching period, but almost no apterous animals appeared during the period of main population development. These data suggest that the hatching of diapausing, fertilized eggs in *P. dolichoptera* is limited to short periods prior to population development and when water temperatures are low. This is consistent with the findings of May, who could hatch the fertilized eggs of this species only at 5°C and 10°C (*see above*).

In studies of the population dynamics of *B. plicatilis* and *B. quadridentatus* in a shallow coastal system in the southern Baltic, Arndt (1988, 1991, pers. comm.) was able to determine when fertilized eggs hatched from the sediment by the occurrence of empty shells in the plankton. The surfaces of these egg shells had not yet been appreciably colonized by bacteria, protists, and fungi; hence, the eggs were estimated to have hatched during the previous 1 or 2 weeks. In *B. plicatilis*, which occurred from May through August, shells of fertilized eggs were found only in May, just before the population began to develop and when water temperatures rose quickly from 10°C to 15°C (Arndt 1988). This hatching date matched the time of a major negative death rate and

hence a period of massive hatching of fertilized eggs. In *B. quadridentatus,* shells of fertilized eggs were observed at various times of the year in the early and late stages of population development when water temperatures exceeded 10°C—April to June and September and October in 1981 and March, May, and August to September in 1982 (Arndt 1991).

Only one study (Hairston et al. 2000) has directly observed hatching of fertilized rotifer eggs from sediments in situ. Emergence traps, carefully designed to keep conditions at the sediment surface as natural as possible, were placed in the sediment at 5- and 13-m depths in Oneida Lake (New York) and monitored every week or two from May to August 1996. Almost all rotifer emergence was in May. *Keratella, Notholca, Synchaeta,* and *Ascomorpha* were found only in May; some *Polyarthra* and *Euchlanis* also appeared in July or June and July, respectively.

Emergence traps are probably the most promising technique for observing a hatch of fertilized eggs from the sediment in natural systems, but great care should be taken to prevent underestimates of hatching. If sediment resuspension facilitates hatching by the transport of eggs into the water column where conditions are more likely to permit hatching, traps in the sediment could reduce the disturbance of the sediments. Also, the enclosed environment in the trap could increase the mortality of recently hatched rotifers because of unsuitable chemical conditions or low food availability.

Allozyme studies of rotifers from natural populations have shown that water-column populations are multiclonal (King and Serra 1998) and hence derived from the hatching of many stem females from fertilized eggs. However, these studies probably greatly underestimated the amount of genotypic diversity because of the low sensitivity of the methodology. Using the more recent technique of polymorphic microsatellite loci, Gómez and Carvalho (2000) demonstrated astonishingly high genotypic variation in a population of *B. plicatilis* from a shallow, temporary pond in Spain. On each of four sampling dates, almost every female belonged to a distinct genotype. Such extensive hatching of fertilized eggs is probably the result of an enormous production of fertilized eggs in the plankton as well as conditions that are favorable for their hatching—a shallow, well-mixed water column and cycles of sediment drying and flooding (*see above*). Negative death rates occurring before population development also suggest that very large numbers of fertilized eggs hatch from the sediment in this and several other species of *Brachionus* that live in shallow systems (Arndt 1988; Mnatsakanova and Polishchuk 1996).

General conclusions—Fertilized eggs in dried sediment probably regularly hatch in large numbers soon after flooding. It is more difficult to know or attempt to predict when these eggs hatch from submerged sediments. There are several reasons for this. First, few eggs may hatch. The number of fertilized eggs that must hatch to found a species population could be very small. Assuming conservative age-specific fecundity, Ruttner-Kolisko (1963) calculated that a single stem female could parthenogenetically produce a clonal population of 10^{12} females in 60 d. Second, the hatching of fertilized eggs may go undetected if stem females soon die

because of predation or inappropriate conditions for survival and growth. Third, stem females hatching from fertilized eggs cannot be morphologically distinguished from parthenogenetically produced females except in *Polyarthra.* For these reasons, it will be difficult to differentiate between models proposing synchronous hatching at one time of the year and intermittent hatching for a more prolonged period (Gilbert 1974; Pourriot and Snell 1983; King and Serra 1998).

Considering the very limited information available to date on when diapausing, fertilized eggs hatch from sediments in permanent waterbodies, it appears that hatching often occurs during a short period that marks the beginning of population growth. As discussed above, the timing of this hatching period could be controlled by temperature at the sediment surface, by physical factors that promote resuspension of diapausing eggs into an oxygenated and illuminated water column of the appropriate temperature, or both. Conditions that encourage hatching may or may not be associated with environments likely to have high concentrations of algal food. In *Daphnia,* the hatching of ephippial eggs appears to be limited to the early spring (Cáceres 1998) when conditions for population growth are assumed to be favorable (Arbaciauskas 1998; Arbaciauskas and Lampert in press).

An alternative pattern of more limited and intermittent hatching of fertilized eggs for a longer period also is possible and may occur after an early burst of hatching that initiates population growth. Although there is now little evidence for such intermittent hatching of fertilized eggs throughout the growing season in rotifers, this scenario seems likely. The fertilized eggs of many species have only a brief latent period and hence an ability to hatch soon after oviposition. As discussed above, this seems to be the case in many brachionids. Therefore, in natural systems, it is reasonable to assume that fertilized eggs produced early in the season, and lying at the surface of the sediment, could hatch days or weeks later. Even if fertilized eggs sink to the bottom in an environment that inhibits hatching, some may be resuspended periodically into the water column where they could hatch. Resuspended bottom sediments could include diapausing eggs produced years earlier as well as those produced earlier in the year. In some species, only $\geq$1-yr-old eggs may be able to hatch because of a long latent period or a need to experience successive temperature signals associated with seasonal changes.

Information on the timing and extent of the hatching of rotifers from fertilized eggs clearly is important for understanding both population dynamics and seasonal patterns of genotypic diversity. In some systems, many diapausing, fertilized eggs may hatch early in the growing season and account for some population growth as well as high genotypic diversity at that time. In other systems, only small numbers of fertilized eggs may hatch throughout the growing season, so that population growth is almost exclusively via female parthenogenesis with a few new genotypes being introduced from time to time. The pattern of emergence from diapausing, fertilized eggs in the sediment has implications regarding the storage effect, particularly the coexistence and diversity of species and genotypes during the growing season. It would be interesting to compare patterns of such hatching

and genotype diversity in a variety of waterbodies differing in parameters that affect the extent and seasonality of hatching, such as size, depth, productivity, and water-level variation.

Suggestions for future research

A better appreciation of rotifer population dynamics and genetics requires much more extensive knowledge about the timing and extent of hatching from diapausing, fertilized eggs in the sediment of natural systems. This is especially true for permanent waterbodies where hatching is unlikely to be associated with the flooding of dried sediments. For all rotifers, the procedure most likely to provide information involves the use of emergence traps, such as those used by Hairston et al. (2000). If possible, the traps should be positioned to allow natural disturbance at the sediment surface, which may be important in suspending eggs into a hatching environment. Also, the traps should be set out and retrieved or monitored at frequent intervals to minimize mortality of hatched rotifers and modification of the sediment environment by the traps.

Toward this same goal, additional studies should take advantage of the unique possibilities offered by *Polyarthra.* In systems with *P. dolichoptera* or *P. remata,* the timing and extent of hatching from fertilized eggs can be determined from the abundance of *aptera* forms, and hence stem females, in plankton samples. Frequent sampling of such ecosystems by Amrén (1964) and Magnien (1983) provided much useful information. It would be useful to separately quantify juvenile and ovigerous *aptera* females. Juvenile abundance would indicate only very recent hatching, and thus would minimize the possibility of counting *aptera* females that hatched during a previous sampling interval.

In addition, laboratory experiments, together with analyses of physical and chemical conditions in natural sediment environments, should continue to investigate factors that inhibit or trigger the hatching of fertilized eggs. In particular, it may be important to determine the extent to which sediment resuspension facilitates hatching by transporting eggs into conditions more favorable to hatching. These conditions could be higher light intensity, higher oxygen concentration, lower concentrations of sulfide and dissolved organic compounds, or a more appropriate temperature. If sediment resuspension proves to be important for hatching, then hatching events may be closely related to water currents that could be predicted by weather, water temperature, and basin morphometry and morphology.

Stem females that hatch from fertilized eggs appear to be especially well adapted for colonizing new habitats in having larger reserves of yolk than females that hatch from parthenogenetic eggs. Future research should test this hypothesis with quantitative microscopic and chemical analysis of eggs and neonates as well as with life-table experiments of females cultured without food or with below-threshold concentrations of food. Results from these experiments could then be compared with those obtained on ex-ephippial and parthenogenetic females of *Daphnia* spp. by Arbaciauskas (1998) and Arbaciauskas and Lampert (in press).

Further attention should be given to the phenomenon of mixis delay reported for some rotifers in early generations from the fertilized egg (Gilbert 2002; Schröder and Gilbert unpubl. data). The decreased propensity of early generations to respond to stimuli that induce mictic females could be very important in the life cycle, because it ensures some generations of female parthenogenesis before a commitment to sexuality and production of diapausing, fertilized eggs. It would be interesting to know if the existence and duration of this mixis delay is correlated with the likely duration of appropriate conditions for population growth. Also, it would be of great interest to elucidate the mechanism responsible for this nongenetic, but apparently programmed, generational effect.

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