Maintenance of androdioecy in the freshwater clam shrimp *Eulimnadia texana*: longevity of males relative to hermaphrodites

Naida Zucker, Brian Stafki, and Stephen C. Weeks

Abstract: The clam shrimp *Eulimnadia texana* exhibits a rare mixed mating system known as androdioecy. In this ephemeral-pond branchiopod crustacean, males coexist with hermaphrodites, which can outcross with males or self-fertilize. We provide an estimate of the longevity of males relative to hermaphrodites ($1 - \sigma$), an important parameter of a model that was developed to explain the conditions under which this system would be stable. Under both optimal rearing conditions and various sex-ratio treatments, hermaphrodites from two study populations lived significantly longer than males. Since various aspects of mating have been found to be costly to males and females/hermaphrodites in other taxa, we explored this possibility as well. Hermaphrodites showed no differences in longevity when kept in groups provided with different mating opportunities. Males, however, lived significantly longer when mating opportunities were increased, a result contrary to what we had expected. Behavioral observations, however, suggested that male–male interactions may have been deleterious to males living in groups with little opportunity to mate. This was confirmed by an additional study in which individual males were maintained in the presence and absence of hermaphrodites. Under these conditions we still detected no longevity cost of mating for males.

Résumé : Le conchostracé *Eulimnadia texana* possède un système d'accouplement très particulier, l'androdioécie. Chez ce crustacé branchiopode des étangs temporaires, les mâles cohabitent avec des hermaphrodites qui peuvent se croiser à des mâles ou s'auto-féconder. Nous avons estimé la longévité relative des mâles par rapport à celle des hermaphrodites ($1 - \sigma$), paramètre important d'un modèle conçu pour expliquer les conditions dans lesquelles ce système peut être stable. Dans des conditions d'élevage optimales où le rapport mâles : femelles peut varier, les hermaphrodites de deux populations expérimentales ont survécu significativement plus longtemps que les mâles. Or, comme certains aspects de l'accouplement se sont avérés coûteux pour les mâles et les femelles/hermaprodites chez d'autres taxons, nous avons examiné cette possibilité également dans ce cas-ci. La longévité des hermaphrodites ne varie pas lorsqu'ils sont gardés en groupes où leurs chances de s'accoupler sont variables. Cependant, les mâles vivent significativement plus longtemps dans des conditions où leurs chances de s'accoupler sont meilleures, un résultat contraire aux prédictions. Les comportements observés indiquent cependant que les relations mâle–mâle peuvent nuire aux mâles vivant dans des groupes où leurs chances de s'accoupler sont faibles. Cela a été confirmé par les résultats d'une recherche additionnelle où des individus mâles ont été gardés en présence ou en l'absence d'hermaphrodites. Néanmoins, dans ces conditions nous n'avons toujours pas détecté de baisse de la longévité comme coût de l'accouplement chez les mâles.

[Traduit par la Rédaction]

Introduction

Androdioecy, a reproductive system in which hermaphrodites and males (but no pure females) coexist, is rare (Jarne and Charlesworth 1993). Recently, the clam shrimp *Eulimnadia texana*, a branchiopod crustacean, was shown to be androdioecious (Sassaman and Weeks 1993; Zucker et al. 1997). In this species, like the soil nematode *Caenorhabditis elegans* (Gem and Riddle 1996), hermaphrodites may outcross with males or self-fertilize, but they cannot outcross with other hermaphrodites. Theory generally suggests that androdioecious organisms are in a transitional state between hermaphroditism and dioecy or vice versa (Charlesworth 1984; Liston et al. 1990). However, a recent model developed specifically for the *E. texana* system proposes that androdioecy in these shrimp could be maintained in a state of equilibrium under appropriate conditions (Otto et al. 1993).

In *E. texana*, unlike *C. elegans*, a simple Mendelian autosomal trait determines the sex. Males are homozygous recessive and hermaphrodites are either homozygous dominant or heterozygous. Thus, two morphologically indistinguishable but genetically different types of hermaphrodites exist: monogenics (SS), which produce only hermaphroditic offspring when selfing, and amphigenics (Ss), which produce 25% males (ss) when selfing (Sassaman and Weeks 1993). Otto et al.'s (1993) model predicts the equilibrium frequencies of the three mating types in *E. texana* (males (u) and monogenic (w) and amphigenic (v) hermaphrodites), based on four relevant parameters: α, the ability of a male to fertilize hermaphroditic eggs; β, the proportion of eggs that are not fertilized by a male which are then self-fertilized by the

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hermaphrodite; $(1 - \sigma)$, the viability of males relative to hermaphrodites; and δ, inbreeding depression experienced by selfed offspring. The model assumes that the outcrossing rate is related to male frequency, u. The parameter α can vary from 0 to ∞, but is constrained so that $0 \leq \alpha \times u \leq 1$ (Otto et al. 1993). The combination of male frequency in the population and relative male mating ability (α) dictates the expected proportion of hermaphroditic eggs that will be outcrossed (i.e., αu). The remaining proportion of eggs (i.e., $1 - \alpha u$) is then available for selfing. The model allows for a proportion, $1 - \beta$, of these non-outcrossed eggs to remain unfertilized. This would occur if some eggs were "earmarked" for outcrossing, or if the hermaphrodites were unable to produce enough sperm to fertilize all of their eggs in the absence of males (as in *C. elegans*; Ward and Carrel, 1979; Hodgkin and Barnes, 1991; Van Voorhies, 1992). The model also incorporates the commonly observed difference in viability between the sexes in conchostracan shrimp, defined as $1 - \sigma$. Finally, the model provides for the commonly documented decrease in viability (δ) observed in self-fertilized offspring (Jarne and Charlesworth 1993; Husband and Schemske 1996). Otto et al.'s (1993) model proposes that if the proportion of eggs fertilized by males (α) multiplied by the viability of males relative to hermaphrodites $(1 - \sigma)$ (i.e., the relative fitness of outcrossing) is greater than 2 times the proportion of eggs not fertilized by males but fertilized by hermaphrodites (β) multiplied by the relative inbreeding depression suffered by selfed offspring $(1 - \delta)$ (i.e., the relative fitness of selfing), then males and both types of hermaphrodites will be maintained in the population. However, so little is known about the reproductive biology and life history of this species that estimates of these parameters are lacking (but see Weeks et al. 1999, 2000*b*) and several assumptions of Otto et al.'s (1993) model remain untested.

We recently set out to test Otto et al.'s (1993) model by collecting the necessary life-history and reproduction data. Here we report on one parameter of Otto et al.'s (1993) model: the life-span of males relative to hermaphrodites $(1 - \sigma)$. We tested the relative longevity of isolated individuals reared under optimal growth conditions by making within-family comparisons. We also reasoned that environmental conditions might influence longevity. Male and (or) female/hermaphrodite longevity has been shown to decrease with increasing mating opportunities in other organisms (*Drosophila* sp., Partridge and Farquhar 1981; *C. elegans*, Van Voorhies 1992; but see Gems and Riddle 1996). We therefore predicted that, given an excess of members of the opposite sex, males and hermaphrodites would exhibit reduced longevity relative to those with few or no mating opportunities. Thus, we also set out to determine whether reduced longevity is a reproductive cost paid by *E. texana* males and (or) hermaphrodites when given excess mating opportunities.

Materials and methods

Study organism and study sites

Eulimnadia texana is a small (carapace length up to 8 mm) branchiopod crustacean belonging to the order Conchostraca (but its classification is still controversial; Sassaman 1995). A carapace folded over the body of clam shrimp that is shaped and sculpted much like a bivalve mollusc shell gives the group its common name. *Eulimnadia texana* is found throughout the southwestern United States and into northern Mexico wherever summer rains temporarily fill natural depressions and cattle tanks. The shrimp typically hatch about 18–30 h after heavy summer rains and reach sexual maturity in about 5 days (Vidrine et al. 1987). They live for an additional 1–2 weeks, or less if the pond dries up. Males (m) use their two pairs of claspers to hold onto a hermaphrodite's (h) carapace during mate guarding and sperm transfer (Knoll 1995). Hermaphrodites lack claspers but can be clearly recognized from the many developing eggs in the ovotestes, which can be seen through the translucent carapace with a hand lens, or by a clutch of eggs being brooded in the fold of the carapace, which can be seen with the naked eye. One clutch is produced and laid in a little less than a day (Weeks et al. 1997). Hermaphrodites do not store sperm between clutches (Weeks et al. 2000*a*). Eggs, or more appropriately "cysts," since early development has taken place prior to laying, typically go through a drying period prior to hatching. Some populations have been known to go 10 years between rain events sufficient to cause hatching (MacKay et al. 1990).

Two populations of *E. texana* were studied. One was located in JT4, which is a natural depression (K. Havstad, personal communication), approximately 32 × 18.5 × 0.3 m deep when filled, on the U.S. Department of Agriculture Agricultural Research Service Jornada Experimental Range in Doña Ana County, south-central New Mexico. The other was located in WAL, which is a cattle tank constructed in the 1950s (W.C. Sherbrooke, personal communication), approximately 25.3 × 26.2 × 1 m deep when filled, near Portal, Cochise County, Arizona. Dry soil was collected from the locations of the two populations and stored for several months prior to use in two laboratories, where the work was done: Stephen C. Weeks' laboratory in Akron, Ohio, which will be referred to as SCW lab, and Naida Zucker's laboratory in Las Cruces, New Mexico, which will be referred to as NZ lab.

Longevity under optimal conditions: SCW lab

Shrimp were reared from cysts by hydrating small amounts of soil in aged tap water. Shrimp can usually be sexed by day 4 or 5 (day 0 = day of hydration). Hermaphrodites from both populations were isolated in 500-mL cups with about 12 g of soil (finely sifted to remove any branchiopod cysts). Hermaphrodites were allowed to produce eggs for up to 7 days, then the hermaphrodites were removed and the cups were dried for 30 days. After drying, the cups were rehydrated with aged tap water and the resulting nauplii were transferred to 37-L aquaria within a few hours of hatching. Aquaria were provisioned with 40 mL of baker's yeast liquid (1 g yeast/100 mL water). Shrimp were reared until sexually mature and then individually isolated. Isolated shrimp (JT4: $N = 96$ m, 133 h; WAL: $N = 100$ m, 112 h) were reared in 500-mL cups under optimal conditions for growth (kept at 25–27°C and fed 1 mL of yeast liquid per day). Day of death was recorded for each isolate. These family isolations were done in a number of temporal blocks. However, blocks were confounded with families, and thus the "family" main effect incorporates block-to-block variation in the ANOVA.

Data were analyzed using JMP version 3 (SAS Institute Inc.). Since males and hermaphrodites were grouped by family, a nested ANOVA was used to test for differences in mortality. Residuals were found to be normally distributed, therefore no data transformations were necessary. Since shrimp could not be sexed until about day 4, we started our experiments at that time but measured longevity from day 1, resulting in possible overestimation of longevity. An α level < 0.05 is considered significant throughout, except where noted.

Longevity under various sex-ratio treatments: NZ lab

Rearing

Shrimp were reared under various sex-ratio treatments, providing them with different numbers of mating opportunities. On day 0,

approximately 100 mL of JT4 soil and 25 mL of WAL soil (which contained many more cysts) was filtered separately through a 270 μm mesh sieve with aged tap water into each of several 4-L plastic tanks. The sieve mesh size allowed the cysts of *E. texana* to pass through but not those of the tadpole shrimp *Triops* sp., a known predator of *E. texana*. The remaining unfiltered soil and particulate matter were collected into 11-L plastic tanks (separated by population) and filled with aged tap water. All tanks were placed under continuously lit 100-W bulbs, resulting in a water temperature of 28–30°C. To ensure that all mature shrimp used in the experiment were the same age, 24 h after hydration (day 1) the water and any hatched larvae were transferred from two 4-L tanks into one 11-L tank for each population. The soil, containing unhatched cysts, was discarded. To create "common garden" conditions, water from the "unfiltered-soil" tanks was poured through a 63 μm mesh sieve to exclude any larvae and equally distributed among tanks containing the other population (i.e., WAL water was added to JT4 tanks and vice versa). Each tank was again placed under the heat lamps and provisioned with 5 mL of yeast liquid (1 mL of Fleischmann's™ dry yeast in 10 mL water) and 12 "shakes" of TetraMin™ Baby "E" fish food. The tanks were also provisioned as above on days 2 and 3.

Relative longevity in groups of shrimp

On day 4, when the shrimp were mature enough to sex but had not yet produced their first clutch, individuals were sexed and focal animals were color-coded with a small drop of Testor's™ model paint. They were distributed to their respective social treatments in clean 4-L tanks containing aged tap water as follows: 5 focal males with no hermaphrodites (5 m : 0 h), 2 hermaphrodites (5 m : 2 h), or 15 hermaphrodites (5 m : 15 h), or 5 focal hermaphrodites with no males (5 h : 0 m), 2 males (5 h : 2 m), or 15 males (5 h : 15 m) for each population. The treatment tanks were placed in a walk-in environmental chamber (1.2 × 2.4 × 2.4 m) maintained at an air temperature of 32°C (28°C H_2O) on a 14 h light : 10 h dark cycle using four 110-W Vita-Lite™ (full solar spectrum) fluorescent bulbs and provisioned daily with an excess amount of ground TetraMin™ fish-food flakes. Remaining food was removed each day and fresh food added. Animals were monitored once daily for deaths. The date of death of each focal animal was recorded; nonfocal animals in each tank that died were replaced in order to maintain the same number of mating opportunities as initially set up. In total there were 5 replicates, or $N = 25$ focal animals per sex per treatment per population.

Data were analyzed using JMP version 3 (SAS Institute Inc.). Since the data were not normally distributed, nor could they be made so through transformations, we used the nonparametric Kaplan–Meier (product–limit) survival estimates and we report log-rank test results. Again, since shrimp could not be sexed until about day 4 but we began measuring longevity from day 1, we may be overestimating longevity.

Behavior of focal shrimp in groups

One 10-min behavioral observation of each of about half the focal animals was made when the shrimp were between 7 and 10 days old, using a computerized event recorder program. Behaviors were grouped into 4 categories: 1, swimming (including the time spent swimming with a partner, where the male propels the hermaphrodite); 2, grazing (actively feeding on the bottom or sides of the tank, on fish flakes, or on microbial or algal growth); 3, resting (lying on their side on the bottom of the tank); 4, other (primarily male–male interactions or hermaphrodites struggling with clasping males). The amount of time each individual spent performing behaviors in each of these 4 categories during the 10-min observation period was determined.

Data were analyzed using JMP version 3 (SAS Institute Inc.). Since the data were not normally distributed, nor could they be made so through transformations, nonparametric tests were used.

Individual male longevity under various sex-ratio treatments

Male longevity under the various social treatments in the above experiment did not conform to our expectations (see Results). Our behavioral observations, however, suggested to us that our methods may have failed to test male longevity relative to mating opportunities per se, but instead may have tested male longevity as a consequence of male–male interactions. We therefore performed a further experiment on male longevity using methods that eliminate male–male interactions as a determining factor.

Since no population differences had been found previously (see Results), only shrimp from the WAL population were used. They were reared as previously described, except that charcoal-filtered, rather than aged, tap water was used. Observation tanks consisted of 15 cm diameter, round translucent plastic containers divided in half by polyester window screening glued to the sides and bottom. White silica sand (200 mL) was placed in the tanks (effectively blocking any gaps along the bottom between the two halves of the tank), and 500 mL of filtered tap water was added. On day 4, shrimp were placed in treatment groups as follows: (*i*) 1 male (m), (*ii*) 1 male on one side of the screen with 9 hermaphrodites on other side (m/h), and (*iii*) 1 male with 9 hermaphrodites on the same side of the screen (m + h). Ten replicates of the 3 treatments were run simultaneously in each of 4 different blocks for a total of 40 replicates of each treatment. Food rations were controlled by providing 30 μL of the yeast liquid per shrimp per container per day. Water was replaced every 4th day, effectively preventing the growth of algae. Shrimp were monitored for deaths 2 times each day, at 08:00 and 20:00, to increase the accuracy of our measure of time of death.

Data were analyzed using JMP version 3 (SAS Institute Inc.). Since the data were not normally distributed, nor could they be made so through transformations, we used the nonparametric Kaplan–Meier (product–limit) survival estimates and we report log-rank test results. Again, since shrimp could not be sexed until about day 4 but we began measuring longevity from day 1, we may be overestimating longevity.

Results

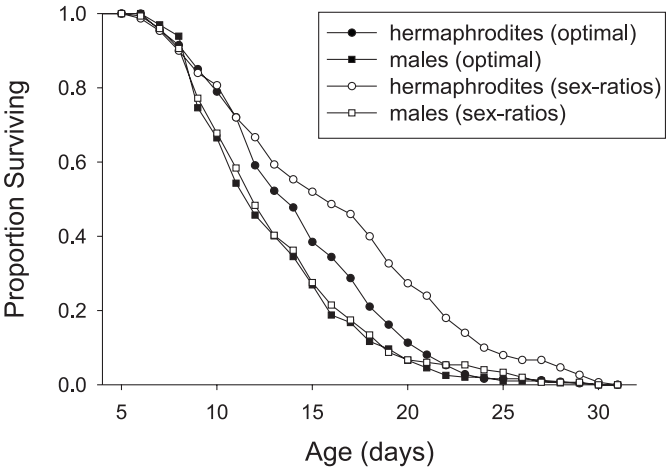
Longevity of males relative to hermaphrodites: SCW lab versus NZ lab

We did not statistically compare the longevity of hermaphrodites and males between laboratories, since the methods of rearing and maintaining the shrimp were somewhat different. Nevertheless, the trends seen for shrimp reared under optimal conditions (SCW lab) were quite similar to those found for shrimp reared under the various sex-ratio treatments and then pooled (NZ lab), with hermaphrodites living longer than males (Fig. 1).

Optimal conditions: SCW lab

There was no significant overall difference in longevity between populations when shrimp were reared under optimal conditions (Table 1). Hermaphrodites, however, lived 2 days longer, on average, than males in both JT4 and WAL (Table 2). This 2-day difference is statistically and, most likely, biologically significant (Table 1). The differences in longevity between the sexes were similar in the two populations (Table 1).

Fig. 1. Survival curve for male and hermaphroditic clam shrimp *Eulimnadia texana* reared under optimal conditions and various sex-ratio treatments. The populations and the various sex-ratio treatments are pooled.



Relative longevity of groups of shrimp in the different sex-ratio treatments: NZ lab

The populations were pooled for analysis because the null hypothesis of no difference in median longevity between populations was not disproved (log-rank test, χ^2 = 2.116, df = 1, P = 0.1462). Hermaphrodites were found to have lived significantly longer than males (log-rank test, χ^2 = 23.6687, df = 1, P < 0.0001; open symbols in Fig. 1; Table 2).

Hermaphrodites exhibited no differences in longevity among the 3 sex-ratio treatments in which they were the focal animals (log-rank test, χ^2 = 1.5097, df = 2, P = 0.4701) (Fig. 2A; Table 2). Males, however, exhibited significant differences among sex-ratio treatments (log-rank test, χ^2 = 21.0268, df = 2, P < 0.0001). Males in tanks with 15 hermaphrodites lived significantly longer than males in tanks with 2 hermaphrodites (log-rank test, χ^2 = 7.0824, df = 1, P = 0.0078) and no hermaphrodites (log-rank test, χ^2 = 20.1846, df = 1, P < 0.0001). (Note: Because two comparisons using the same data set are made here, the significance value should be adjusted to α = 0.025 for both tests.) (Fig. 2B). Males in tanks with 2 hermaphrodites lived significantly longer than males in tanks with no hermaphrodites (log-rank test, χ^2 = 4.2980, df = 1, P = 0.0382) (Fig. 2B). Thus, male longevity increased significantly with greater mating opportunities, especially when hermaphrodites were in excess relative to males.

From these results we can determine the viability of males relative to hermaphrodites ($1 - \sigma$) for each population in each laboratory (Otto et al. 1993). For shrimp under optimal conditions and in various sex-ratio treatments, relative viability ranged from 0.80 to 0.87 in JT4 and from 0.67 to 0.94 in WAL (Table 2).

Behavioral differences between the sexes and among sex-ratio treatments: NZ lab

There was a marginally significant difference between populations only for the infrequently exhibited behavioral category "other" in hermaphrodites (Wilcoxon's ranked sums, df = 1, P = 0.048): JT4 hermaphrodites spent 20.1 s versus 18.1 s for WAL hermaphrodites. All male and all other hermaphroditic behaviors (swimming, grazing, and resting) showed no differences between populations (Wilcoxon's ranked sums, df = 1, P ranged from 0.08 to 0.91). Therefore, behavioral data for the two populations were also pooled. Males spent significantly more time swimming (462 s out of a possible 600 s) and exhibiting "other" behaviors (mostly male–male interaction; 68 s) than did hermaphrodites (72 and 19 s, respectively). Hermaphrodites spent significantly more time grazing (320 s) and resting (189 s) than did males (53 and 17 s, respectively) when sex-ratio treatments were pooled (all tests: Wilcoxon's ranked sums, df = 1, P = 0.0001) (Fig. 3). Thus, males spent 77% of their time swimming compared with only 12% for hermaphrodites. Conversely, hermaphrodites spent 53% of their time actively feeding (grazing) compared with only 9% for males.

Within a sex, no significant differences were seen between sex-ratio treatments for any of the behaviors. However, some interesting trends were evident (Fig. 4). Hermaphrodites spent a small but increasing amount of time in "other" behaviors as the number of males increased, from 1 s with no males present to 20 s with 2 males and 37 s with 15 males present. This increase is marginally significant (Kruskal–Wallis test, df = 2, P = 0.05) and represents mainly nonreceptive hermaphrodites struggling with clasping males (Fig. 4A). As the number of hermaphrodites increased, males averaged less time resting (38 s for 0 h vs. 18 s for 2 h vs. 0.5 s for 15 h). Males with no hermaphrodites present spent, on average, 130 s in "other" behaviors, which consisted mainly of male–male interaction, while males with 2 and 15 hermaphrodites present spent only 22 and 51 s, respectively, in "other" behaviors (Fig. 4B).

Table 1. Results of ANOVA of optimal conditions for longevity.

Source	df	Sum of squares	F ratio	$P > F$
Population	1	0.004	0.009	0.9251
Sex	1	0.795	12.440	0.0005
Population × sex	1	0.049	0.774	0.3795
Mother (population)*	25	16.660	10.432	<0.0001
Error	369	23.572		

*Nested factor for families, associated by mother, nested within the two populations.

Longevity of individual males under various sex-ratio treatments: NZ lab

Males kept by themselves in experimental tanks (m; N = 39) lived a median of 10.5 days, while individual males kept physically (but not visually or chemically) isolated from 9 hermaphrodites in a tank (m/h; N = 39) and individual males kept with 9 hermaphrodites on the same side of the tank (m + h; N = 40) lived for 11.5 days (Fig. 5). None of these treatments were significantly different from any of the others (log-rank test, χ^2 = 1.7785, df = 2; P = 0.4110).

Discussion

The fact that longevity of males relative to hermaphrodites was found to be similar between laboratories despite considerably different rearing and maintenance methods (estab-

Table 2. Median[a] longevity (in days) of males and hermaphrodites by population and the resulting parameter $1 - \sigma$ from Otto et al.'s (1993) model.

	JT4			WAL		
Rearing conditions	Males	Hermaphrodites	$1 - \sigma$	Males	Hermaphrodites	$1 - \sigma$
Optimal	11	13	0.85	13	15	0.87
Social[b]						
With no other shrimp	12	14	0.86	11	16	0.69
With 2 other shrimp	12	14	0.86	12	18	0.67
With 15 other shrimp	13	15	0.87	16	17	0.94

[a]Because of the exponentially decaying nature of survival data (Peto et al. 1977), median scores rather than mean values are reported here.

[b]The three sex-ratio treatments show the number of individuals of the other sex present in the tank with the 5 focal animals.

Fig. 2. Survival curves for hermaphroditic (A) and male *E. texana* (B) under various sex-ratio treatments; h = hermaphrodites, m = males.

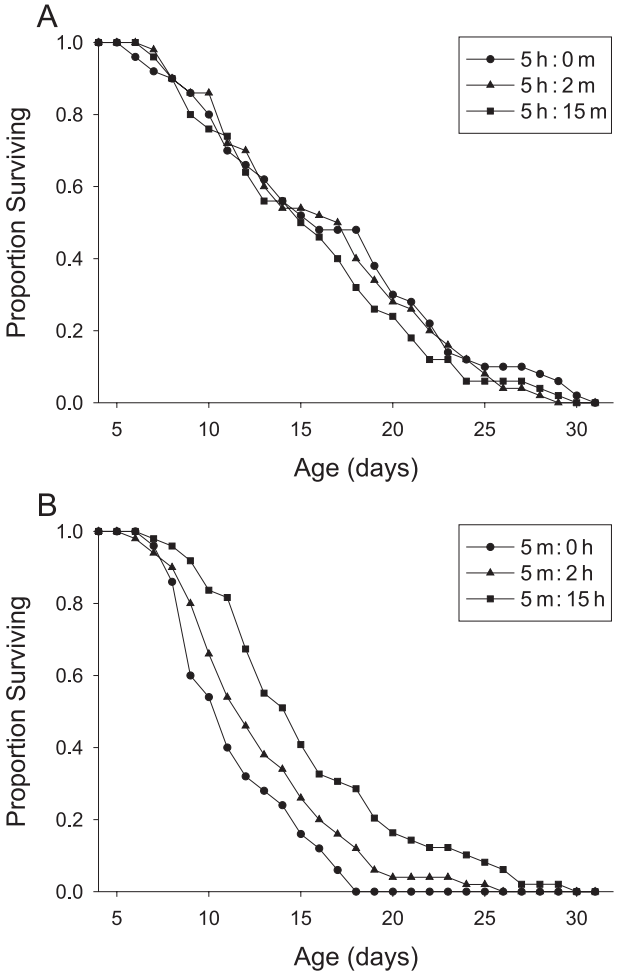
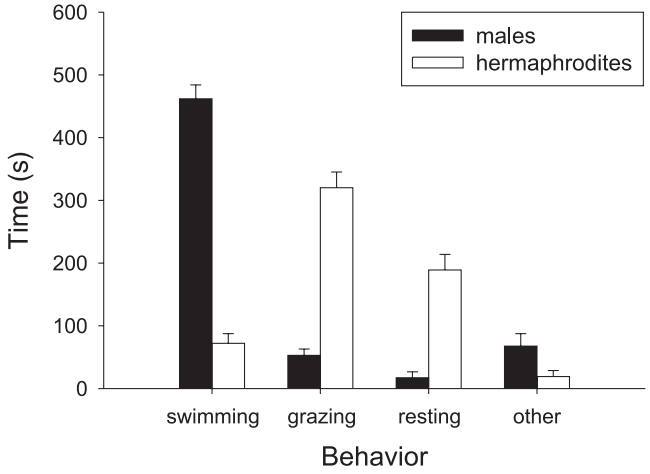


Fig. 3. Time budget (mean ± SE) for male and hermaphroditic *E. texana*. Both populations and all three sex-ratio treatments are pooled for each sex.

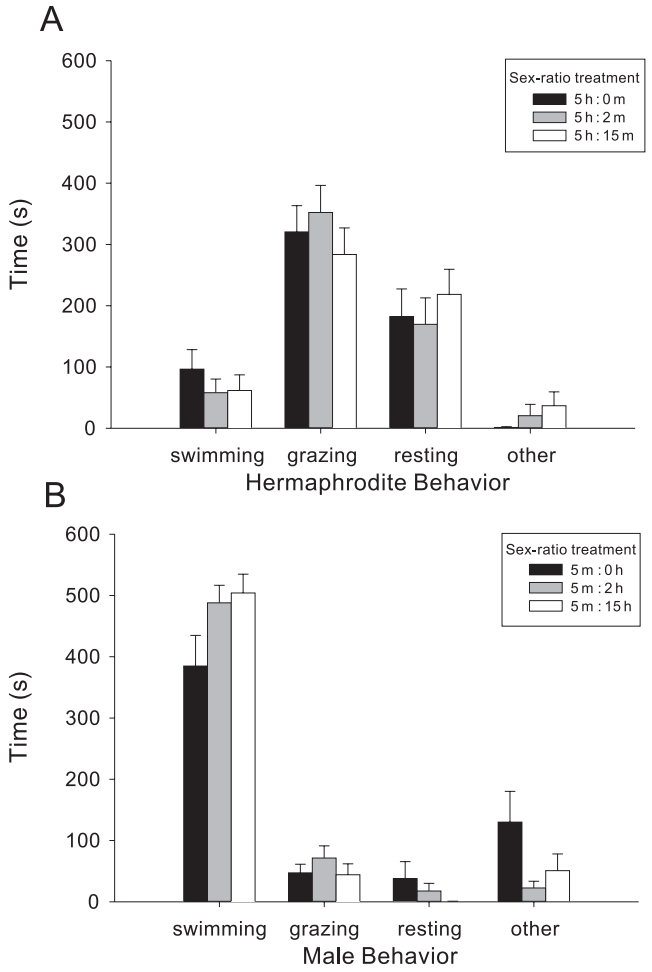


lished prior to our collaboration) implies that the longevity differences between the sexes are robust.

It came as no surprise that male longevity was less than that of hermaphrodites. While ours is the first systematic study of the relative longevity of *E. texana* males and hermaphrodites, several previous studies had suggested that hermaphrodites outlive males. Strenth (1977) found a decreasing proportion of males over time in two naturally flooded ponds in Texas, as did Knoll (1995) in small experimental laboratory populations. Our results suggest, however, that the viability of males relative to hermaphrodites is still quite high, ranging from 67 to 94%.

A possible explanation for the discrepancy in longevity between males and hermaphrodites can be gleaned from the behavioral observations. Males spent far more (88%) of their time in energy-consuming activities (swimming and interaction with others) than did hermaphrodites (15%). Consequently, males spent far less time in energy acquisition (grazing, 9%) and low energy expenditure activities (resting, 3%) than did hermaphrodites (53 and 32%, respectively). The imbalance between male and hermaphrodite energy expenditure is probably even greater, since males also swim significantly faster than hermaphrodites (Medland et al. 2000). An increased energetic cost of swimming rates in males relative to females (hermaphrodites) has been suggested in another, dioecious, clam shrimp (Eriksen and Brown 1980). Thus, males may simply have less total energy available, and use it faster, than hermaphrodites, resulting in a shorter life-span. However, while swimming, clam shrimp are filtering food particles from the water column (Pennak 1989) so the energy intake of males is greater than appears from the small amount of time they spend actively grazing. Furthermore, we currently do not know the relative investments in gamete production made by the two sexes. It is likely that this investment is greater in hermaphrodites, which would tend to decrease or

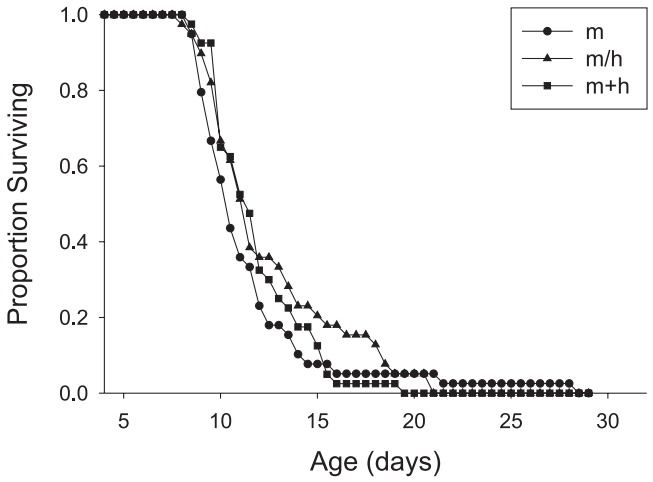
Fig. 4. Time budgets (mean ± SE) for hermaphroditic (A) and male *E. texana* (B) (populations pooled) maintained in different sex-ratio treatments.



equalize the apparent difference in energy expenditure seen in the behavioral data.

Differences in survival between the sexes appear to be evolutionarily anomalous. Such differences between the sexes will skew sex ratios towards hermaphrodites over the aging of a pond (as was found in a wild population by Strenth 1977). Since mating opportunities for males should increase as the sex ratio is skewed towards hermaphrodites, one might expect natural selection to favor equality of longevity between the sexes in order to equalize the lifetime sex ratios; this is similar to the arguments in favor of the evolution of equal sex ratios (Fisher 1958; Bodmer and Edwards 1960). This argument is further strengthened when one considers that generations do not overlap in this species, hermaphrodites continue to grow with age (Weeks et al. 1997), fecundity is positively correlated with size (Knoll and Zucker 1995) up to a point (Weeks et al. 1997; see below), and sperm storage is not observed in this species (Weeks et al. 2000*a*). Thus, it seems that survival parity in males and hermaphrodites would be advantageous because older hermaphrodites would be of "higher quality" (i.e., larger, with more eggs), and any male that lived longer than other males would have lower mate competition. Therefore, natural selection should increase the average male life-span, and the observation that males have shorter life-spans than hermaphrodites appears paradoxical.

Fig. 5. Survival curves for male *E. texana* from the WAL population maintained in tanks with no other shrimp (m), with 9 hermaphrodites across a screen barrier (m/h), and with access to 9 hermaphrodites (m + h).



One potential explanation for the discrepancy in survival concerns reproductive senescence in hermaphrodites. Egg production in hermaphrodites initially increases with age and size, but then plateaus and eventually decreases as the shrimp continue to age (Weeks et al. 1997). Thus, even though hermaphrodites may survive for longer than males, mating "quality" (in terms of quantity and quality of eggs produced) may actually decline near the end of a hermaphrodite's lifetime. If this is true, and males do not show equivalent reproductive senescence, it would explain the "paradox" of relative male survival: reproductive competency would be equivalent even though somatic-degradation rates would be different. Currently, studies are planned to assess this possibility.

Most studies of female/hermaphrodite longevity relative to mating opportunities reveal that various aspects of courtship and mating are costly. Male mating behavior has been reported to reduce female growth and survival rates in a related conchostracan (Roessler 1995). In tsetse flies (*Glossina morsitans morsitans*; Clutton-Brock and Langley 1997), harassment caused by an excess of males apparently significantly reduced female longevity. A study of the androdioecious nematode *C. elegans* by Gems and Riddle (1996) showed that mated hermaphrodites suffered a significant cost of mating in terms of longevity, but this was not due to harassment by males (or to the other factors that they tested). Their results contrast with those of a study by Van Voorhies (1992) in which no longevity cost of mating was observed for hermaphrodites of a different strain of the same species. Like Van Voorhies (1992), we detected no longevity cost of mating for hermaphroditic clam shrimp housed in groups with some (5 h : 2 m) or excess (5 h : 15 m) mating opportunities relative to those with no mating opportunities (5 h : 0 m). Nevertheless, the opportunity for male harassment of hermaphroditic clam shrimp does exist. Male clam shrimp often attempt to clasp nonreceptive hermaphrodites that then struggle to free themselves (Knoll 1995). The likely increase in

male harassment of hermaphrodites in tanks with 3 times more males (5 h : 15 m; note that more time was spent in "other" behaviors) did not lead to any detectable change in longevity in this study.

The finding that males with greater mating opportunities (5 m : 15 h) lived significantly longer than those kept with no (5 m : 0 h) or few hermaphrodites (5 m : 2 h) stands in stark contrast to previous findings for other taxa, where an increase in mating opportunities exacted a significant survival "cost." For example, male survival in the androdioecious nematode with a similar mixed mating system (*C. elegans*) decreased when males were housed with 3 times more hermaphrodites (Van Voorhies 1992) (but remained the same in a similar experiment carried out by Gems and Riddle 1996). Similarly, an increase in mating opportunities led to decreased survival of male fruit flies (Partridge and Farquhar 1981) and male tsetse flies (Clutton-Brock and Langley 1997).

Our behavioral observations suggest that in the absence of hermaphrodites, male–male interactions might have exacted a cost. Males tend to clasp any shrimp they encounter; when hermaphrodites are absent or rare, male–male encounters become more likely. These interactions occasionally lead to physical damage when a clasping male forcefully pushes another into the bottom of the tank (K. Wilson, personal communication). Natural populations of clam shrimp are typically hermaphrodite-biased (Sassaman 1995) and thus, males will generally encounter hermaphrodites and not other males. We therefore redesigned the experiment to eliminate male–male interaction by placing only a single male in each tank and providing him either with no opportunity to mate (m tanks and m/h tanks) or with the opportunity to mate with 9 hermaphrodites throughout their lives (m + h tanks). Under these conditions no difference in longevity was observed between males with and without mating opportunities, which suggests that male–male interactions were detrimental to male longevity in the first study. Nevertheless, we still did not detect a "longevity cost" of increased mating opportunities as has been found for other taxa (see above). A possible reason why the *E. texana* system differs from most others is that the absence of hermaphrodites does not appear to eliminate male "mating" behavior. Male "mating" behavior consists of locating a hermaphrodite, clasping it until it becomes receptive (several minutes to an hour or more), and thrusting for several seconds while eggs are extruded from the hermaphrodite's ovotestes. No preliminary courtship is involved. Mate searching, in the form of a high level of swimming activity, continued in the absence of hermaphrodites. If mate-searching behavior (i.e., swimming) is the most energetically costly portion of "mating" for males, there would be little difference in energy expenditure by males in the presence and absence of mating opportunities. Other studies have also shown that longevity costs associated with reproduction can occur as a result of preliminary activities such as courtship rather than gamete production or the mating act itself (Partridge and Farquhar 1981; Cordts and Partridge 1996; Clutton-Brock and Langley 1997).

Future studies should examine energy costs of swimming behavior and gamete production in order to more fully understand the differences in longevity between the sexes. For a species like *E. texana*, in which adults live for only about a week or so, hermaphrodites do not store sperm, males may mate multiple times per day, and clutch sizes increase dramatically with increasing size (age) (Knoll 1995; Weeks et al. 1997), even a slight decrease in longevity might prove to be biologically significant to males.

Testing Otto et al.'s (1993) model

When we combine our estimates of relative male viability ($1 - \sigma$) with previous estimates of inbreeding depression (δ) (Weeks et al. 1999, 2000*b*), we can begin to address the stability of *E. texana's* mixed mating system using Otto et al.'s (1993) model. The estimates of relative male viability reported here were found to range from 0.80 to 0.87 for JT4 and from 0.67 to 0.94 for WAL (Table 2). The lifetime cumulative inbreeding depression (δ) in these populations has been estimated as 0.50 for JT4 and 0.68 for WAL (Weeks et al. 2000*b*). Plugging these low (0.80 and 0.67) and high (0.87 and 0.94) estimates of relative male viability for JT4 and WAL, respectively, into Otto et al.'s (1993) model, we suggest that for the mixed mating systems found in these two populations to be evolutionarily stable, the following conditions must be met:

low: $\alpha > 1.25\beta$ (JT4)

and

$\alpha > 0.96\beta$ (WAL)

high: $\alpha > 1.15\beta$ (JT4)

and

$\alpha > 0.68\beta$ (WAL)

The parameter α (the proportion of eggs fertilized by the male) can vary between 0 and ∞, with the constraint that $\alpha \times u \leq 1$ (u is the frequency of males in the population and $\alpha \times u$ is the proportion of eggs fertilized by males; Otto et al. 1993). If we assume that male fertility is not frequency-dependent (i.e., α is constant, as modeled in Otto et al. 1993), and that male frequency is unlikely to exceed 50%, then the maximum value that α can reach is 2.0. Therefore, the "best case scenario" for the maintenance of males would be $\alpha = 2$ (i.e., males fertilize the equivalent of the lifetime egg production of two hermaphrodites). Because β (the proportion of remaining eggs fertilized by hermaphrodites) ranges from 0 to 1, the combination of the current estimates of relative male viability and inbreeding depression with the best case scenario for male mating rates (i.e., $\alpha = 2$) suggests that males can be maintained in either population even if all eggs not fertilized by males can be self-fertilized by hermaphrodites (i.e., $\beta = 1$). In *C. elegans*, it is estimated that hermaphrodites can only fertilize approximately 80% of their eggs when not mated to a male (Ward and Carrel 1979; Hodgkin and Barnes 1991), which if true for *E. texana*, would make the maintenance of males even more likely. However, preliminary estimates of β suggest that hermaphrodites can fertilize 100% of their eggs even when not mated to a male (i.e., $\beta = 1$). This suggests that in WAL, α must be greater than ~0.7–1.0 and in JT4, α must be higher than 1.2 for males to be maintained.

A second mechanism for the maintenance of males would be if α was not constant, but was frequency-dependent (Otto et al. 1993). If α is greater when males are rare, then males

are more likely to be maintained. Under this scenario, α could be very large (i.e., »1.25) when males are rare, thus allowing male maintenance. Mating systems like that of *E. texana* and *C. elegans*, where hermaphrodites do not cross-fertilize, are especially likely to exhibit such frequency-dependent selection, since rare males experience no competition when attempting to mate with a hermaphrodite.

Clearly, further work needs to be conducted to better estimate the remaining parameters of Otto et al.'s (1993) model. Experiments in progress will provide better estimates of α (V.G. Hollenbeck, A.D. Stafford, N. Zucker, S.C. Weeks, unpublished data) and β (S.C. Weeks, J.A. Hutchison, N. Zucker, unpublished data). Studies are planned to determine the dependence of α on male frequency. With these data, we will be able to more fully assess the selective forces maintaining males in *E. texana*.

The greatest relevance of the current system is to that of other androdioecious branchiopod crustaceans. Androdioecy has been inferred in three other conchostracans (Sassaman 1995) as well as a notostracan branchiopod (Sassaman 1991). Additionally, sex ratios described for several conchostracan species in the family Limnadiidae suggest that an additional nine species are androdioecious (Sassaman 1995), which would make androdioecy the most prevalent reproductive mode in this family. If these other conchostracans are also truly androdioecious, and if the genetic mechanism of sex determination in these species is found to be analogous to *E. texana*'s (genetic evidence from another conchostracan species suggests sex-linkage relationships similar to those described in *E. texana*; Sassaman, 1990), then our current examinations of Otto et al.'s (1993) model should further help to explain the preponderance of androdioecy in this crustacean family.

In conclusion, explaining why sex and outcrossing are so ubiquitous is one of the great unsolved puzzles in evolutionary biology. Studies of species in which two separate sexes are neither essential nor always maintained allow useful comparisons that should lead to an eventual understanding of this puzzle. The androdioecious mating system of *E. texana* is ideal for investigating the costs and benefits of males and the evolutionary forces that preserve them. A comprehensive understanding of these forces should clarify how and why outcrossing evolves, both in this species and generally.

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