Behavioral Cost of Reproduction in a Freshwater Crustacean (*Eulimnadia texana*)

Stephen C. Weeks, Thomas F. Sanderson, Bethany F. Wallace & Brian Bagatto

Program in Integrated Bioscience, Department of Biology, The University of Akron, Akron, OH, USA

Correspondence
Stephen C. Weeks, Program in Integrated Bioscience, Department of Biology, The University of Akron, Akron, OH 44325, USA.
E-mail: scw@uakron.edu

Abstract

It is commonly noted that investments in reproduction, both physiological and behavioral, can trade off with other life-history traits, such as growth and survival. In males, behavioral reproductive activities (e.g., copulations) are associated with weight loss, increased predation risk, reduced future reproductive output, and decreased lifespans. It is uncommon to find species in which increased copulations actually increase survival. Herein, we examine one such species, the androdioecious (males + hermaphrodites) crustacean *Eulimnadia texana*, in which increased copulations have been associated with increased lifespan. We examined two potential causes of this association: (1) males not copulating actually expend significant energy by searching for mates and (2) males are experiencing shorter lifespan primarily because they are more inbred than hermaphrodites. We found that isolated males did indeed expend more energy than hermaphrodites, consistent with previous studies showing that males swim over twice as much as hermaphrodites when isolated. Additionally, although inbreeding was associated with reduced lifespan, outcrossed males still had shorter lifespans relative to outcrossed hermaphrodites. Thus, isolated males consistently show decreased lifespans relative to isolated hermaphrodites, which is not explainable only on the basis of level of inbreeding. We conclude that the costly searching behavior of these males is the likely underlying cause of this observed difference in lifespan between the sexes, as previously suggested.

Introduction

The processes associated with reproduction have long been known to exact costs from organisms engaging in the behavior. Because females often invest more energy and time in yolking eggs, gestation, and birthing offspring than do males, it is commonly inferred that females should concomitantly endure a higher reproductive cost than males (Trivers 1972; Maynard Smith 1977). However, this is not universally true. Males can expend a great deal of energy in mating behavior, and in some cases, this energy investment can be comparatively high. One of the best documented examples of this is in the fruit fly *Drosophila melanogaster*. In a series of elegant experiments, Prowse & Partridge (1997) found that *D. melanogaster* males have approx. 30% higher mortality when performing mating behaviors with receptive females than when raised with no mating opportunities. Additionally, males immediately decreased their mortality rate when they were removed from receptive females, and control males almost immediately increased their mortality rate when switched into conditions with receptive females. Clearly, in these flies, the behaviors performed by the males to entice the females to mate are costly enough to reduce survivorship significantly (see also Partridge & Farquhar 1981).

Many subsequent studies have documented a variety of costs associated with mating behavior in males. Males performing more copulations with females have increased mortality in fish (Fleming 1996) and insects (Oliver & Cordero 2009). In fish (Vandenberghe 1992) and elephant seals (Galimberti et al. 2007), reproductively active males exhibit significant weight loss compared with males with lower or no reproductive activity. The act of copulation increases predation risk by reducing the effectiveness of escape behavior in butterflies (Almbro & Kullberg 2009), and males with high current investment in mating (both physiologically and behaviorally) have decreased future reproductive success compared with males with lower reproductive investment (Hughes et al. 2000; Lauwers & Van Dyck 2006). The act of copulation can even cause bodily harm: in crickets, females can damage males' wings and genitalia, and the risk of this type of injury increases with duration of copulation in these insects (Kuriwada & Kasuya 2009). In all of these examples, male costs of reproduction increase as copulation with females increases.

In one species of freshwater crustaceans, the opposite pattern has been reported: male costs of reproduction decrease when males experience increased copulations in the clam shrimp *Eulimnadia texana* (Zucker et al. 2001). In this species, males appear to experience an increase in lifespan when in the presence of receptive mates. Because *E. texana* is androdioecious (males and hermaphrodites, Sassaman & Weeks 1993), males mate with hermaphrodites. Male clam shrimp mate guard hermaphrodites (Knoll & Zucker 1995), and when they have greater mating opportunities, their lifespan increases by approx. 25% (Zucker et al. 2001). Zucker et al. (2001) speculated that this increase in survival was because males were expending more energy searching for mates than when mate guarding. When males are searching for receptive mates, they are known to swim over twice as fast and cover greater distances than hermaphrodites (Medland et al. 2000), and thus the notion that they are expending a great deal of energy in the process is a reasonable suggestion. If this proposed increased energy expenditure had an analogous effect on survival, as previously reported in other species (Fleming 1996; Oliver & Cordero 2009), then male clam shrimp would also have reduced survival because of behaviors associated with reproduction (i.e., the commonly termed 'cost of reproduction'; Reznick 1985). However, in *E. texana*, males would experience higher rather than lower survival in the presence of receptive mates.

Although Zucker et al. (2001) hypothesis is a logical extension of their findings, there is an alternative hypothesis that is equally logical for these shrimp. The unique sex determining system of these shrimp reveals that there are actually three reproductive types in these crustaceans: two genetically distinct but morphologically indistinguishable hermaphrodites ('monogenics' and 'amphigenics') and males (Sassaman & Weeks 1993). Sex appears to be determined by a sex chromosomal system in which maleness is recessive to hermaphroditism (Weeks et al. 2001b, 2010). Thus, monogenics are WW, amphigenics are ZW, and males are ZZ. Because hermaphrodites can self-fertilize or mate with males, there are actually five offspring types produced in these shrimp (Weeks et al. 2010): selfed monogenics (WW-S), selfed amphigenics (ZW-S), selfed males (ZZ-S), outcrossed amphigenics (ZW-O), and outcrossed males (ZZ-O; Table 1). Weeks et al. (2001a) noted that WW-S hermaphrodites had significantly lower survival than ZW-S hermaphrodites, even within the same clutch from the same parents. They suggested that this was likely due to associative overdominance wherein a suite of deleterious recessive alleles embedded within the sex chromosomes were likely expressed in WW-S but masked in ZW-S hermaphrodites. If this is true, then one would expect a similar decrease in survival for ZZ-S relative to ZW-S shrimp, which would imply that the previously reported lower survival rates of males (Zucker et al. 2001) could be primarily a reflection of high levels of ZZ-S and low levels of ZZ-O males being compared to primarily ZW-S hermaphrodites. Natural populations of *E. texana* are highly skewed toward amphigenic (ZW) hermaphrodites (Weeks et al. 1999), although the levels of ZW-S to ZW-O are not known. Nonetheless, it is certainly conceivable that lower male survival in this species is more a reflection of the differing ratios of these five

Table 1: Five offspring types possible in *E. texana* populations

| | Hermaphroditic parent | | | |
| | Selfing | | Outcrossing with ZZ | |
Offspring	WW	ZW	WW	ZW
Monogenic (WW)	WW-S	WW-S		
Amphigenic (ZW)		ZW-S	ZW-O	ZW-O
Male (ZZ)		ZZ-S		ZZ-O

Selfed offspring noted with a -S while outcrossed offspring noted with a -O.

offspring types than any survival cost associated with increased male mate-searching behavior.

Herein, we test both the notion that lower relative male survival is primarily attributable to high levels of ZZ-S males as well as assessing whether male swimming behavior indeed is more energetically demanding than hermaphrodite swimming behavior. We then use these results to assess whether decreased relative male survival can be explained by high reproductive costs, as originally suggested by Zucker et al. (2001).

Methods

Natural History of *Eulimnadia texana*

Eulimnadia texana inhabit temporary ponds, ditches, and many other ephemeral freshwater habitats throughout the southern United States, throughout Central America and into northern South America (Sassaman 1989; Pereira & Garcia 2001). Hermaphrodites produce desiccation-resistant cysts that they bury within the top several millimeters of the soil. These cysts hatch rapidly following hydration under spring and summer conditions (at water temperatures above 18°C), releasing nauplius larva. Growth is rapid, with shrimp reaching reproductive size (5–10 mm total length) in 4–7 d, both in the laboratory and in the field (Vidrine et al. 1987; Weeks et al. 1997). Total lifespan is only 2–4 wks (Weeks et al. 1997; Zucker et al. 2001). The hermaphrodites produce hundreds of eggs per day, generating clutches once or twice daily (Knoll 1995; Weeks et al. 1997). Sexual dimorphism is pronounced: thoracic appendages of hermaphrodites are unmodified, but in males, the first two pairs of thoracic appendages undergo differentiation into claw-like claspers which are used to hold onto the hermaphrodite's carapace during mate guarding (Dumont & Negrea 2002). Consistent with their shorter lifespans, mate guarding in the clam shrimp is also short, ranging from a few minutes to several hours (Knoll 1995; Weeks & Benvenuto 2008; Benvenuto et al. 2009). *Eulimnadia texana* is an omnivore, being able to filter feed and also forage along the pond bottom.

Project 1 – Survival Costs because of Inbreeding Depression

The experimental setup made use of the unique sex determining mechanism of *E. texana*. All offspring used in the current study were derived from amphi-

genic (ZW) hermaphrodites. When hermaphrodites are paired with males, they continue to self some proportion of their eggs (Weeks et al. 2004, 2010), so that each clutch of offspring from such matings contains the above-noted five identifiable categories of offspring (Table 1) in clutches produced by pairing a male with an amphigenic hermaphrodite. These five categories were then compared for differences in survival.

Shrimp rearing procedures followed those previously published (Weeks et al. 1999). The parental generation was reared from soil collected from Portal, Arizona, from a cattle tank earlier defined as WAL, which is known to contain *E. texana* cysts. After the shrimp matured, they were separated by sex into holding tubs for 24 h to ensure that any eggs the hermaphrodites had in their brood chambers were expelled, which ensured no eggs were produced via outcrossing before isolation (hermaphrodites cannot store sperm, Weeks et al. 2000). After this waiting period, male and hermaphrodite shrimp were paired in individually labeled, 500-ml cups to mate. After 7 d, the shrimp were frozen (−80°C) for allozyme surveys.

Cellulose acetate electrophoresis was run for five loci: Idh-1, Idh-2 (isocitrate dehydrogenase, EC 1.1.1.42), Mpi (mannose-phosphate isomerase, EC 5.3.1.8), Pgm, (phosphoglucomutase, EC 5.4.2.2), and Fum (fumarate hydratase, EC 4.2.1.2). Matings in which the parents were homozygous for alternate alleles at any locus and in which the hermaphroditic parent was heterozygous at one or more of the sex-linked loci (Fum, Idh-1, or Idh-2) were chosen for further analyses. The alternately homozygous loci allowed the detection of outcrossing, and any heterozygote at a sex-linked locus distinguished the amphigenic hermaphrodites from the monogenics (Weeks et al. 1999).

The isolation cups from mated parents with the above allozymic characteristics were then hydrated. The resulting nauplii were transferred to larger isolation tubs for 24 h. The offspring were left in these tubs until they matured and were then moved into individual 500-ml isolation cups for the duration of their life. Immediately after the offspring died, their total lifespan was noted before they were frozen for electrophoretic scoring.

Cellulose acetate electrophoresis was run on the offspring as described above for each locus previously identified from the parents. This information, combined with sexual identification (which is easily scored morphologically, Mattox 1959), allowed identification of the five mating types noted above.

Project 2 – Metabolic Costs of Swimming

Shrimp were reared from WAL soil in 37-l glass aquaria as noted above. When the shrimp attained sexual maturity (in approx. 7 d), their metabolism was assayed by placing them in 20-ml plastic syringes filled with water. After the shrimp acclimated for 5 min, we recorded an initial oxygen reading for each individual in the trial. We then set the timers for 15 min and opened the chamber every minute for 15 min to measure remaining dissolved oxygen using Powerlab ADInstruments (Colorado Springs, CO, USA). At the conclusion of data collection, dry weight was recorded for all experimental shrimp by drying them in an oven for 24 h at 50°C. Metabolism was calculated as the amount of oxygen consumed in a 15-min period divided by the dry weight of the shrimp (Bagatto et al. 2001).

Statistical Analyses

In project 1, lifespan was analyzed using the statistical package JMP (SAS 2009). Lifespan needed square-root transformation to normalize residuals and was analyzed in a blocked, one-way ANOVA. Offspring family was considered the blocked effect. Note that a two-way ANOVA using mating type (WW, ZW, and ZZ) and parental type (selfed and outcrossed) is not appropriate because WW hermaphrodites can only be produced via selfing (Sassaman & Weeks 1993), and thus, we can have no WWs that are outcrossed. The five offspring types (WW-S, ZW-S, ZZ-S, ZW-O, and ZZ-O; Table 1) constituted the main effect. Two *a priori* contrasts were performed in the ANOVA: a comparison of males to amphigenic hermaphrodites in the outcrossed group and males to amphigenic hermaphrodites in the selfed group.

In project 2, metabolism was compared between male and hermaphroditic shrimp using a simple one-way ANOVA, with oxygen consumption per unit dry weight as the dependent variable and sex as the independent variable. Oxygen consumption needed square-root transformation to normalize residuals.

For both analyses, means and standard errors were back-transformed to portray the results in the original units (see Figs 1 and 2).

Results

Lifespan differed significantly among the five offspring types (Table 2, Fig. 1). Lifespan was highest in the outcrossed amphigenic hermaphrodites (ZW-O)
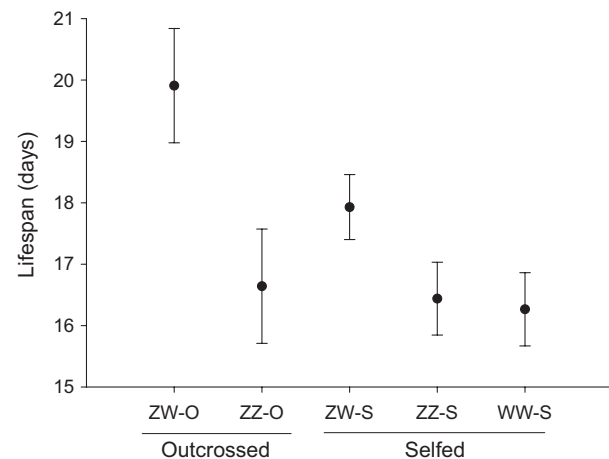

Fig. 1: Relative lifespans of offspring from the five mating types (see Table 1 for definition of mating types) reared in isolation cups. Error bars portray one standard error of the mean.
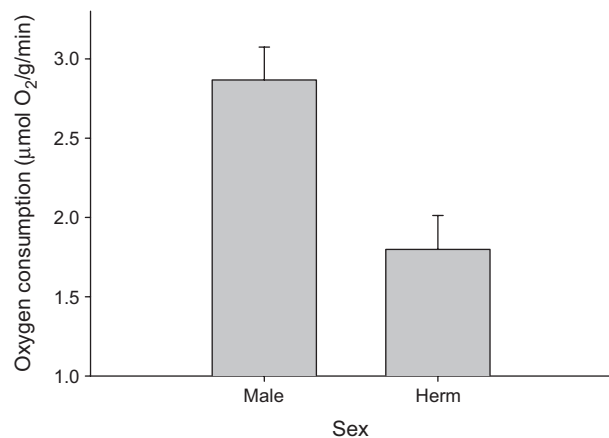

Fig. 2: Oxygen consumption of males and hermaphrodites (Herm) in isolation.

Table 2: ANOVA results for lifespan (square-root transformed) for the five offspring classes outlined in Table 1

Source	df	Sum of squares	F-ratio	p-value
Family	14	72.967	22.703	<0.0001
Offspring type	4	6.211	6.764	<0.0001
Error	504	115.702		
Contrasts				
(1) ZZ-O vs. ZW-O	1	1.706	7.428	0.0066
(2) ZZ-S vs. ZW-S	1	2.438	10.620	0.0012

and low for both categories of males (ZZ-O and ZZ-S; Fig. 1). Male survival was significantly lower than their amphigenic counterparts in both the outcrossed and selfed offspring types (Table 2, contrasts 1 and 2,

respectively). Clearly, when the effect of inbreeding was removed (i.e., by comparing only the males and hermaphrodites in the outcrossing offspring types; Table 2, contrast 1), males continued to have significantly lower survival than did hermaphrodites (Fig. 1).

In the oxygen consumption experiment, metabolic rates differed significantly between the sexes ($F_{1,58} = 12.954$; p = 0.0007; Fig. 2): metabolic rates of swimming were over 60% higher in males (2.70 μmol O_2/g/min) relative to hermaphrodites (1.71 μmol O_2/g/min).

Discussion

There is much evidence that reproduction is costly to both males and females (Reznick 1985; Vandenberghe 1992; Hughes et al. 2000; Galimberti et al. 2007; Kuriwada & Kasuya 2009). Commonly, it is assumed that increased contact between the sexes will lead to increased mating opportunities, which can then lead to increased costs of reproduction (e.g., Partridge & Farquhar 1981; Fleming 1996; Paukku & Kotiaho 2005; Brown et al. 2009; Oliver & Cordero 2009; Dao et al. 2010). However, in clam shrimp, increased mating opportunities have been associated with decreased costs of reproduction (Zucker et al. 2001), which is counter-intuitive.

Zucker et al. (2001) suggested that this counter-intuitive observation could be explained by noting the time budgets of males relative to hermaphrodites. When hermaphrodites were not paired with males, they spent the majority of their time grazing and filter feeding, both energetically efficient behaviors. On the other hand, when males were not paired with hermaphrodites, they spent their time swimming, most likely seeking out a mate, and spent very little time feeding. Medland et al. (2000) noted that male swimming behavior was three times as fast as hermaphrodite swimming, and thus, Zucker et al. (2001) suggested that unpaired males likely expended quite a bit of energy seeking out mates, and that this increased energy expenditure was the driving force reducing the survival of unpaired relative to both hermaphrodites and paired males.

Herein, we verified that unpaired males do indeed expend approx. 60% more energy when isolated compared to isolated hermaphrodites (Fig. 2). Thus, Zucker et al.'s (2001) suggestion of higher costs to males than hermaphrodites when seeking a mate is upheld in this study. When noting that males do not spend as much time feeding (grazing and filter feeding) when unpaired, the assumption that unpaired males may indeed have lower survival than paired males because of higher energy expenditure is quite reasonable. Zucker et al. (2001) reported that male lifespan became more equivalent to hermaphroditic lifespan as the sex ratios were more hermaphrodite-biased. Because paired males commonly lie on the bottom of the pool when clasping hermaphrodites (S. C. Weeks, pers. obs.), male behavior when paired is more similar to hermaphroditic resting/grazing behavior (Zucker et al. 2001) than when males are actively seeking a mate. If the increased energy expended during faster swimming manifests in shorter lifespan, as is noted in several other species (Buttemer et al. 2010; Criscuolo et al. 2010), then it is reasonable to suggest that the positive correlation of lifespan with increased mating opportunities in E. texana is because of reduced activities of males when in pairs, as suggested by Zucker et al. (2001).

There are other studies that have noted a positive relationship between mating activity and male longevity. In bighorn sheep, a positive correlation between male longevity and mating behavior was documented (Pelletier et al. 2006). However, Pelletier et al. suggested that this positive correlation was more because of the social dynamics of these sheep in that larger, more robust males were more likely to garner mates, and thus the correlation was driven by a dominance hierarchy rather than a difference in energetic output between mating vs. searching behaviors noted in these clam shrimp. Similar results for other vertebrates have also suggested a positive correlation between successful intraspecific competition for mates and longevity (Alatalo et al. 1991; Petrie 1992; McElligott & Hayden 2000). However, we are unaware of any other study showing that increased mating opportunities directly lead to increased survival rates in males.

Because of the unique mating system found in E. texana (Sassaman & Weeks 1993), it is possible that previously reported lower survival of males raised in isolation (Zucker et al. 2001) may have been an artifact of comparing inbred males to 'amphigenic' hermaphrodites which do not fully express inbreeding depression because of 'associative overdominance' on the sex chromosomes (Weeks et al. 2001a, 2010). In these shrimp, sex is determined by a Z-W sex chromosomal system (Weeks et al. 2010). In this system, males are homogametic (ZZ) while hermaphrodites can be heterogametic (ZW; termed 'amphigenic') or homogametic (WW; 'monogenic'). The amphigenic hermaphrodites exhibit associative overdominance in that they do not express deleterious recessive alleles

embedded on the heterogametic sex chromosomes (ZW). Because the sex chromosome is predicted to carry the bulk of the alleles causing inbreeding depression in these shrimp (Pannell 2008), comparing males to hermaphrodites that are produced via self fertilization could confound any fitness effects of sex with expression of inbreeding depression. Herein, we uncoupled the fitness effect of sex from inbreeding depression by specifically noting which offspring had been produced via selfing vs. outcrossing (Table 1) and noting the relative survival of all five offspring types (Fig. 1). As noted previously (Weeks et al. 2001a), selfed amphigenic hermaphrodites (ZW-S) were not as negatively affected by inbreeding depression as the other two sexual types (Fig. 1; Table 2), which is consistent with the notion of overdominance. However, when only considering the outcrossed offspring (ZZ-O and ZW-O), males continued to show lower survival than hermaphrodites (Fig. 1; Table 2 contrast 1). Thus, being male has some survival cost that is independent of any inbreeding effect, at least when the clam shrimp are raised in isolation.

In conclusion, the current study reveals an increased energetic output for males relative to hermaphrodites in isolation, which is consistent with arguments by Zucker et al. (2001) that male *E. texana* show increased lifespan with increased mating opportunities because of a high cost of mate searching relative to mate guarding. A direct comparison of longevity in isolated males and hermaphrodites known to be derived from outcrossing revealed a survival cost to maleness, which again is consistent with Zucker et al.'s argument of a searching cost to males in this species.

Acknowledgements

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