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The Effect of Temperature on the Reproductive Success of Male Waterbugs (Belostoma flumineum)

Michael Philip Dmytriw

Abstract

Sexual selection theory predicts that the sex that shows a greater parental investment in the offspring should be limiting to the reproductive success of the opposite sex. Males of the giant waterbug *Belostoma flumineum* exhibit greater parental investment than females by providing all of the parental care for the eggs, which are firmly attached to their dorsal surface. While male backspace availability can be limiting to female reproductive success, it has previously been found to be more limiting during the spring than during the fall, due to newly emerged males being able to breed more quickly than newly emerged females. In light of different thermal regimes in the two breeding seasons, this study examined the relationship between ambient temperature and male backspace availability. Males were exposed to one of four temperature treatments within the laboratory: mating and brooding at 20°C, mating at 20°C and brooding at 26°C, mating at 26°C and brooding at 20°C, or mating and brooding at 26°C. Overall, the size of the egg pad was larger and the time until mating was shorter for males that mated at 26°C, while the time spent brooding was longer for males that brooded at 20°C. The proportion of males that received an additional egg pad, when they had the opportunity, was found to be greater than that of males that received any egg pads at all. While the temperature treatment and the number of brooding attempts were not associated with the result of brooding, the rate of brooder mortality was found to be higher than that of non-brooders when males were exposed to different mating and brooding temperatures. The high rate of brooder mortality, in addition to the delayed date of mating events and relatively long brooding periods observed in this study, are not consistent with previous studies and may be due to a recent change in the natural population of *B. flumineum*.

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The Effect of Temperature on the Reproductive Success of Male Waterbugs (*Belostoma flumineum)*

by

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Introduction

In many species the reproductive success of males is limited by the number of females available to mate with, while the reproductive success of females is limited by the number of eggs that she can produce (Bateman, 1948). As a result, such species often exhibit significant variation in reproductive success among males, as reproduction is positively correlated with the number of successful mating attempts for each male. Since female reproductive success is dependent on the number of eggs that each female is able to produce, it does not increase much with additional matings and is therefore less variable than that of males (Bateman, 1948). While Bateman (1948) attributed this difference in reproductive success between the sexes to a difference in the energetic investment involved in gamete production (eggs are more expensive to produce than sperm), Trivers (1972) demonstrated that total parental investment, in gamete production as well as in the care of the offspring, determines the reproductive success of each sex. Consequentially, females may not always be limiting to male reproductive success, but rather, the sex that makes the larger parental investment in the offspring becomes a limiting resource to the reproductive success of the other sex and should exhibit less variation in reproductive success than the other (non-limiting) sex (Trivers, 1972).

Belostoma flumineum, a predaceous insect commonly found in ponds throughout the United States (Smith, 1976a), is an organism in which males make a greater parental investment than females. Females deposit their eggs as a cohesive pad on the dorsal surface of males, who provide all of the postcopulatory parental care for the fertilized eggs (Smith, 1976a) until they either hatch or become detached prior to hatching (Smith, 1976a; Kight and Kruse, 1992). Males exhibit unique brooding behaviors which serve, in part, to prevent desiccation of the eggs as well as to facilitate embryonic gas exchange (Smith, 1976a). Egg pads that are exposed to the air for an extended period of time become brittle and are likely to detach from the male's dorsal surface (Smith, 1974). Male brooding behavior is critical to the successful hatching of the eggs, as egg pads that have been removed from the dorsal surface of the male (Smith, 1976a; Smith, 1976b; Kraus et al., 1989) or that have been laid on a female's dorsal surface (Kruse and Leffler, 1984; Estévez et al., 2006) are more likely to be inviable than those that remain fixed to the male.

Since males of *B. flumineum* provide all of the care towards the developing eggs (Smith 1976a), theory predicts that males should be limiting to female reproduction (Trivers, 1972). Male backspace is a finite resource, and male backspace availability has previously been found to limit female reproductive success in several species of giant waterbugs (Smith, 1979; Ichikawa, 1989; Kraus, 1989; Kruse, 1990). However, populations of *B. flumineum* in temperate North America have two distinct breeding seasons, one in the spring and one in the fall (Kruse, 1990; Kight et al., 2011), and Kruse (1990) found that the availability of male backspace varies between these two breeding seasons. During the spring, male backspace appears to limit female reproduction, as almost all males are encumbered with eggs and the majority of these males are completely encumbered; their backspace is completely filled with eggs. On the other hand, during the fall breeding season male backspace appears to be less limiting, as almost half of the males are not carrying an egg pad, and of those that are a smaller proportion of them are fully encumbered compared to during the spring breeding season (Kruse, 1990).

Several factors could contribute to this discrepancy in male backspace availability throughout the year, such as the amount of time required until sexual maturation. *Belostoma flumineum* fully emerge as adults prior to the fall breeding season, and newly emerged males have been found to become sexually mature earlier, and are therefore able to mate sooner, than newly emerged females (Kruse, 1990). Since more males than females would be available to mate early in the fall breeding season, this relative increase in sexually available males could account for the lower proportion of encumbered males during the fall (Kruse, 1990).

Perhaps one of the most important variables that could influence the observed differences in male backspace availability (Kruse, 1990) is the difference in ambient temperature between the spring and fall breeding seasons. Changes in temperature affect several aspects of the biology of an ectotherm, including metabolic rate (Gillooly et al., 2001), energy assimilation (Angilletta, 2001) and required energy intake (Dorcas et al., 2004). Changes in temperature have also been found to influence the length of the brooding period in giant waterbugs (Kraus et al., 1989; Kruse, 1990; Ichikawa, 1993) as well as the rate of egg production in giant waterbugs (Kraus, 1989) and other ectotherms (Hirche et al., 1997).

Warmer temperatures, as found during the fall breeding season (Kruse, 1990), have previously been demonstrated to increase the rate of egg development in giant waterbugs, thereby decreasing the amount of time males spend brooding an egg pad (Kraus et al., 1989; Kruse, 1990; Ichikawa, 1993). Males have been found to be sexually unavailable and refuse to accept additional eggs from females while brooding (Smith, 1979). Shorter brooding periods would therefore reduce the amount of time that males are unavailable to females, which could result in male backspace becoming less limiting to female reproduction at warmer temperatures.

Although warmer temperatures may produce an increase of sexually available males as a result of a reduction in required brooding time (Kraus et al., 1989; Kruse, 1990; Ichikawa, 1993), female sexual availability may also increase due to an increase in the rate of egg production (Kraus, 1989; Hirche et al., 1997). The relationship between the effect of temperature on both egg development and egg production is important in influencing the availability of male backspace. For example, if the reduction in brooding time due to increased temperatures is equal to the reduction in the time required for females to produce a clutch of eggs, then available backspace is not likely to change with shifts in ambient temperature. However, Kraus (1989) demonstrated that in a related species of giant waterbug, *Abedus indentatus*, increases in temperature result in a larger reduction in the time required for egg development compared to that for egg production, suggesting that despite the positive effect of temperature on both processes, an increase in temperature could result in male backspace becoming less limiting to female reproduction.

In this study, I examine the effect that changes in temperature may have on male backspace availability as a result of temperature dependent changes in both brooding time and egg production. If ambient temperature is important in shaping the previously observed temporal variation in male backspace availability within *B. flumineum* (Kruse, 1990), then I expect to find more available backspace when waterbugs are exposed to warmer temperatures. Alternatively, if temperature is not an important contributor to the differences in backspace availability between the spring and fall breeding seasons, and other factors such the rate of sexual maturation are more important, I expect to find little variation in available backspace between different temperatures.

Methods

Waterbugs used in this study were captured using aquatic dip nets from ponds throughout Essex, Passaic and Sussex Counties, NJ from August to October, 2012. Silver Lake (Hardyston Township, Sussex County, NJ) served as the primary collection site for this study. After collection, individuals were transported to the laboratory in plastic coolers containing pond water and vegetation.

Belostoma flumineum shows little sexual dimorphism, and the sex of bugs was determined in the laboratory through examination of the genital plate; females exhibit two apical tufts of setae, while males lack this trait (Lauck and Menke, 1961). Males and females were kept at room temperature (approximately 23°C) in separate 18.9-liter aquaria containing dechlorinated tap water and plastic vegetation until the start of the study.

On September 25, after a sufficient number of waterbugs had been collected, four experimental populations of 20 males and 20 females were established in four separate 37.9-liter aquaria, each filled with approximately 16.7-liters of dechlorinated tap water. The water level within these aquaria was adjusted periodically to maintain a relatively constant volume and was replaced approximately every two to three months in order to minimize algal and fungal growth. Plastic vegetation was provided for use as perching sites and waterbugs were fed dragonfly naiads and crickets *ad libitum*.

Males within each aquarium were individually marked on the pronotum using a coding system of colored nail polish. Marks were observed to become dislodged after two to three weeks, however, and a layer of Gorilla™ Super Glue was then applied over the colored marks, which was found to be more effective.

The four experimental aquaria were housed in environmental chambers on a photoperiod of 14L:10D hours and set at either 20°C or 28°C (two of the aquaria were kept at 20°C and the other two at 28°C), which approximate water temperature during the spring and fall breeding seasons, respectively (Kruse, 1990). In an effort to minimize unexpectedly high rates of mortality the chamber initially set at 28°C was lowered to 26°C by the time breeding had begun on November 19.

Each of the aquaria was checked three to five times per week for the presence of eggs. When a male was observed carrying an egg pad, the number of eggs was counted, the length of time until mating was recorded, and the individual was removed from the aquarium and placed in a small plastic container containing approximately 500 ml of dechlorinated tap water and plastic vegetation. Depending on which aquarium the encumbered male was removed from, the isolated male was either kept within the same environmental chamber (at the same temperature) or transferred to the other chamber, thereby creating four different temperature treatments: mating and brooding at 20°C (20/20), mating at 20°C and brooding at 26°C (20/26), mating at 26°C and brooding at 20°C (26/20), or mating and brooding at 26°C (26/26). Isolated males were checked periodically until brooding was complete, either due to the death of the brooding male, removal/dislodging of the egg pad or hatching of the eggs, at which time the male was returned to the main aquarium from which he was previously removed. The length of the brooding period and the result of brooding were recorded.

The sex ratio of each of the four experimental aquaria was initially 1:1 (20 males and 20 females). The isolation of brooding males, however, produces a temporary shift in the sex ratio in favor of females until these males are returned to the population upon the completion of brooding, thereby restoring the equal sex ratio. Smith (1979) showed that while brooding, males do not accept additional eggs from females, and are therefore temporarily removed from the sexually available population, resulting in a change in the operational sex ratio. In this experiment, the shift in the operational sex ratio due to the isolation of brooding males mirrors that which occurs under natural conditions. Therefore, no further adjustments to the experimental populations were made upon the removal of encumbered males.

Individual waterbugs were replaced upon mortality, thereby maintaining populations of 20 males and 20 females within each experimental aquarium. These replacement waterbugs were generally taken from reserve aquaria that served to acclimate individuals to the experimental treatment temperatures. No replacement males were observed to have previously mated within these reserve aquaria. One reserve was kept within each of the two environmental chambers and was identical to the experimental aquaria in all ways except that both the number of individuals and the sex ratio within these reserves were not controlled and varied over the course of the experiment, depending on the number available bugs that had been collected.

Not all waterbugs used in this experiment were first acclimated to their new environment within a reserve aquarium, however. The reserves were not established until October 5, and any individuals used in the experiment before this date - including the initial populations - were introduced directly from aquaria housed at room temperature. Additionally, several times during the experiment, the number of males within the reserve aquaria reached zero, and replacement individuals were either obtained from the other reserve (not at the experimental temperature), from aquaria kept at room temperature or were collected in the field.

The populations within each of the four experimental aquaria were maintained at 20 males (including any isolated brooding males) and 20 females until January 15, at which time no more males were available either in the reserves or at room temperature and the collection of additional males was not possible due to winter conditions. After this date, as waterbugs perished, individuals were added or removed from the experimental aquaria as needed, so that the sex ratio remained at 1:1. However, as no replacement males were available, the total number of individuals within each of the experimental aquaria steadily declined.

26/26 and 26/20 reached a population of zero males on March 5 and April 23, respectively, thereby concluding data collection for these temperature treatments. Data collection for 20/20 and 20/26 concluded on May 20, several weeks after the last observed mating event. At this time, there were seven males remaining in 20/20 and five males remaining in 20/26.

All statistical analysis was performed through the use of vassarstats.net, SAS 9.3 (for ANOVA post-hoc comparisons) or JMP Pro 10 (for two-way ANOVAs). All assumptions of the performed statistical tests were untested and were assumed to be met.

Results

Egg pad size

There were a total of 70 mating events in the aquaria housed at 26°C (all matings occurred after the temperature was lowered from 28°C) (26/20: n = 34; 26/26: n = 36) and 35 matings in the aquaria housed at 20°C (20/20: n = 12; 20/26: n = 23). The size of the resulting egg pad from these matings was significantly larger in the warm aquaria (Figure 1) than in the cold aquaria (Figure 2) (Student-t: $t_{(103)} = 5.20$, $p = 1.06*10^{-6}$).

Only 51 of 98 different males took part in the 70 mating events within the warm aquaria. 38 of these males received only one egg pad, seven males received two egg pads and six males received three egg pads. When comparing among males who mated either one, two or three times, there was no difference in the number of eggs within the first pad (ANOVA: $F_{(2,48)} = 0.892$, $p = 0.416$). There was also no difference in the number of eggs within the second pad (ANOVA: $F_{(1,11)} = 0.0794$, $p = 0.783$) compared among males that mated two or three times.

28 of 54 males took part in the 35 matings that occurred within the aquaria housed at 20°C. 23 of these males received only one egg pad, four males received two egg pads and one male received four egg pads (no males received only three egg pads). There was no difference in the number of eggs received within the first pad of males who mated once, twice or four times (ANOVA: $F_{(2,25)} = 0.476$, $p = 0.627$) or within the second pad of those who mated two or four times (ANOVA: $F_{(1,3)} = 0.270$, $p = 0.639$).

Considering only those males that mated multiple times (at either 20°C or 26°C), there is a positive correlation between pad size and each subsequent brooding attempt ($r = 0.328$, $n = 44$, $p = 0.0297$; Figure 3). However, when these same pad size data are compared to the date of each mating there is no relationship ($r = -0.175$, $n = 44$, $p = 0.257$; Figure 4). There is a negative correlation when egg pad sizes from all mating events are compared across time ($r = -0.248$, $n = 105$, $p = 0.0108$; Figure 4). For only those matings that took place at 26°C ($r = -0.0699$, $n = 70$, $p = 0.565$) or at 20°C ($r = 0.0182$, $n = 35$, $p = 0.917$) there was no significant relationship between pad size and the date of mating.

Time until mating

Considering only those waterbugs introduced to the experimental aquaria on September 25, at the start of the experiment, males in the warm aquaria ($n = 14$) received eggs significantly faster than those in the cold aquaria ($n = 20$) (Student-t: $t_{(32)} = 3.96$, $p = 4.36*10^{-4}$). Only the initial populations of males were included in this analysis, as the date on which a male was added to the aquarium may have influenced the time until reproduction, since the first observed mating was not until November 19 and January 10, within the warm and cold aquaria, respectively.

For males that received multiple egg pads, there was no difference in the number of days between each subsequent egg pad (ANOVA: $F_{(2,23)} = 0.311$, $p = 0.736$). Additionally, males that had a shorter interval between broods appeared to receive a larger pad, although this effect is not significant ($r = -0.259$, $n = 26$, $p = 0.202$; Figure 5). The removal of an outlier for inter-brood intervals (69 days) which was twice as long as the next largest interval (Dixon's test: $p < .010$), resulted in no change in the length of time between successive pads (ANOVA: $F_{(2,22)} = 1.37$, $p = 0.274$), but did produce a negative correlation between inter-brood interval and pad size ($r = -0.371$, $n = 25$, $p = 0.0675$).

Brooding results

There were three possible results to any brooding event: death of the brooding male, removal/dislodging of the egg pad or hatching of the eggs. There were 105 total brooding attempts: 71 resulted in the death of the brooding male, 28 resulted in removal/dislodging of the egg pad and six resulted in hatching of a portion of the egg pad (Table 1). Both the size of the egg pad (ANOVA: $F_{(2,102)} = 13.3$, $p = 7.14*10^{-6}$) and the length of the brooding period, in days (ANOVA: $F_{(2,102)} = 69.2$, $p = 1.09*10^{-19}$), differed among brooding events that ended in the three different possible results. In both cases, post-hoc, pair-wise comparisons using Duncan's multiple range test showed that pad size is significantly larger and that the brooding period is significantly longer for broodings that resulted in the hatching of the eggs, compared to those that resulted in either the death of the brooding male or in the removal of the eggs pad (Table 1).

Temperature effects on pad size

A two-factor ANOVA comparing mating and brooding temperatures to egg pad size showed that among all brooders, males that mated at 26°C had larger egg pads than those that mated at 20°C ($F_{(1,101)} = 27.2$, $p < 0.0001$; Table 2). There was no relationship between pad size and brooding temperature ($F_{(1,101)} = 6.69*10^{-2}$, $p = 0.796$) or between pad size and the interaction of mating and brooding temperature ($F_{(1,101)} = 1.65$, $p = 0.202$; Table 2).

Among only brooding events that resulted in the death of the brooding male, a two-factor ANOVA showed that males that mated at 26°C had larger egg pads than males that mated at 20°C ($F_{(1,101)} = 16.8$, $p = 0.0001$; Table 2). Again, brooding temperature ($F_{(1,101)} = 1.38$, $p = 0.244$) and the interaction between mating and brooding temperature ($F_{(1,101)} = 0.347$, $p = 0.558$) had no significant relationship to pad size (Table 2).

Among those that aborted their egg pad while brooding, males that mated at 26°C ($F_{(1,101)} = 9.58$, $p = 0.0049$) and males that brooded at 20°C ($F_{(1,101)} = 4.48$, $p = 0.0450$) had larger egg pad sizes than those that mated at 20°C and brooded and 26°C, respectively (Table 2); the only case in which brooding temperature had a significant relationship to pad size. There was no significant interaction between mating and brooding temperatures in this case ($F_{(1,101)} = 0.526$, $p = 0.475$; Table 2).

No males that mated at 20°C successfully brought their eggs to hatching. Five males that brooded at 20°C and one that brooded at 26°C (all mated at 26°C) successfully hatched a portion of their egg pad, but there was no relationship between brooding temperature and egg pad size among these males (Student-t: $t_{(4)} = 0.903$, $p = 0.418$; Table 2).

Temperature effects on brooding length

A two-factor ANOVA comparing mating and brooding temperature to the length of the brooding period showed that males that brooded at 20°C brooded for a significantly longer period of time than those that brooded at 26°C ($F_{(1,101)} = 4.32$, $p = 4.02*10^{-2}$; Table 2). Mating temperature ($F_{(1,101)} = 0.615$, $p = 0.435$) and the interaction between mating and brooding temperature had no significant relationship to brooding time ($F_{(1,101)}$ = 0.935, p = 0.336; Table 2).

Among brooding events that resulted in the death of the brooding male, a two-factor ANOVA showed that males that brooded at 20°C had a longer brooding time than males that brooded at 26°C ($F_{(1,101)}$ = 7.14, p = $9.5*10^{-3}$; Table 2). Again, there was no relationship either between brooding time and mating temperature ($F_{(1,101)}$ = 0.634, p = 0.429) or between brooding time and the interaction of mating and brooding temperatures ($F_{(1,101)}$ = 1.12, p = 0.294; Table 2).

For only those males that aborted their egg pad while brooding, there was no significant effect of mating temperature ($F_{(1,101)}$ = 1.48, p = 0.235) or of brooding temperature ($F_{(1,101)}$ = 1.83, p = 0.188) on the length of the brooding period; the only situation in which brooding temperature did not have a significant relationship with the length of brooding. Although not significant, males that brooded at 26°C appeared to have had longer brooding times compared to those that brooded at 20°C (Table 2); a reversal in the trend shown in all other cases in which males brooded for a longer period of time at 20°C than at 26°C. The interaction between mating and brooding temperature had no relationship to the length of brooding ($F_{(1,101)}$ = 0.0250, p = 0.876; Table 2).

No males that mated at 20°C successfully brought their eggs to hatching. Five males that brooded at 20°C and one that brooded at 26°C hatched a portion of their egg pad. Of these males, those that brooded at 20°C brooded for a longer period of time than males at 26°C (Student-t: $t_{(4)}$ = 3.64, p = 0.0220), although sample size was very small (Table 2).

First-time brooders

Out of the 105 total brooding events, 79 were cases in which the male was brooding his first egg pad. Within these first-time broodings, when comparing males who brooded at warm (26°C) vs. cold (20°C) temperatures (Figure 6) and males who mated and brooded at the same (20/20 and 26/26) vs. at different (20/26 and 26/20) temperatures (Figure 7), there were no differences in the proportion of males that died while brooding (fisher's exact test: warm vs. cold, $p = 0.623$; same vs. different, $p = 0.326$), aborted their egg pad (fisher's exact test: warm vs. cold, $p = 0.290$; same vs. different, $p = 0.185$) or successfully hatched their egg pad (fisher's exact test: warm vs. cold, $p = 0.317$; same vs. different, $p = 0.630$). A subsample of only those males that did not die while brooding (i.e. were able to complete brooding either via removal or hatching of the egg pad) shows no difference in the proportion of males that aborted or hatched their egg pad (fisher's exact test: warm vs. cold, $p = 0.260$; same vs. different, $p = 0.317$).

Multiple brooders

Of all 79 first-time brooders, only 21 males survived to later be returned to the experimental aquarium and had the opportunity to receive a second pad. Of these 21 males, 18 received a second pad (the other three were still alive at the end of data collection). Only nine of these 18 males survived to be returned to their respective experimental aquarium after brooding, and seven of them received a third pad (the other two were still alive at the end of data collection). Of the seven males that received a third pad, only one male survived to be returned to the main aquarium. This male received a fourth pad, but ultimately died while brooding it. No males, therefore, had the opportunity to receive a fifth egg pad (Figure 8). Males that brooded a pad and were later returned to the experimental aquarium were significantly more likely to receive an additional egg pad, compared to the proportion of all males that received a first pad. (79/152 males received a first pad; 26/31 males received an additional pad) (fisher's exact test, $p = 1.17 * 10^{-3}$). There was no difference in the proportion of males that either died while brooding (fisher's exact test, $p = 0.289$), removed their egg pad (fisher's exact test, $p = 0.275$) or hatched a portion of their egg pad (fisher's exact test, $p = 0.432$) between brooding attempts (Figure 8).

Mortality

The rate of mortality while brooding was extremely high. 71 of 105 brooding attempts ended with the death of the brooding male (Table 1) and in three of the six cases in which the male hatched a portion of his egg pad, he died soon afterwards. The proportions of males that died with and without previous brooding experience were calculated on a weekly basis from the time that mating had begun (20/20: Jan 10; 20/26: Jan 10; 26/20: Nov 24; 26/26: Nov 19) until the end of data collection (20/20: May 20; 20/26: May 20; 26/20: April 23; 26/26: March 5) for each of the aquaria. A comparison of these proportions showed that in 20/26 (paired samples t-test: $t_{(18)} = 5.85$, $p < 0.0001$; Figure 9) and in 26/20 (paired samples t-test: $t_{(22)} = 1.99$, $p = 0.0592$; Figure 10) the proportion of males that died with brooding experience was greater than that of males that had not brooded. There was no difference in the proportions of males that died in 26/26 (paired samples t-test: $t_{(16)} = 1.13$, $p = 0.274$; Figure 11). In 20/20, the proportion of males that died with no previous brooding experience was actually greater than that of males with brooding experience (paired samples t-test: $t_{(18)} = 3.26$, $p = 4.21*10^{-3}$; Figure 12). The total number of males with and without brooding experience at the end of the experiment was small in some cases, however, especially within the aquaria housed at 20°C (20/20: brooding experience, n = 9, no brooding experience, n = 19; 20/26: brooding experience, n = 19, no brooding experience, n = 7; 26/20: brooding experience, n = 26, no brooding experience, n = 19; 26/26: brooding experience, n = 25, no brooding experience, n = 28).

Discussion

Variations in temperature have a substantial effect on metabolic rate (Gillooly et al., 2001), particularly within ectotherms (Dorcas et al., 2004). Warmer temperatures, which result in higher metabolic rates (Gillooly et al., 2001), have previously been shown to increase the rate of egg production in giant waterbugs (Kraus, 1989) and other invertebrate ectotherms (Hirche et al., 1997). Such an increase in egg production suggests that females should be able to not only lay a greater number of eggs, but to do so more quickly, when housed at warmer temperatures. Data from the present study show that the size of the egg pads carried by males was larger (for all males, as well as for only those males that died while brooding or discarded their egg pad) and that the time taken to receive these egg pads was shorter for males housed at 26°C, compared to those housed at 20°C. Ichikawa (1989) demonstrated a similar effect of temperature on egg pad size, as males were found to carry smaller egg pads earlier in the breeding season; a finding attributed to reduced fecundity of females due to lower temperatures earlier in the year. Similarly, Kruse (1990) found that females of *B. flumineum* were able to remate a few days sooner in July than in May, suggesting that warmer temperatures in July may increase the rate of egg production. However, in contrast to the present study, both of these previous studies were conducted in the field and it is possible that additional factors such as seasonal variation in food availability may have also contributed to their findings.

While egg pad size differed between males housed at 20°C and at 26°C, the data from this study show no differences in the size of the first or second egg pad between males who received either one, two or 3+ pads (at each of the two mating temperatures) or in the time elapsed between each subsequent egg pad. It should be in the interest of males to brood the maximum number of eggs that can be carried, and once brooding is complete to receive any additional egg pads as quickly as possible, thereby maximizing their fitness for the breeding season. If all males that received an egg pad behaved in this way, there should be no differences among males in the size of the pad received or in the interbrood interval, assuming that egg availability did not vary during this study. Alternatively, sample size may have been too small to accurately differentiate between various potential reproductive strategies, as only 18, seven and one males received a second, third and fourth egg pad, respectively, out of 79 males that received a first pad.

Although there was no difference in the size of the first or second egg pad among males that received either one, two or 3+ pads, I did find an increase in the size of each successive pad for males that received multiple egg pads. Ichikawa (1989) discovered that egg pad size increased with subsequent matings in the field, likely due to reduced female egg production at the cooler temperatures (Kraus, 1989) earlier in the breeding season. However, since female waterbugs were housed at constant temperatures in the present study, a change in the rate of egg production over time is unlikely to explain the observed increase in pad size.

Such an increase could be explained by males attempting to maximize their overall fitness, however. Each brooding event places significant costs upon the brooding male, such as reduced swimming speed (Kight et al., 1995), reduced foraging efficiency (Crowl and Alexander, 1989) and increased risk of predation (Smith, 1976b; Kraus et al., 1989). These costs are evident in that brooding males have previously been found to have a shorter life span than virgin males (Gilg and Kruse, 2003). Larger egg pads may result in a slightly greater cost to the brooding male, due to increased drag (Smith, 1976b) for example, and males brooding larger pads would therefore be incurring a greater total cost. Male waterbugs may be more likely to accept these increased costs of brooding towards the end of the mating season, when they are older. The residual reproductive value of older males is smaller than that of younger males, due to a greater risk of mortality and lesser future fecundity (Williams, 1966; Hirshfield and Tinkle, 1975). Older males may accept a larger egg pad, and the greater cost associated with it, in order to maximize their fitness as they are less likely to secure additional matings.

Although egg pad size increased with successive matings, there was no relationship found between the same pad size data (for males that mated multiple times) and the date of mating. This is likely due to the fact that matings were staggered across time; at the same time that some males were receiving a relatively larger second or third pad, others were receiving a smaller first pad. On the other hand, pad size from all 105 mating events showed a negative correlation when compared across time. This is likely due to the fact that males in the aquaria housed at 20°C began mating later and received smaller egg pads, on average, compared to males housed at 26°C (as described above), thereby resulting in a greater number of small egg pads later in the study. It is unlikely that this negative relationship was due to any temporal shift in reproductive strategy or in female egg production, as no relationship was found for those matings that occurred only at 20°C or only at 26°C.

Upon the completion of brooding, males that were able to quickly receive another egg pad appeared to receive larger pads than males that took a longer time to remate. Again, this may indicate that males attempt to maximize the total number of eggs that they receive throughout the breeding season, thereby maximizing their fitness. The eventual acceptance of a smaller egg pad suggests that after an extended period of time without a successful mating, males may seek to receive any number of eggs that are available to them.

On the other hand, this trend could be a result of females attempting to maximize their own fitness. Smith (1976b) suggested that in the related species of giant waterbug, *Abedus herberti*, male brood-pumping behaviors are energetically expensive, and males of this species have been found to rapidly perform these pumping behaviors during courtship (Smith, 1979). This courtship behavior could potentially serve as an honest signal of the male's ability to brood an egg pad, and those males that quickly remated may be more attractive to females, perhaps due to an increased frequency or longer duration of this expensive pumping behavior. While female choice of a mate could explain the observed variation in interbrood intervals, it appears unlikely to also explain the differences in egg pad size. Males normally have control over the female during copulation (Smith, 1979) and it should be in the interest of males (and likely of females) to produce as large an egg pad as possible.

Alternatively, the inverse relationship between pad size and interbrood interval may not be an effect of male or female reproductive strategies, but rather of female egg production. If after brooding, males were returned to the aquaria at a time when females had a large number of eggs available, males would likely soon receive these eggs, as is in the interest of both sexes. However, if males were returned to the aquaria at a time when females had few eggs available (if, for instance, they had recently deposited clutches on other males) then that male may have to wait for females to produce another clutch, thereby accounting for the longer inter-brood interval. This could also explain the smaller pad size found on such males, as females rarely produce full additional clutches (Kruse, 1990). Females, however, were almost always housed in the presence of several other unencumbered males, and would therefore be unlikely to have a full clutch of eggs available.

While each of these potential mechanisms only partially explains the negative correlation found between pad size and interbrood interval, together they could more fully account for this trend. The data from the current study, however, are not sufficient to determine which of these mechanisms, if any, may be at play, and other explanations not described above could be more important in shaping this trend. Alternatively, the observed relationship may be a result of small sample size, especially for longer interbrood intervals.

Kight and Kruse (1992) found that there is a greater chance of males discarding small, rather than large egg pads, while brooding, and the present study supports this finding, as discarded egg pads were significantly smaller than those that hatched. Males that died while brooding (and therefore neither removed nor hatched their brood pad) had egg pads significantly smaller than those that hatched, but slightly larger than those that were discarded. This likely indicates that the brooding mortality observed in this study is independent of the size of the egg pad being carried, although alternative explanations may be possible.

Although the risk of mortality may have no relation to the size of the egg pad being carried, Gilg and Kruse (2003) demonstrated an increased chance of mortality in male brooders compared to virgin males, as the former were found to have a shorter life span. Data from the present study show that the proportion of males with brooding experience that died was greater than that of virgin males in both 20/26 and 26/20. In 20/20, the proportion of virgins that died was greater than that of brooders, and there was no difference in 26/26. The variation in results between the four aquaria may be due to the unknown life history of the males used in this study. Males were collected from the field as adults and it is possible that some of the bugs labeled as virgins had previously brooded in the field prior to collection (for example, if the mating seasons had shifted to earlier in the year, as described below). Gilg and Kruse (2003), on the other hand, collected bugs as last instar nymphs which emerged as adults within the laboratory.

While differences in the life stage of the captured animals make it difficult to directly compare the findings of the present study to those of Gilg and Kruse (2003), data from the present study show that the proportion of brooder deaths was greater only in the two aquaria in which brooding males were exposed to a rapid shift in temperature while virgin males were kept at a constant temperature. It is possible that a rapid temperature change increased the stress level of the animal (Crawshaw, 1979), thereby increasing the risk of mortality. The two aquaria in which brooding males were kept at constant temperature did not show a higher proportion of brooder mortality. In fact, one of these aquaria (20/20) showed a higher proportion of virgin male deaths, perhaps as a result of small sample size.

Of those males that discarded their egg pad while brooding, waterbugs that brooded at 20°C had larger egg pads than those that brooded at 26°C. Males have previously been found to be more likely to abort smaller egg pads (Kight and Kruse, 1992); an effect more pronounced at warmer ambient temperatures (Kight et al., 2000). Kight et al. (2000) suggested that this behavior may be due to a terminal investment strategy, in which males increase reproductive effort at cooler temperatures, which indicate the end of the fall breeding season. If smaller pads are more likely to be removed at warmer temperatures, then the average size of those egg pads that are discarded at warmer temperatures should be smaller than that of those discarded at colder temperatures.

Data from the present study show that the length of the brooding period was longest for those males that successfully hatched their egg pad. While it is not surprising that males that finished brooding via successful hatching of the eggs spent a longer time brooding than those that discarded their egg pad or died while brooding, the length of the brooding period was different from expected. Kruse (1990) reported that *B. flumineum* brooded for 12-14 days at 20°C and 6-7 days at 28°C, while Kight and Kruse (1992) reported that males that aborted their brood pad did so after approximately two days. Waterbugs in the current study brooded for almost twice as long as previously reported, however, as males took over 20 days, on average, to successfully hatch their brood pad and those that discarded their egg pad did so, on average, after brooding for over four days.

Warmer temperatures increase the rate of egg development in giant waterbugs (Kraus et al., 1989; Kruse, 1990; Ichikawa, 1993), resulting in a shorter brooding period. The data from the current study show that for all brooding events, as well as for only those that ended with the death of the brooding male or the hatching of the egg pad, males had a shorter brooding time at 26°C than at 20°C. For those males that discarded their egg pads, it appears that males actually brooded for a longer time at 26°C, compared to at 20°C, prior to removal of the egg pad. While differences in ambient temperature may initiate different reproductive strategies (Kight et al., 2000), this apparent reversal in the relationship between temperature and the length of the brooding period was not found to be a significant effect, and could be an artifact of the small sample size.

Although I found that brooding temperature was, in some cases, related to the size of the egg pad and to the length of the brooding period, data from this study show no relationship between either brooding temperature, or between the difference in the mating and brooding temperature, and the proportion of first-time brooders that died, discarded their egg pad or hatched their eggs while brooding. I also found no differences among subsequent brooding attempts in the proportion of males that died, discarded or hatched their eggs while brooding. However, the data do show a significant difference between the proportion of males that only mated once and those that were able to receive an additional egg pad; consistent with theory for when females have a greater parental investment (Trivers, 1972). Under such circumstances, access to females is expected to limit male reproductive success and males should compete among themselves for mates. Females, on the other hand should be selective in their choice of a male (Trivers, 1972). More competitive males should be more attractive to females and therefore have a higher reproductive success than less competitive males. Such circumstances are consistent with the findings of Kruse (1990), who demonstrated that the reproductive success of males appeared to be limited by females during the fall breeding season.

Belostoma flumineum, however, exhibit sexual role reversal, as males provide all of the postcopulatory care for the eggs developing on their dorsal surface (Smith, 1976a) and therefore might be expected to be limiting to female reproduction (Trivers, 1972). Despite this role reversal, Smith (1979) suggests that males and females may have a similar investment in the offspring, as the cost of brooding by males may be equal to the cost of egg production by females. When both males and females show comparable parental investment, one would expect a similar variation in reproductive success to exist in both sexes and for male choice in a mate to be equally as important as female choice (Trivers, 1972). Males have been shown to occasionally refuse to mate with receptive females (Smith, 1979), indicating the significance of male choice during courtship. In addition, the lack of sexual dimorphism in this species suggests that sexual selection is not prominent and that both sexes have similar reproductive success. Although female reproductive success was not measured in this study, it is possible that it was equally variable to that of males, and that neither sex is limiting to the other's fitness.

Several findings from the present study differed from expectations. As described above, the length of the brooding period was found to be longer than previously reported for males that discarded (Kight and Kruse, 1992) or hatched their egg pad (Kruse, 1990). In addition, the rate of mortality for both brooding and non-brooding males was extremely high. These discrepancies may be a result of an unknown variable within the laboratory, although this is unlikely as animal husbandry methods closely followed those of Kight et al. (2011). Alternatively, the life history of waterbugs used in this study may have differed from those used in previous studies in Illinois (Kruse, 1990; Kight and Kruse 1992) and New Jersey (Kight et al., 2000; Kight et al., 2011).

Belostoma flumineum in temperate North America have two distinct breeding seasons; adult waterbugs emerge in the fall to breed, before overwintering and reproducing again in the spring (Kruse, 1990; Kight et al., 2011). Collection of bugs used for the present study began in early August and continued until further collection was not possible due to winter conditions. With the exception of one male found in the field, no encumbered males were observed until November 19, when mating began in the laboratory. Therefore, it appears that the natural population did not breed during the normal fall mating season.

Recent alterations to the habitat, including recent climate change, may have shifted the timing of the breeding seasons for natural populations. Warmer spring temperatures could potentially induce earlier mating, thereby shifting the time at which adults emerge as well as the normal fall breeding season, to earlier in the year. If so, the mating events observed throughout this experiment could have been part of a third breeding season, thereby accounting for the delayed matings in the laboratory. Such a shift could have also contributed to the high rates of mortality which were observed. Gilg and Kruse (2003) reported an average life span of approximately six months for males kept in the laboratory. Despite the high death rate, adults in the current study may have had comparable lifespans, although it is possible that a significant portion of it was spent in the field, as adults may have emerged several months prior to capture. Further study of natural populations is necessary in order to determine if a temporal shift in the bugs' life cycle has occurred and what mechanisms may be responsible for such a shift.

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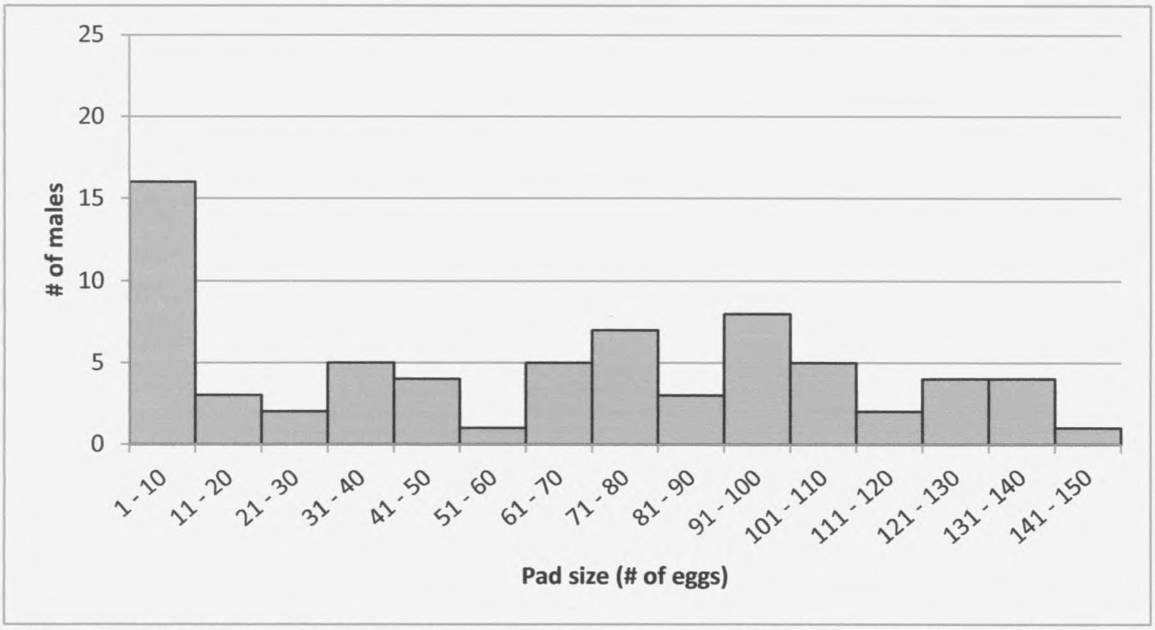

Figure 1. Distribution of the number of eggs per egg pad for all matings (n = 70) that occurred at 26°C.

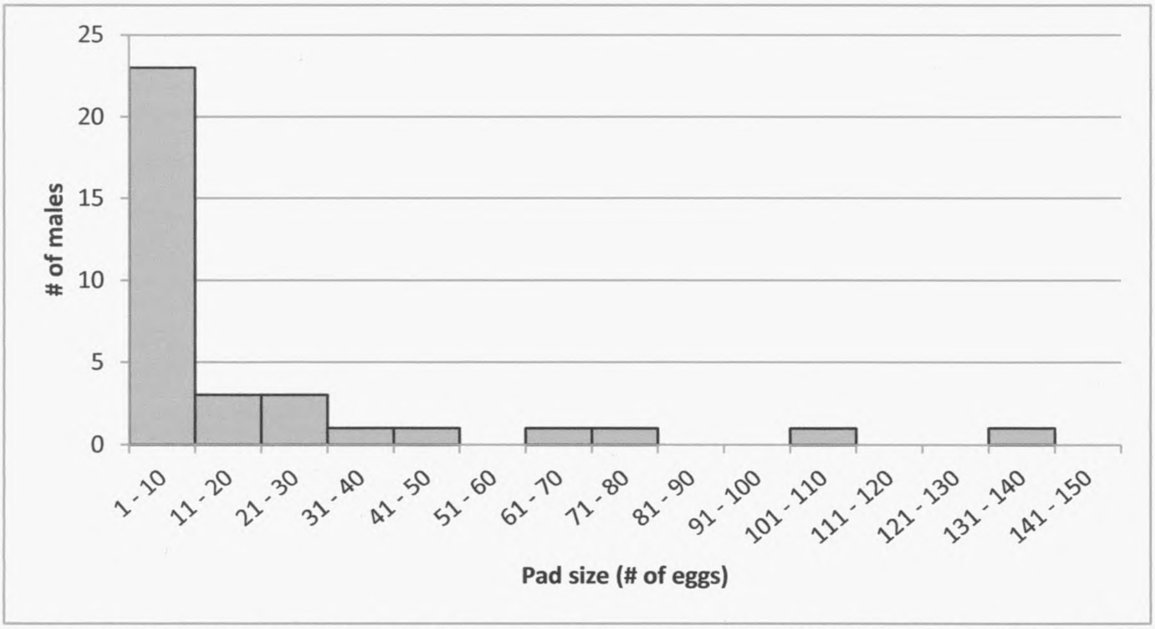

Figure 2. Distribution of the number of eggs per egg pad for all matings (n = 35) that occurred at 20°C.

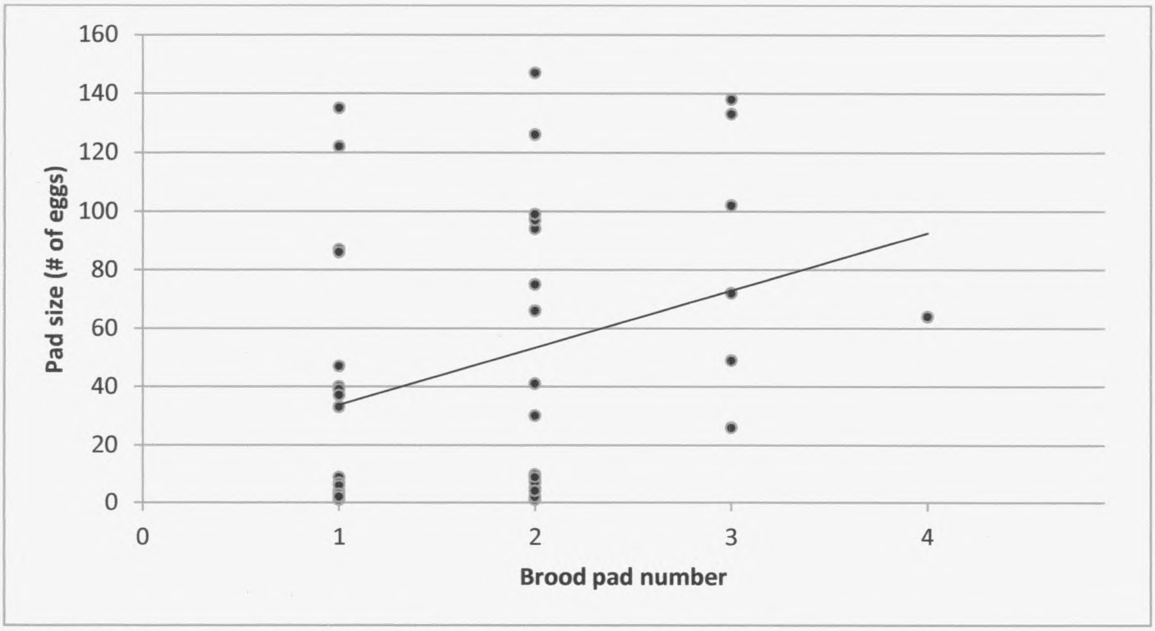

Figure 3. For all males that mated more than once, pad size increases with each subsequent mating (r = 0.328, n = 44, p = 0.0297). Sample size for first mating = 18; second mating = 18; third mating = 7; fourth mating = 1.

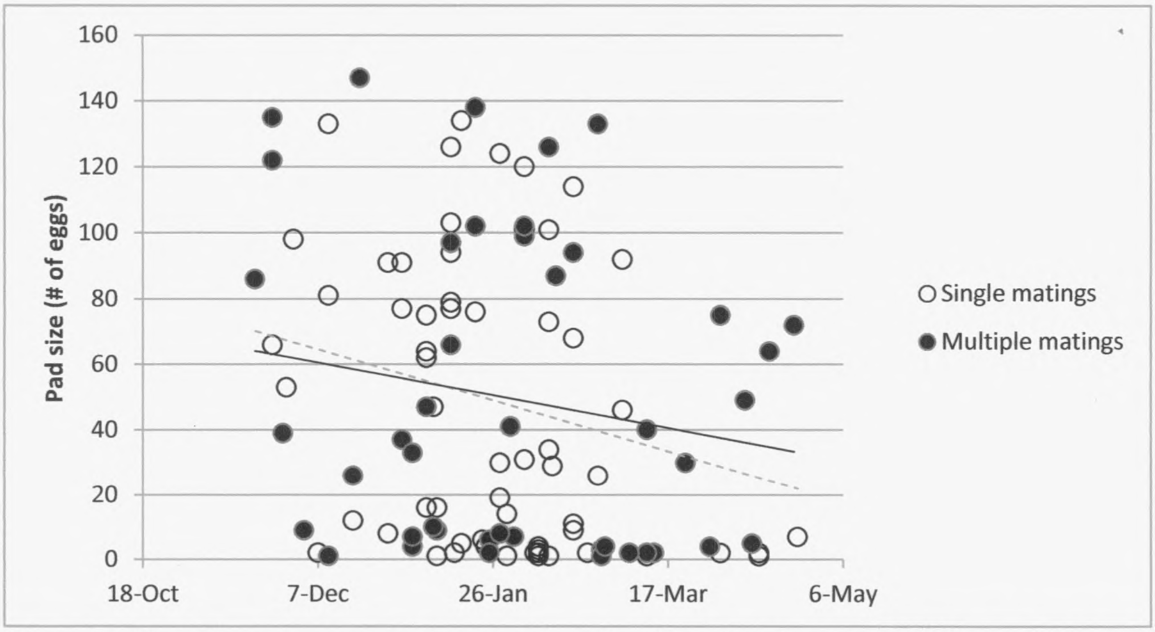

Figure 4. Pad size compared to the date of mating for all males (r = -0.248, n = 105, p = 0.0108) and for only those males that mated more than once (r = -0.175, n = 44, p = 0.257). Open circles represent data from males that mated only once. Closed circles represent data from males that mated more than once. The date of the first mating was November 19 in both cases. The last mating was on April 23 for all males and April 22 for those who mated multiple times. The dashed linear trend line corresponds to data for all males, while the solid linear trend line corresponds to data for males that mated multiple times.

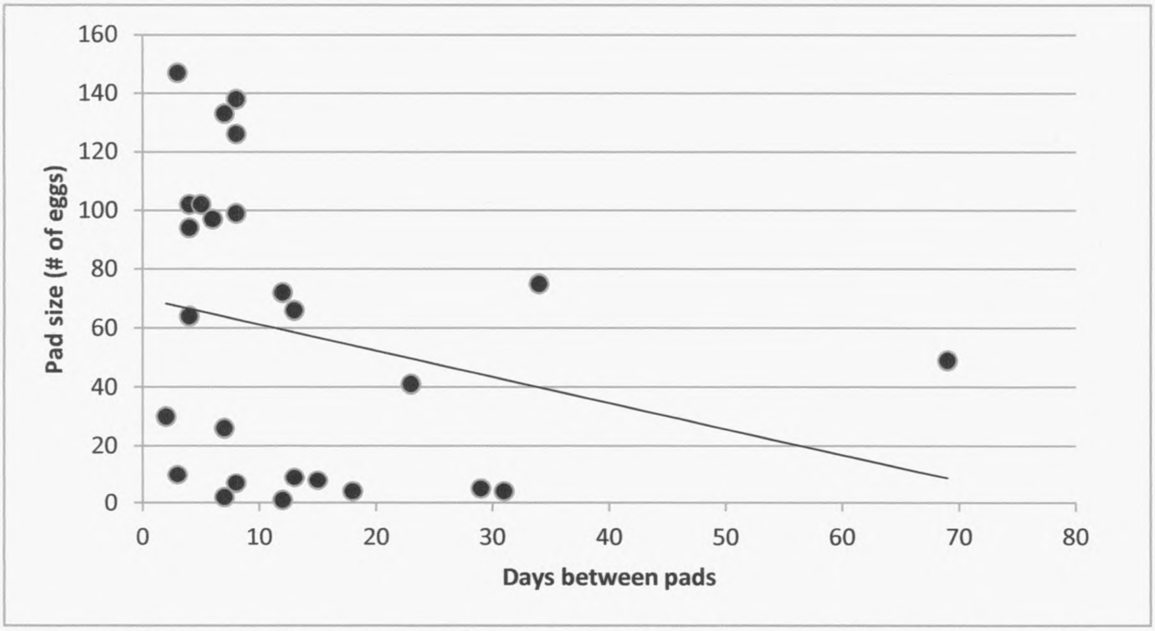

Figure 5. Days spent between brood pads compared to the size of the resultant pad (r = -0.259, n = 26, p = 0.202). Removal of the outlier (69 days, 49 eggs) resulted in a significant relationship (r = -0.371, n = 25, p = 0.0675).

Table 1. Average egg pad size and length of brooding for each of the three possible results of all brooding events. Identical letters indicate no statistically significant difference in pad size or brooding time between the different brooding results (according to Duncan's multiple range test at $\alpha = 0.05$).

	N	average pad size (# of eggs)	standard deviation		average brooding time (# of days)	standard deviation	
died	71	52.7	44.73	*a*	4.7	3.06	*a*
removed	28	22.6	28.51	*a*	4.3	3.33	*a*
hatched	6	111.8	36.71	*b*	20.7	4.84	*b*

Table 2. Average egg pad size and length of brooding for males that mated at either 20°C or 26°C and for males that brooded at either 20°C or 26°C. Data are presented for all males as well as for each of the three possible results of brooding.

		N	average pad size (# of eggs)	standard deviation	average brooding time (# of days)	standard deviation
mating temperature						
20°C	*died*	23	24.9	35.74	4.8	3.46
	removed	12	7.8	13.44	5.2	4.57
	hatched	0	-	-	-	-
	all brooders	35	19.0	30.87	4.9	3.81
26°C	*died*	48	66.0	42.67	4.7	2.89
	removed	16	33.7	31.98	3.7	1.92
	hatched	6	111.8	36.71	20.7	4.84
	all brooders	70	62.6	44.42	5.9	5.41
brooding temperature						
20°C	*died*	31	51.2	46.23	5.7	3.63
	removed	10	34.1	34.06	3.3	1.25
	hatched	5	118.0	37.40	22.4	2.61
	all brooders	46	54.8	48.26	7.0	6.34
26°C	*died*	40	53.9	44.08	4.0	2.33
	removed	18	16.1	23.56	4.9	3.98
	hatched	1	81.0	-	12.0	-
	all brooders	59	42.8	42.41	4.4	3.08

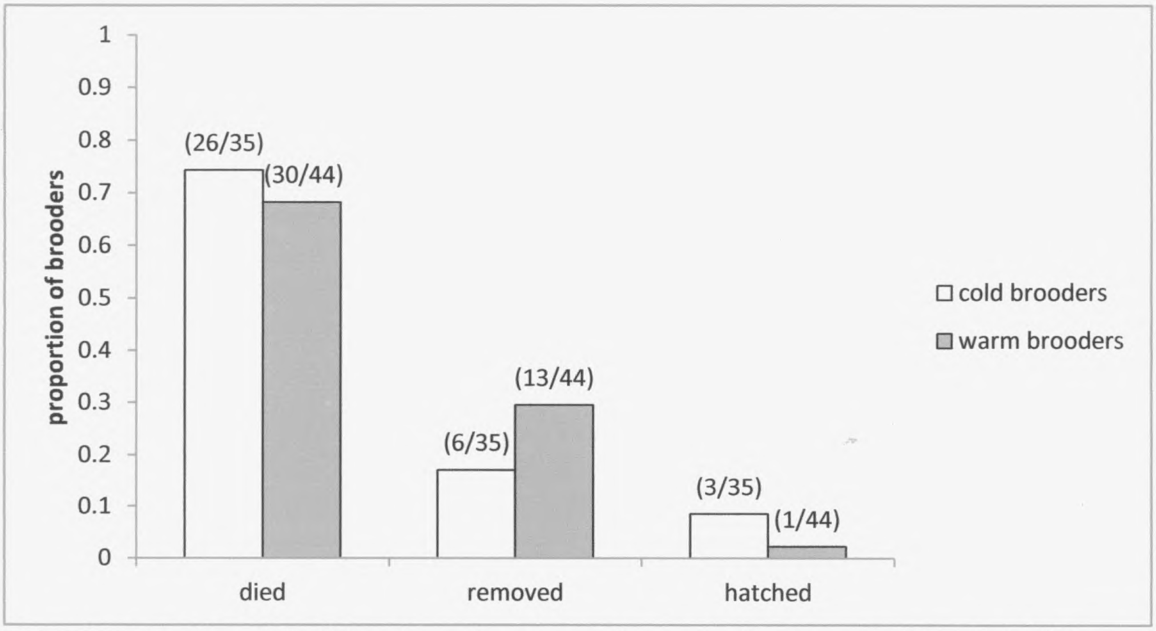

Figure 6. Results of first-time brooding events for males that brooded at cold (20°C; open boxes) and warm (26°C; shaded boxes) temperatures. There were no differences in the proportion of males that died ($p = 0.623$), removed ($p = 0.290$) or hatched ($p = 0.317$) their egg pad.

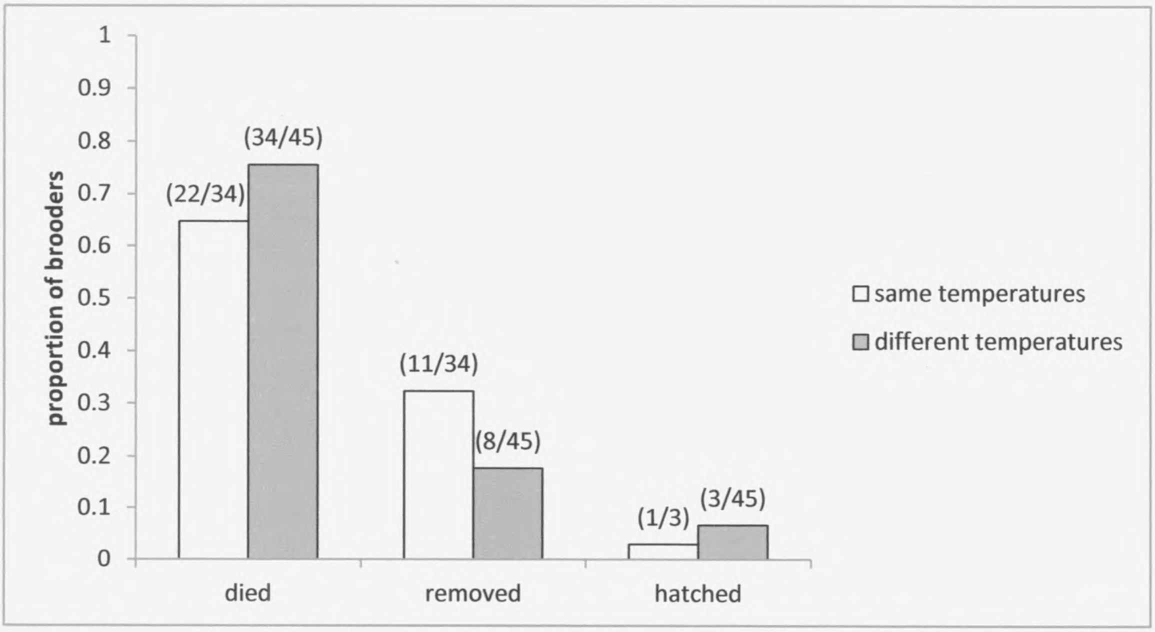

Figure 7. Results of first-time brooding events for males that mated and brooded at the same (20/20, 26/26; open boxes) or different temperatures (20/26, 26/20; shaded boxes). There were no differences in the proportion of males that died (p = 0.326), removed (p = 0.185) or hatched (p = 0.630) their egg pad.

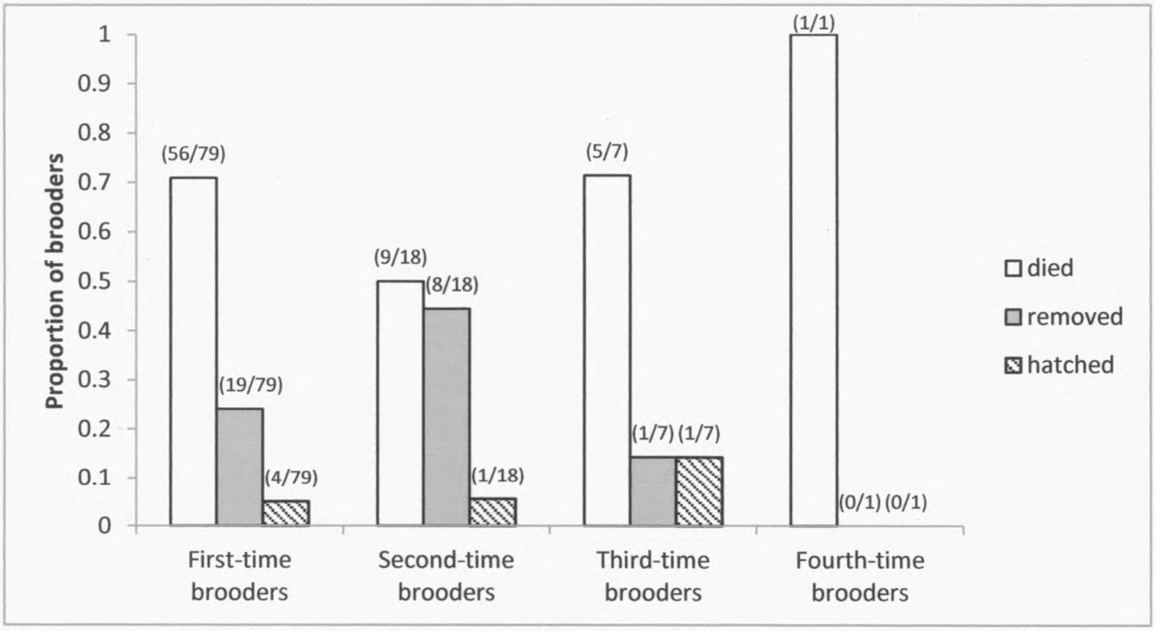

Figure 8. Results of all first-time, second-time, third-time and fourth-time brooding attempts. Open bars represent the proportion of males that died while brooding, shaded bars represent the proportion of males that removed their egg pad while brooding and diagonally hatched bars represent the proportion of males that hatched their egg pad. There were no differences in the proportion of males that died ($p = 0.289$), removed ($p = 0.275$) or hatched ($p = 0.432$) their egg pad between brooding attempts.

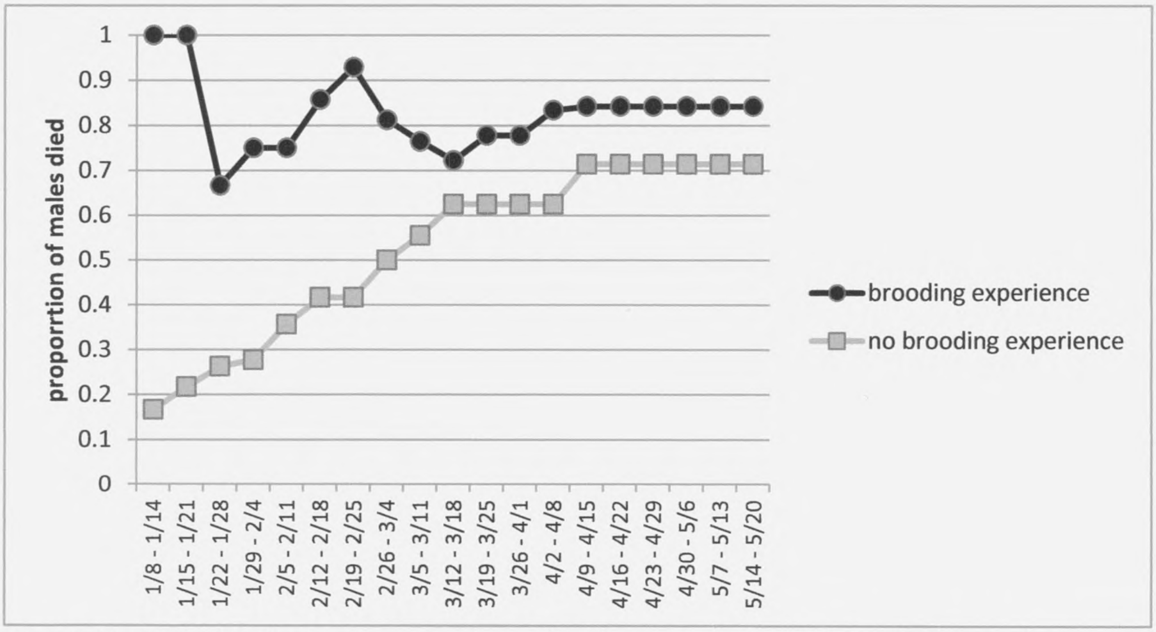

Figure 9. In 20/26, the total proportions of males that died with brooding experience (black circles) were greater, compared over time, than that of males with no brooding experience (grey squares) ($p < 0.0001$). Dates analyzed range from the time of the first mating (Jan 10) to the end of data collection (May 20).

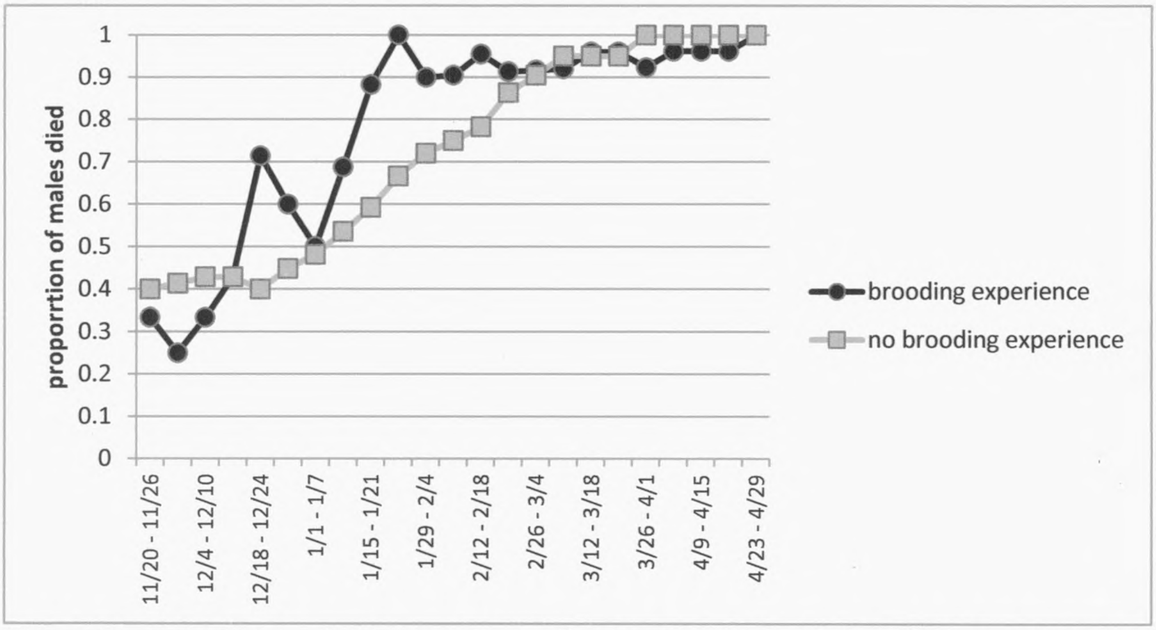

Figure 10. In 26/20, the total proportions of males that died with brooding experience (black circles) were greater, compared over time, than that of males with no brooding experience (grey squares) (p = 0.0592). Dates analyzed range from the time of the first mating (Nov 24) to the end of data collection (April 23).

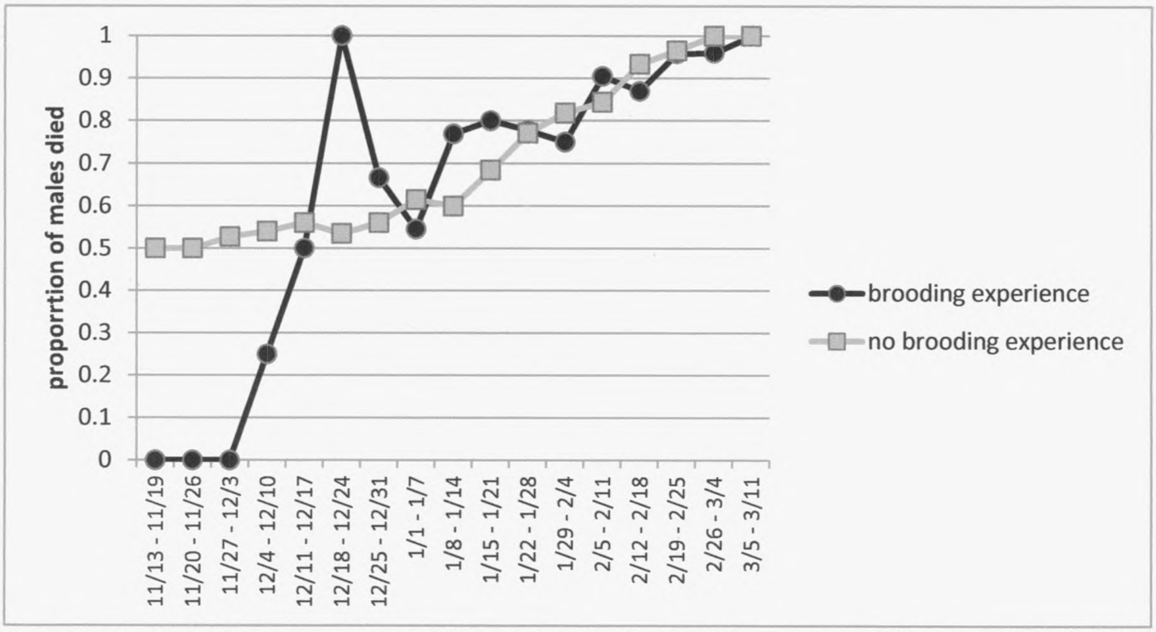

Figure 11. In 26/26, there was no difference in the total proportions of males that died with brooding experience, compared over time, and that of males with no brooding experience (grey squares) ($p < 0.274$). Dates analyzed range from the time of the first mating (Nov 19) to the end of data collection (March 5).

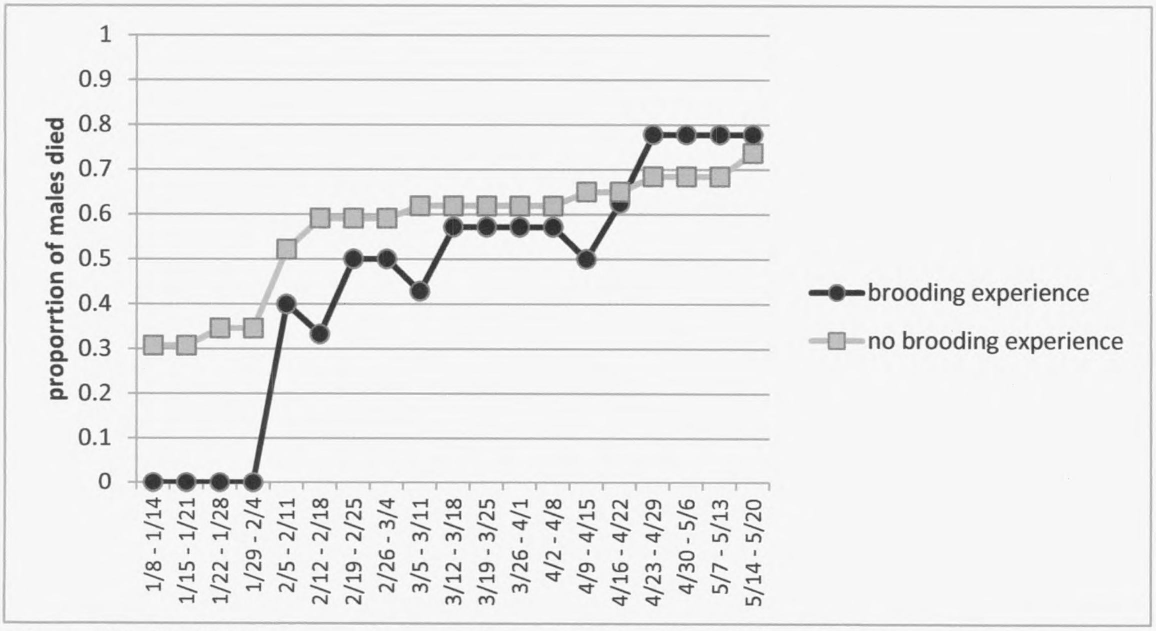

Figure 12. In 20/20, the total proportions of males that died with no brooding experience (grey squares) were greater, compared over time, than that of males with brooding experience (black circles) ($p = 4.21*10^{-3}$). Dates analyzed range from the time of the first mating (Jan 10) to the end of data collection (May 20).