ASSOCIATIONS BETWEEN SIZE AND FITNESS OF ADULT FEMALES IN THE MODEL ODONATE: *ENALLAGMA CIVILE* (ODONATA: COENAGRIONIDAE)

**Kelly S. Baker and Nancy E. McIntyre***

*Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409-3131
*Correspondent: nancy.mcintyre@ttu.edu
ASSOCIATIONS BETWEEN SIZE AND FITNESS OF ADULT FEMALES IN
THE MODEL ODONATE: ENALLAGMA CIVILE
(ODONATA: COENAGRIONIDAE)

KELLY S. BAKER AND NANCY E. MCINTYRE*

Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409-3131
*Correspondent: nancy.mcintyre@ttu.edu

ABSTRACT—During June 2009–June 2010, we collected 561 actively mating female familiar bluets Enallagma civile. Although only ca. 25% of these subsequently laid eggs in the laboratory, size of clutch averaged 250 eggs (range, 1–1,047). Overall, there was a high average rate of hatching success (75.8%). Size of females, in terms of width of head capsule, a non-labile trait in adults, was not significantly associated with metrics of fitness. Hatching success was associated positively with length of eggs (indicating that size of eggs may be an indicator of quality of eggs) and negatively related to duration of hatching.

RESUMEN—Entre junio del 2009 y junio del 2010, colectamos 561 hembras de caballitos del diablo Enallagma civile que estaban en época de apareamiento. Aunque sólo ca. 25% de ellas pusieron huevos en el laboratorio, el tamaño promedio de la nidada fue de 250 huevos (rango de 1–1,047). En total, hubo un promedio alto de eclosión (75.8%). El tamaño de la hembra, en términos del ancho de la cápsula de la cabeza, una característica no lável en adultos, no fue asociado significativamente con la capacidad reproductiva. El éxito de eclosión fue asociado positivamente con la longitud del huevo (indicando que el tamaño del huevo puede medir la calidad del huevo) y negativamente relacionado con la duración de la incubación.

Size of body in odonates (Insecta: Odonata: Anisoptera—dragonflies and Zygoptera—damselflies), as in many animals, can be influenced by several environmental factors, including availability of food or temperature during maturation (Reece, 2009). Differences in these factors between locations or times suggest that a cohort of odonates can exhibit differences in size. Moreover, time-stress has been documented in odonate larvae of several species (Stoks et al., 2008), whereby species can sense the approach of winter (cued by photoperiod, changes in temperature, or other environmental factors), and either accelerate their growth and development to reach maturity before winter or completely halt growth and development and wait as larvae for spring. If they accelerate growth and development to reach maturity before winter, the tradeoff may be a smaller adult (Stoks et al., 2008). The implications of differences in size of body within populations are not well-understood, but one possibility may be effects on aspects of fitness, such as number of eggs laid, hatching success, size of eggs, time between oviposition and hatching, and length of time for a clutch to hatch.

In odonates, fitness of females may be influenced positively by size of body because larger females have accrual of resources to support production (and ballast) of larger clutches. The literature, however, is conflicting on this topic. Some studies have reported a positive relationship between mass and fitness (e.g., Harvey and Corbet, 1985; Banks and Thompson, 1987; Koenig and Albano, 1987; Gribbin and Thompson, 1990; Harvey and Walsh, 1993; Cordero, 1995; Sokolovská et al., 2000), whereas others have shown a negative relationship, or none at all (Fincke, 1986, 1988; Anholt, 1991; Richardson and Baker, 1997). However, mass is only one assay of size, and it is far more plastic than some other metrics of size, such as total length of body or width of head capsule. Considerable problems arise when using either total length of body or mass. Odonates can telescope their abdomen, manipulating their overall length and effectively impede accurate measurement. Mass is highly variable and directly influenced by recent activity of individuals (i.e., feeding or bowel movements) or age (Anholt, 1991). In contrast, width of head capsule is a fixed measurement in adults that is not subject to any immediate environmental conditions. In light of these contradictory studies on a labile assay of size, it appears that our understanding of the effect of size of females on fitness in odonates is still incomplete.

The familiar bluet Enallagma civile (Coenagrionidae) is distributed from southern Canada to Central America and from the Atlantic to Pacific oceans (Westfall and May, 1996). In the southern High Plains of Texas, E. civile is the most common odonate (Reece and McIntyre, 2009). This species is non-territorial and gregarious (Bick, 1963; Bick...
and Bick, 1980), in which males engage in scramble competition for mates (Corbet, 1999). On average, life span from hatching to emergence as an adult is \( \geq 21 \) days in shallow, ephemeral wetlands in Texas (Booker, 2002); lifespan of adults typically is \(<1\) week (Bick and Bick, 1963).

Objectives of our study were two-fold. We sought to test the hypothesis that larger female odonates have higher fitness (lay more eggs, have higher hatching success) than smaller females and we gathered baseline reproductive and fitness information on *E. civile* (including width of head capsule of mating females, size of clutch, time to hatching, duration of hatching, mean length of eggs, and hatching success). This is a widespread and abundant, but under-studied model species, thereby providing information that may be of comparative value in trying to detect overall trends in life-history patterns of odonates from species-specific idiosyncrasies.

**Materials and Methods**—Samples were collected from one locality to ensure that there was no confounding effect between geographic variability in sites and sizes of damselflies. Individuals were collected from the northeastern portion of Canyon Lake Number 5 in Mae Simmons Park in Lubbock County, Texas (33.57779°N, –101.82596°W). Canyon Lake Number 5 is an impoundment of the Brazos River. Grass grows to the edge of much of the site and is mowed periodically by personnel of the city of Lubbock. A variety of aquatic reeds and plants such as cattails (*Typha*) also are present at the site, providing additional perching and ovipositioning material. Fish (most notably carp *Cyprinus carpio* and bass *Micropterus*) are present which may depredate larval odonates. Canyon Lake Number 5 was chosen because of its prolific population of *E. civile* as well as its accessibility.

*Enallagma civile* begins to hatch in Lubbock County in mid-to-late spring. Tandem pairs of familiar bluets generally can be collected May-September in this area. In 2009, females were collected on 16 days during June-September (23–24 June; 3–4, 6–7, 13–16 July; 27–29, 31 August; and 2, 8 September). In 2010, females were collected over a shorter time (28 May–1 June and 7–20 June) to concentrate sampling over a smaller window of time; thereby reducing any potential biases from time-stress, which may result in different-sized cohorts emerging early in summer versus in autumn. Individuals collected during the 4-month period in 2009 likely belonged to \(\geq 3\) cohorts (assuming \(\geq 21\) days from hatching to emergence of adult; Booker, 2002), whereas individuals collected in 2010 likely belonged to the same generation.

Samples were collected midday (1100–1530 h). Aerial nets were used to collect females in tandem (while actively copulating in the wheel formation, if possible). Females were individually placed in small, clear, specimen envelopes within a shaded container in the field and transported to the lab. In the lab, each individual was placed into a separate oviposition chamber. The chamber was a 0.95-L glass Mason jar filled with ca. 2.5 cm of pond water. An oviposition apparatus (wooden rod wrapped in damp paper towels) was placed in the chamber at a 45° angle. The oviposition apparatus allowed females to perch while in the chamber and mimicked stems of vegetation on which female *E. civile* naturally lay their eggs.

Each female was left undisturbed in the chamber for 36–48 h, with the lab maintained at 27°C with a 16L:8D cycle. After this time, the female was removed from the chamber and placed into a labeled specimen envelope for preservation. Next, the oviposition apparatus was checked for eggs. If eggs were present, the section of paper towel containing the eggs was cut out and placed into a Petri dish filled with pond water. A Zeiss Stemi 2000 dissecting microscope (Carl Zeiss Microscopy, Thornwood, New York) was used to count eggs laid by each female. Furthermore, for samples collected in 2010, 10 eggs/clutch were chosen at random and their length was measured as an additional potential metric of fitness. For clutches with \(<10\) eggs, all eggs were measured. Petri dishes containing eggs were incubated under UV lights with a 16L:8D cycle until the clutch hatched. Once hatching commenced, each Petri dish was checked daily to quantify the number of larvae, which were removed daily to reduce cannibalism of younger individuals. Therefore, time to hatching consisted of the number of days from when eggs were laid to when the first larva was observed. After the last active day of larval emergence, the dish was checked for an additional 14 days to ensure that no other larvae emerged. If a larva was found after several dormant days, the 14-day count restarted. After a 2-week period with no further hatching, the sample was considered complete. Duration of hatching consisted of the number of days between the observation of when the first larva hatched and the last. Hatching success refers to the percentage of eggs that hatched into larvae.

After oviposition, each female was euthanized via refrigeration at 4°C for \(\geq 48\) h, and width of head capsule was measured from outermost edge of one eye to outermost edge of the other eye, using an ocular micrometer. Each individual was measured once, and a subsample of 20 individuals for each set (egg-laying and non-egg-laying for 2009 and 2010) was chosen at random and remeasured to gauge precision. There was \(<5\%\) difference in all remeasured values.

We measured fitness in *E. civile* in terms of size of clutch and hatching success. Ideally, we would have included number of clutches in fitness, but logistical constraints (e.g., lab-rearing, collection of all eggs per clutch) of doing so are immense. Furthermore, we would have liked to follow larvae through all developmental stages until reaching maturity, but high lab-related mortality precluded this.

Mean and median are reported for all variables (width of head capsule, size of clutch, length of eggs, time to hatching, duration of hatching, hatching success). SAS version 9.2 (Statistical Analysis Systems software) was used for all analyses. Two-tailed *t*-tests and Pearson and Spearman correlations were employed to measure how size of female affects fitness. Data were checked for normality using a Shapiro-Wilk test. In all but one instance, data were distributed normally. In the one instance with non-normal data, a Spearman correlation was used (instead of Pearson). Furthermore, *t*-tests assume that both populations being tested have equal variance. Folded *F*-tests for equality of variance were run prior to each test and showed that, in all cases, both groups being tested had satisfied the assumption of equal variance.

We analyzed five dependent (fitness) variables. Raw data are in Baker (2011). Size of clutch is the number of eggs laid and is a direct assay of fitness. Hatching success measures the percentage of eggs that hatch. Because this variable is a proportion, it was
transformed with the arcsine-square-root function for inclusion in correlation statistics (Sokal and Rohlf, 1981). Length of eggs, time to hatching, and duration of hatching are indirect assays of fitness that may be affected by size of female.

**RESULTS**—We collected 561 actively mating females (187 in 2009, 374 in 2010). Their average width of head capsule was 3.73 ± 0.01 mm (n = 530), median = 3.75 mm. Of females captured in 2009 and 2010, only 27.3% (153 of 561) laid eggs. Of those, the mean (±SE) number of eggs laid was 250 ± 18 eggs (n = 151), with a range of 1–1,047 eggs and a median of 212 eggs. Mean time to hatching was 11.9 ± 0.2 days (n = 116), median = 12.0 days. Range of time to hatching was 10–34 days. However, >98% of data were within the range of 10–14 days. There were two outliers at 17 and 34 days; excluding these outliers, the mean was 11.6 ± 0.1 days. Range in duration of hatching was 1–25 days, with a mean of 7.1 ± 0.4 days (n = 115) and a median of 6.0 days. Mean hatching success was 75.8% ± 2.1% (n = 122), median = 83.5%. In 2009 and 2010, eight females laid eggs that did not hatch. Excluding these eight individuals, the mean hatching success was 81.1 ± 0.0% (n = 114).

Size of eggs within and among clutches varied. In 2010, overall range in length of eggs was 0.60–0.90 mm (n = 807), mean length of eggs was 0.76 mm (median = 0.76 mm), and range of mean length of eggs was 0.71–0.83 mm (n = 82 clutches). Within a clutch, the largest difference between minimum and maximum lengths was 0.23 mm (0.60–0.83 and 0.67–0.90 mm, respectively). Most clutches expressed such variation in length of eggs, but there were nine clutches that had no variation (all 10 eggs were the same length).

There was no significant difference in width of head capsule between egg-laying and non-egg-laying females (means = 3.74 ± 0.01 and 3.72 ± 0.01 mm, respectively; Table 1). When considering only egg-laying females, width of head capsule had no detectable effect on size of clutch. Females laying >500 eggs had the same width of head capsule as females laying fewer eggs (Table 1). Similarly, females who laid <100 eggs had the same width of head capsule as females laying more eggs (Table 1). Furthermore, size of females does not appear to affect size of eggs or hatching success because no significant correlation was detected between width of head capsule and mean length of eggs or between width of head capsule and hatching success (Table 2) when excluding the eight outliers (clutches in which no egg hatched). Finally, there was no significant correlation between width of head capsule and duration of hatching (Table 2). However, hatching success was significantly greater in clutches with larger eggs (Table 2).

A post-hoc Tukey’s comparison of data collected in 2009 (June–September) for width of head capsule between all egg-laying and non-egg-laying females collected early in summer (23 June–7 July; n = 42) and those collected later in summer (2–8 September; n = 23) revealed no significant difference between groups (critical value = 2.826, P > 0.05). In 2010, we collected all females in a shorter time (<3 weeks); thereby reducing any effect seasonality may have on size of females. Thus, we believe that our results are not skewed due to time-stress.

**Table 1**—Results of Tukey’s t-tests of relationships of fitness in female familiar bluets *Enallagma civile*. Variable 1 and variable 2 represent the two variables being compared (years 2009 and 2010).

<table>
<thead>
<tr>
<th>Variable 1</th>
<th>Variable 2</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Width of head capsule of non-egg-laying females (n = 384)</td>
<td>Width of head capsule of egg-laying females (n = 146)</td>
<td>−1.05</td>
<td>0.29</td>
</tr>
<tr>
<td>Width of head capsule of females laying &lt;500 eggs (n = 127)</td>
<td>Width of head capsule of females laying &gt;500 eggs (n = 17)</td>
<td>−1.38</td>
<td>0.17</td>
</tr>
<tr>
<td>Width of head capsule of females laying &lt;100 eggs (n = 47)</td>
<td>Width of head capsule of females laying &gt;100 eggs (n = 97)</td>
<td>1.08</td>
<td>0.28</td>
</tr>
</tbody>
</table>

**Table 2**—Correlations (r) related to fitness of female familiar bluets *Enallagma civile*. All are Pearson correlations except for assessment of the relationship between mean length of eggs and duration of hatching (Spearman correlation).

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatching success and mean length of eggs</td>
<td>81</td>
<td>0.242</td>
<td>0.054</td>
</tr>
<tr>
<td>Duration of hatching and hatching success</td>
<td>114</td>
<td>−0.186</td>
<td>0.048</td>
</tr>
<tr>
<td>Mean length of eggs and width of head capsule</td>
<td>80</td>
<td>0.108</td>
<td>0.342</td>
</tr>
<tr>
<td>Mean length of eggs and duration of hatching</td>
<td>78</td>
<td>−0.097</td>
<td>0.397</td>
</tr>
<tr>
<td>Number of eggs and width of head capsule</td>
<td>144</td>
<td>0.067</td>
<td>0.428</td>
</tr>
<tr>
<td>Hatching success and number of eggs (without clutches of eight females where no egg hatched)</td>
<td>114</td>
<td>0.055</td>
<td>0.561</td>
</tr>
<tr>
<td>Mean length of eggs and number of eggs</td>
<td>81</td>
<td>−0.041</td>
<td>0.720</td>
</tr>
<tr>
<td>Hatching success and width of head capsule (without clutches of eight females where no egg hatched)</td>
<td>110</td>
<td>−0.032</td>
<td>0.744</td>
</tr>
<tr>
<td>Width of head capsule and duration of hatching</td>
<td>111</td>
<td>0.031</td>
<td>0.747</td>
</tr>
</tbody>
</table>
DISCUSSION—In *E. civile*, there was no tradeoff observed between size of clutch and length of eggs, as there was no significant correlation between number of eggs laid and mean length of eggs or between duration of hatching and mean length of eggs (Table 2). Furthermore, number of eggs laid was not correlated to hatching success (Table 2). However, there was a strong positive correlation between mean length of eggs and hatching success (Table 2), indicating that size of eggs laid may be related to survivorship of larvae. Survivorship of larvae may also be related to the length of time it takes to emerge. Hatching success was correlated negatively with duration of hatching (Table 2), suggesting that females whose clutches take longer to hatch tend to have fewer larvae that emerge.

The percentage of egg-laying females in our study was relatively low (27.3%). Along with unnatural laboratory conditions, behavioral mating patterns may help explain this phenomenon. Copulation by *E. civile* includes three distinct behavioral phases: exploratory, underwater oviposition, and terminal (Corbet, 1999). During at least the first and third stages, females are in tandem. Insemination occurs during the first stage and oviposition during the second. During the second stage, the female submerges herself beneath the surface of the water and may be released by the male. After oviposition, the pair may remain in tandem and repeat the process, or they may separate. In our study, all females were collected while in tandem, but we do not know during which behavioral stage the individuals were collected. Because the first stage lasts significantly longer than the terminal stage, we assumed that most individuals were collected during the first stage, still having eggs to oviposit. However, some individuals were likely collected in stage three. It also is possible that tandem pairs are more active, and thus, more visible (and more likely to be collected) in later than in earlier stages, or that the large aggregations of familiar bluets at our study site induced lowered efficiency of oviposition (a density-dependent effect seen elsewhere for this species; Moss, 1992).

No significant effect of time (early versus late in the season) on width of head capsule was seen in 2009, and the sampling regime in 2010 was compressed into a smaller window to minimize this potential effect of time-stress. In our study, width of head capsule was slightly smaller than formerly reported for females. Córdoba-Aguilar (2008) reported mean width of head capsule of female *E. civile* as 3.80 mm. In contrast, average width of head capsule we measured for female *E. civile* was 3.73 ± 0.01 mm. The difference between these averages may be attributed to size of sample, or possibly variation in sites, environmental conditions, or age. The relatively narrow range in width of head capsule we noted may be due to constraints imposed by urban land use or any other unique factors at our study site; further research would be needed to determine whether this is so.

Despite consistencies in time to hatching, there was considerable variation in duration of hatching (1–25 days). This could indicate that more than one strategy is being employed within this species. Some clutches had all eggs hatching in a single day. By hatching all at once, this clutch could take advantage of optimal environmental conditions. Other clutches in our study contained eggs that hatched over >20 days. By extending duration of hatching, it is possible that survivorship may increase because the clutch is less vulnerable to unforeseen, changeable conditions (e.g., weather, predators), and with a lower concentration of individuals, reductions in intraspecific competition and risk of cannibalism occurs.

We expected to find a negative correlation between mean length of eggs and size of clutch (as a female lays more eggs, size of eggs would be expected to decrease because of the finite resources available to allocate to either size of clutch or to size of eggs). However, no relationship was detected between these variables. If such a relationship existed, it could indicate that there was an average reproductive mass that could be achieved through size or number of eggs. However, no such average reproductive mass appears to exist.

Hatching success was affected by mean length of eggs and duration of hatching. Females with high hatching success tended to have clutches that consisted of eggs with larger mean lengths, and their eggs hatched more quickly. Notably, hatching success was independent of size of female.

There have been studies on the effect that body mass has on fitness of odonates, with variable effects seen depending on species, sex, and territoriality (Anholt, 2008), but none using the less-labile metric of width of head capsule. For example, larger-bodied females produce larger clutches in the dragonflies *Plathemis lydia* (Koenig and Albano, 1987) and *Pyrrhosoma nymphula* (Gribbin and Thompson, 1990). Furthermore, with more mass, females produce more clutches in some species (Cordero, 1991; Leung and Forbes, 1997; Marden and Rowan, 2000; De Block and Stoks, 2005), but not in others (Koenig and Albano, 1987; Michiels and Dhondt, 1989; Anholt, 1991). In some species, smaller males may actually be associated with higher lifetime reproductive success (Anholt, 1991; Carchini et al., 2000). Finally, responses also differ with respect to territoriality; greater mass is associated with higher reproductive success in territorial species (Sokolovska et al., 2000), but no such effect has been seen in non-territorial species (Banks and Thompson, 1985; Stoks, 2000). Our evaluation of size of females, in terms of a metric other than mass, relative to fitness (i.e., in terms of size of clutch and hatching success) in *E. civile* adds to the understanding of the complex phenomenon of life-history tradeoffs.

It appears that fitness (in terms of size of clutch and hatching success) in *E. civile* is influenced by a variable other than width of head capsule. Although it has been
speculated that in odonates the fixed non-mass size (width of head capsule) of an individual “must set some upper limit to clutch size” (Anholt, 2008:168), we found that width of head capsule was not associated with size of clutch. Females that laid eggs were the same size as those that did not lay eggs. Among females who laid eggs, those with the largest and smallest clutches averaged the same width of head capsule as did all other egg-laying females. Similarly, we failed to detect an effect of width of head capsule on success, as eggs of both large and small females experienced similar rates of larval emergence.

In light of our results, the ultimate effects of variation in size of *E. civile* may be minimal. Barring indirect influences (i.e., accrual of resources), if this species physically is able to survive a set of environmental conditions, *E. civile* can inhabit the area without a reduction in fitness and exhibit variation in size. In conjunction with its high level of tolerance for several environmental variables, independence of size and fitness may help explain the ubiquitous nature of *E. civile*.

As a result of our study, new information on life history and fitness for *E. civile* has been obtained. However, future research is needed in several areas. First, this study assumed that observed differences in size were due largely to factors other than the surrounding environment because all individuals were collected from the same locality, but it is possible that some adults we collected had not emerged from that site and dispersed there instead. Environmental differences among sites may amplify differences in size between females, and thus, amplify differences in fitness as well. Until recently, the southern High Plains was a nearly homogeneous grassland. Therefore, pressures of anthropogenic activities on the landscape are relatively recent. Potential effects of such activities on a playa and its wildlife are, evolutionarily speaking, novel and recent, and thus, may account for the lack of a significant effect of size detected on fitness in *E. civile*. Future research is needed to understand the contributions of environmental variables on fitness. Second, fitness also includes lifetime reproductive success, or total number of clutches per female. Future research could examine the number of times a female mates and differences in size of clutch per hatching success per copulation. In fact, some studies (Fincke, 1988; De Block and Stoks, 2005) reported that the number of clutches laid was more important for determining lifetime fitness than was size of clutch. Female familiar bluets typically leave their natal wetland to forage in surrounding uplands, whereas males tend to stay by the water, patrolling for females (Bick and Bick, 1963). When a female returns to the water to reproduce, she encounters males that engage in scramble competition for an opportunity to copulate with her; it is unknown whether she exerts some kind of choice of mates. Following oviposition, she normally retreats from the males, again moving away from the wetland to forage.

Accumulating resources is risky, and because lifespan of adult familiar bluets is ca. 1 week (Bick and Bick, 1963), the possibility for multiple copulations is low. Therefore, having a mechanism to maximize fitness on a per-clutch basis (such as sizes of eggs or clutches) is likely to be selected for. Third, a more comprehensive understanding of fitness and life history of females must include information about when they reach reproductive maturity. It is possible that there are differences in size between reproductive and non-reproductive females; females may have to attain a certain critical mass before reproducing. If so, what is that critical mass, and how long, on average, does it take for a female to attain that size? Fourth, we did not consider the role of males in the reproductive process and their potential influence on fitness. Size of males could influence fitness through their ability to acquire a mate and the condition and amount of sperm. Finally, we detected a large amount of variation in duration of hatching. Future research could explore factors that account for this variation.

This study adds to existing literature on effects of size on fitness in female odonates. Obviously, the relationship between these two variables is not straightforward, as evidenced by the accumulating research. Previously proposed as a potential new angle for gaining insight into fitness of odonates (Anholt, 2008), our study failed to support a relationship between width of head capsule and fitness in *E. civile*.

Funding was provided by Texas Tech University Department of Biological Sciences, an AT&T Chancellor’s Fellowship, and the Sandy Land Underground Water Conservation District through the Water Conservation Research Fellowship. We also acknowledge B. Reece and J. Baker for assistance in the lab and field, respectively, and Y. Sepulveda for the Spanish translation of the abstract. Comments from two anonymous reviewers and the Associate Editor improved the manuscript.

**Literature Cited**


Bick, G. H. 1963. Reproductive behavior in *Enallagma civile* and


Submitted 7 August 2011. Accepted 9 November 2012.

Associate Editor was Jerry L. Cook.