

Effects of Zebra Mussel Colonization  
on Dragonfly Larvae Burying Behavior

by

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## Abstract

Invasive species have caused massive ecological and economic damage throughout the world. In North America, zebra mussels (*Dreissena polymorpha*) native to Eastern Europe invaded aquatic ecosystems in the 1980s, altering ecological communities and harming human infrastructure. Zebra mussels have been found attached to dragonfly larvae, decreasing the likelihood of successful emergence as adults. This study assesses the negative impacts zebra mussel colonization has on dragonfly larvae by testing the effects of colonization on dragonfly burying behavior. *Macromia illinoensis* larvae and zebra mussels were collected and tested at Douglas Lake, Michigan in July and August 2009. Weather and water temperature affected uncolonized burial time, but not uncolonized burial depth. Uncolonized burial time, head width, and body area were predictors of which individual dragonflies got colonized. Once individuals were colonized, their burial depth was impaired, which could lead to early mortality. Because dragonflies link aquatic and terrestrial ecosystems, increased early mortality of dragonflies could cause cascading effects across ecosystems.

# 1. Introduction

## 1.1 Zebra Mussels

Zebra mussels (*Dreissena polymorpha*) are freshwater bivalves native to the Black, Caspian, and Azov Seas. They were first seen in North America in 1988 in Lake St. Clair, a lake that lies between Lake Huron and Lake Erie on the Canadian-American border (Hebert et al. 1989). Zebra mussels are believed to have been carried to North America in a ship's ballast water as free-floating larvae from eastern Europe arriving in Lake St. Clair in 1986 (Hebert et al. 1989; Griffith et al. 1991). By 1990, they had been found in all of the Great Lakes. In 1991, they were found in the Illinois River, from which they invaded the Mississippi River and the rest of the eastern US. By 1994, they had been sighted in rivers, streams, inland lakes, and other water bodies of 20 states, and that number has increased to 27 states today (United States Geological Survey 2010; Figure 1.1). Some sightings have been made as far from Lake St. Clair as Huntington Creek, UT and San Justo Reservoir, CA (USGS 2010).

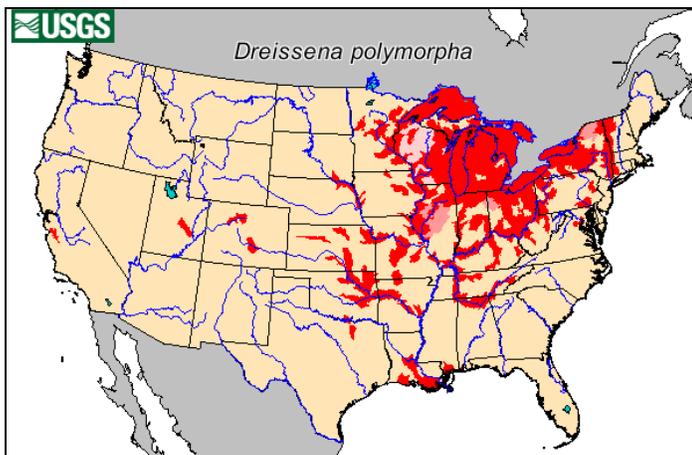


Figure 1.1: Current distribution of zebra mussels in the United States. Red represents invaded areas. Source: Benson, A. J. (2010). Zebra mussel sightings distribution. Retrieved 4/11/10.

Zebra mussels are named because of their light and dark striped shells, although their shells can be entirely white or black (Figure 1.2). Adult shells are typically 2.5 – 3 cm in length (Mackie & Schloesser 1996). They are most similar in appearance to the invasive freshwater quagga mussel, *Dreissena bugensis*, and the native brackish water false dark mussel, *Mytilopsis leucophaeata*. Zebra mussels are easily identified, however, by their ability to stand up on their flattened side where the two shells hinge, which no other freshwater mussel can do. This unique shell shape gives zebra mussels more stability when attaching to hard surfaces with byssal threads, a function discussed in more detail later, and allows for increased density of mussels on hard substrates.



Figure 1.2: White-shelled, black-shelled, and striped-shelled zebra mussels. Notice the unique shape of the shell that makes it easy to stand up on the flattened side where the two shells hinge. Photo from United States Geological Survey.

### **Why are zebra mussels invasive?**

Zebra mussels have been able to colonize North America because they differ biologically from most native North American freshwater species, hereafter referred to as native unionids because they belong to the family Unionidae (Mackie 1991). Zebra mussels have been characterized as successful invaders because of their rapid population growth, widespread dispersal, and high genetic variation (Hebert et al. 1989; Mackie 1991; Ackerman et al. 1994).

Zebra mussels show high reproduction rates leading to rapid population growth. Both zebra mussels and native unionids are dioecious, but only zebra mussels use external fertilization (Mackie 1991). Because many bivalves use internal fertilization, each female is limited in how many larvae she can brood (Mackie 1991). Native unionids, however, are not limited by the use of internal fertilization because they produce tiny larvae known as glochidia, with a single female producing up to 2,000,000 glochidia (Mackie 1991). Instead, the survival of glochidia is very low – with only 1 in 10,000 glochidia surviving to adulthood – because glochidia are parasitic and must attach to host organisms, often a fish species, to develop. Once released from their host, they must land in a specific substrate to grow to adulthood. In contrast, zebra mussels are not dependent on a host species and are not substrate-specific. Females are able to produce many more successful larvae, with fecundity rates ranging from 30,000-40,000 eggs per female per year and calculations suggesting females can produce up to  $1.6 \times 10^6$  eggs in a lifetime (Hebert et al. 1989; Mackie 1991; Mackie & Schloesser 1996). This extraordinarily high fecundity rate allows for rapid population growth, creating more individuals that can disperse.

Zebra mussels disperse widely allowing their populations to spread and invade new habitats. Their primary mode of dispersal is as pelagic larvae known as veligers that are free-floating, living in the water column rather than attaching themselves to host organisms (Hebert et al. 1989; Mackie 1991; Ackerman et al. 1994). Zebra mussel veligers are easily and rapidly dispersed via water currents (Griffiths et al. 1991; Mackie 1991; Mackie & Schloesser 1996), allowing veligers to disperse far more widely and rapidly than those of native species.

Zebra mussel dispersal is further increased because zebra mussels also disperse as post-larvae and adults. Zebra mussels produce byssal threads composed of DOPA (3,4-dihydroxyphenyl-alanine) that individuals use to attach to hard substrates at the bottom of lakes (Griffiths et al. 1991; Mackie 1991; Mackie & Schloesser 1996; Figure 1.3). While some native unionids can also produce byssal threads as larvae, zebra mussels retain this ability throughout adulthood. Juvenile zebra mussels can use byssal threads for dispersal by using them as drag lines to passively drift with water currents (Griffiths et al. 1991; Mackie 1991; Mackie & Schloesser 1996). In addition, juvenile and adult zebra mussels may settle on mobile substrates such as floating debris, again allowing for passive dispersal (Mackie & Schloesser 1996).



Figure 1.3: A zebra mussel with exposed byssal threads. Photo from Ohio Sea Grant.

While both types of natural dispersal allow for wider dispersal ranges than those of most native unionid species, as with most exotic species invasions, human influences have also increased zebra mussel dispersal. Larvae and juveniles passively drift through artificial waterways and in the ballast water of ships, while adults attached to boats and recreational equipment are carried between water bodies (Mackie & Schloesser 1996). Such human-mediated dispersal has greatly aided the spread of zebra mussels to and throughout North America.

Zebra mussels show extremely high genetic variability. The variability is so high that Hebert et al. (1989) suggest the initial zebra mussel population in Lake St. Clair could not have been founded by only a few individuals and could not have suffered from a bottleneck after establishment. High genetic variability potentially allows for more adaptability and may be the reason that the original North American zebra mussel population has been able to successfully colonize a wide range of habitats, allowing for their widespread dispersal throughout the country.

### **What effects do zebra mussels have on their environment?**

Zebra mussels are probably most well-known for their effects on human development and infrastructure. Because of their ability to strongly attach to hard substrates with byssal threads, zebra mussels have attached to lots of human infrastructure causing large economic damage (Mackie 1991; MacIsaac 1996). For example, large masses of zebra mussels have attached to navigational buoys, making them sink from the extra weight. Zebra mussels have attached to fishing nets, making them non-functional. Such massive aggregates of zebra mussels have collected on the hulls of boats that sailing efficiency has become impaired. Zebra mussels have even clogged industrial and municipal pipelines and impaired components of hydropower plants (Mackie 1991; MacIsaac 1996).

In addition to harming human infrastructure, zebra mussels alter abiotic characteristics of their freshwater habitats. As filter feeders, they ingest particles suspended in water, thereby cleaning water. This may seem beneficial, and in the past, zebra mussels have been introduced as management tools to improve water quality in the Netherlands (MacIsaac 1996). By making water more transparent,

however, zebra mussels may induce a shift in ecological communities from pelagic to benthic energy production, thereby altering entire ecosystem structures and functions (Mackie 1991; MacIsaac 1996; Miehl et al. 2009).

Zebra mussels affect all levels of biological communities. Phytoplankton is negatively affected as individual phytoplankton are either ingested or enveloped in buoyant pseudofecal pellets (MacIsaac 1996). Zooplankton is also negatively affected, although it is unclear whether the effects are direct from zebra mussels ingesting larvae and small individuals or indirect from zebra mussels limiting zooplankton food resources (MacIsaac 1996). Because zooplankton is reduced, planktivorous fish, such as yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum*), decrease (MacIsaac 1996). Other fish species, like freshwater drum (*Aplodinotus grunniens*), eat zebra mussels, but this new food resource is not beneficial because fish that eat high proportions of zebra mussels are shorter in length (French 1996). Finally, zebra mussels biomagnify metal and organochlorine contaminants, threatening all of their predators, including fish and waterfowl, by transferring high levels of contaminants up the trophic levels (MacIsaac 1996).

Probably the most well studied biological damage caused by zebra mussels is their effect on native unionids. Because biofouling is a trait common to many marine organisms, marine mussels have adapted defense mechanisms against fouling (Haag et al. 1993). Freshwater mussels, however, have evolved without the presence of foulers and therefore lack such defense mechanisms. Thus, the introduction of fouling zebra mussels has been detrimental to North American unionids. Zebra mussels have been documented to colonize unionid beds at densities as high as 11,550 zebra

mussels per live unionid and 14,393 zebra mussels per dead unionid (Schloesser & Nalepa 1994). After the invasion of zebra mussels began in Lake St. Clair, native unionids suffered high mortality rates, and some populations were wiped out within five years; even more extreme, it took only two years after zebra mussels invaded Lake Erie for unionids to suffer high mortality (Schloesser et al. 1996). Mechanisms for the causes of mortality include restricting valve movement, impairing travel mobility, limiting food resources, and increasing overall stress on individuals (Mackie 1991; Haag et al. 1993; Van Appledorn & Bach 2007).

While zebra mussels have many negative biological impacts, some studies have shown positive effects on benthic invertebrates. Several studies have found that the presence of zebra mussels in soft sediments increased benthic invertebrate richness and abundance because zebra mussels increased food resources by depositing rich particles of organic matter as pseudo-feces and feces (Greenwood et al. 2001; Beekey et al. 2004). Zebra mussels also increased habitat complexity by forming aggregates of clumped adults, creating ideal hard-substrate environments for benthic invertebrates (Botts et al. 1996; Greenwood et al. 2001; Beekey et al. 2004). Not all benthic macroinvertebrates, however, respond positively to zebra mussels; one study in Lake Ontario found gastropods decline in the presence of zebra mussels (Haynes et al. 1999).

## **1.2 Dragonflies**

### **Basic biology**

Dragonflies, or their ancestors, are one of the most ancient known insects in the world (Corbet 1999). Dragonflies are members of the order Odonata, which is

divided into two suborders, Anisoptera (commonly referred to as dragonflies) and Zygoptera (commonly referred to as damselflies). Dragonflies have been well studied because they link trophic levels within and between ecosystems (Knight et al. 2005; Remsburg & Turner 2009; Wesner unpublished). At all life stages, dragonflies eat live prey and are prey for other animals, so they act as a trophic link between micro- and macrofauna. In addition, adult dragonflies are terrestrial while the larvae are aquatic, so they connect two ecosystems. An ecological study on trophic cascades by Knight et al. (2005) found that the presence of fish in a pond increased nearby plant pollination indirectly through dragonflies – fish in the pond ate aquatic dragonfly larvae, resulting in fewer adult dragonflies, decreasing the number of pollinators that were eaten, allowing more plants to be pollinated. Similarly, a study by Wesner (unpublished) found the presence of insectivorous fish decreased emerged dragonfly abundance, thereby altering the terrestrial insect community. Dragonflies thus play a vital role in trophic cascades both within and across ecosystems.

Dragonflies spend the majority of their lifetime as larvae, with many temperate species spending one to four years as larvae and only one summer as adults (Rensburg & Turner 2009). Throughout their life as larvae, individuals molt anywhere from 9 to 15 times depending on the species (Corbet 1999). Larvae are generalists, eating whatever is available from freshwater invertebrates to fish and amphibian larvae to other odonates, changing their diets seasonally. They can be found in a variety of habitats, most commonly in lakes, ponds, rivers and streams, but also in groundwater litter, tree holes, and marine tidal marshes (Corbet 1999). At the end of the larval stage, they metamorphose and leave their aquatic habitats. They

crawl up shores and beaches, sometimes even up plant stems and tree trunks, and undergo a final molt (emergence), when they become terrestrial, winged adults.

Anisoptera larvae have been grouped into four classes based on morphology and behavior (Corbet 1999). *Claspers* have elongated abdomens and cling to surfaces such as plant stems and hanging roots, a behavior not performed by larvae in other classes. *Hiders* have dorsoventrally flattened abdomens and cover themselves in leaf litter and detritus. *Sprawlers* have long legs that they extend laterally to support their bodies on or within a matrix, usually composed of detritus or macrophytes. *Burrowers* use their legs to dig into sediment and bury themselves. This last category is split into two sub-classes: *shallow burrowers* that leave their eyes above sediment while their bodies are buried, and *deep burrowers* that live in self-made burrows, usually leaving to forage at night and then returning to their burrow.

### **Study species**

*Macromia illinoiensis*, the Swift River Cruiser, was the species used in this study. *M. illinoiensis* belongs to the family Macromiidae, the Cruisers, of which there are two genera and ten species known from North America (Needham et al. 2000). Macromiidae are identified by a large, flat abdomen, long legs, and a unique horn between the eyes. They live on the bottom of shallow lakes and wide beds of larger streams. Like most dragonfly larvae, they are sit-and-wait predators and will eat any live prey of suitable size they find.

Although Corbet (1999) classifies Macromiidae as sprawlers, several sources have observed their burrower-like behavior. Williams (1978) observed several species of *Macromia* in aquaria noting that *M. taeniolata* often “burrowed” in the bottom and

*M. pacifica* was “always on the bottom, covering its body with sand during the day so that only the outline was visible” (cited in Corbet 1999, 152). He observed that both species “spread the legs and lay flat on the sand and gravel during the daytime; at night they retracted the legs slightly and elevated the body, adopting what was apparently a foraging posture” (cited in Corbet 1999, 152). This diel cycle was also observed in *M. illinoensis* by Tylczak (unpublished), who found individuals under a layer of sand during the day and above the sand while waiting for prey at night. Finally, Needham (2000) described Macromiidae burrowing behavior, and Remsburg & Turner (2009) classified *M. illinoensis* larvae as displaying both sprawler and burrower behavior.

It is unclear exactly what evolutionary purpose the larval burying behavior serves. Corbet (1999) discusses the camouflage provided by burying as an antipredation mechanism, while Needham (2000) discusses the camouflage as a way to hide from prey while foraging. Because *M. illinoensis* sprawl above the sand while waiting for prey at night (Tylczak unpublished), the burying behavior most likely serves as camouflage from predators during the day. Therefore, if larval burying behavior is altered, it may be detrimental because individuals would be more vulnerable to predation.

### **1.3 Threats by Zebra Mussels**

Zebra mussels were first observed colonizing a dragonfly larva in North America in the Mississippi River in Jersey County, Illinois in 1993. Tucker & Camerer (1994) collected a twig covered in zebra mussels and found a *Gomphus vastus* larva attached to the twig by the byssal threads of two zebra mussels. Nine

years later, Weihrauch & Borcharding (2002) published a short paper summarizing the 28 colonized larvae and exuviae consisting of ten species that had been documented worldwide. They discuss some potential mechanisms as to how the zebra mussels colonized the dragonflies, but they do not address the frequency at which individuals within a population are colonized or the effects on dragonfly fitness due to colonization.

The first study to quantify the frequency of zebra mussel colonization in an invaded population of dragonflies was McCauley and Wehrly (2007). They collected 51 individual larvae of which 32 (63%) were colonized by one or more zebra mussels. Varying rates of colonization were found across species, so it was hypothesized that body shape and area may affect colonization. In addition, they predicted potential harms that zebra mussels may cause to dragonfly larvae: a mechanical block from emergence as adults, high energetic costs due to increased biomass, limited mobility and burying behavior, and even early mortality from zebra mussels attaching larvae to benthic substrates using byssal threads. This last hypothesis was observed once by McCauley and Wehrly, their only documented observation of how zebra mussels may affect dragonfly larvae fitness.

Fincke et al. (2009) published a follow-up study documenting zebra mussel colonization rates of more species and testing the effects of colonization on dragonfly fitness, specifically on mobility and survivorship (Figure 1.4). Zebra mussels colonized species non-randomly, with increased colonization on individuals with larger body areas and sprawler behavior over individuals with smaller body areas and burrower behavior. They also found that colonization by zebra mussels impaired

mobility and decreased survivorship of several of the dragonfly species. For three of the sprawler species, increased mussel load decreased the likelihood that individuals would leave the water for emergence, while for the two burrower species, increased mussel load decreased the distance traveled up the beach for emergence. For one of the sprawler species, increased mussel load increased the likelihood of being preyed upon. Finally, colonization by zebra mussels slowed or even prevented larvae from flipping over, a skill necessary to correct for being overturned by currents when leaving the water.

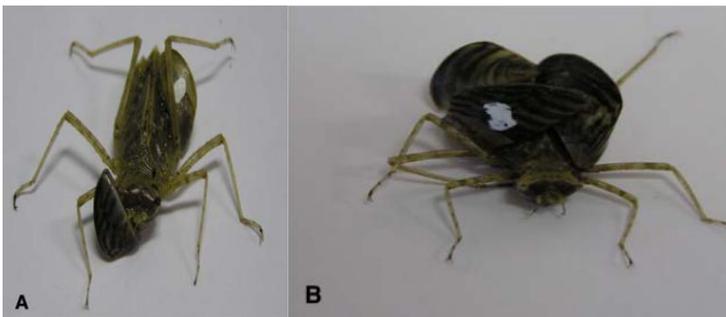


Figure 1.4: *Didymops transversa* larvae colonized by zebra mussels. A: A zebra mussel on the head may impair ability to feed. B: Multiple zebra mussels on the thorax may prevent emergence. Photos from Ola Fincke.

The most recent study performed by Tylczak (unpublished) looked at the effects of zebra mussel colonization on dragonfly larvae feeding behavior. While colonized individuals killed fewer mayfly larvae than uncolonized individuals, the apparent relationship was not statistically significant, and it was concluded that zebra mussel colonization does not impair larval feeding behavior.

#### **1.4 Study Objectives**

There have now been several studies documenting colonization of dragonfly larvae by zebra mussels, and some of those studies have looked at fitness effects

caused by colonization. Little is known, however, about dragonfly larvae burying behavior or how colonization affects this behavior. The purpose of this study was to document dragonfly larvae burying behavior and to test the effects of zebra mussel colonization on burying behavior. The study asks three questions. What factors affect burying behavior of uncolonized larvae? Does an individual's burying behavior affect its probability of being colonized by zebra mussels? Does zebra mussel colonization, specifically the presence or absence of attached zebra mussels, affect burying behavior?

We hypothesized that several internal and external factors would affect burying behavior. Weather and water temperature were expected to affect burying behavior because dragonflies, like all invertebrates, are poikilothermic (their body temperature varies with their external environment). Morphology, including head width and body area, was also expected to affect burying behavior because different body sizes and shapes may make burying easier or harder. We hypothesized that slower, shallower buriers would be more likely to get colonized because they would be more exposed to zebra mussels. Finally, we hypothesized that being colonized would increase burial time and decrease burial depth because increased biomass would impede burying behavior.

## 2. Methods

This study was performed along the shore of Douglas Lake at the University of Michigan Biological Station (43°35'N, 84°42'W) near Pellston, Michigan. Douglas Lake is a mesotrophic kettle lake with a sandy bottom and clear water. Zebra mussels invaded Douglas Lake in 2001 and have since devastated the local unionid populations (Fincke et al. 2009).

Individual *Macromia illinoensis* were collected using a dip net between the shore and 5 meters into the lake along the South Bay to Pine Point of Douglas Lake. Collection took place on the afternoon of 19 July 2009. Individuals were brought back to the Lakeside Lab boatwell, where body length, abdomen width, and head width were measured with calipers to the nearest 0.1 mm. Of the collected individuals, 15 were determined to be F-2 instars based on size. To determine natural colonization rates of F-2 instars, all F-2 individuals naturally colonized by zebra mussels were recorded, and mussels were removed before the beginning of experimentation. Previously colonized individuals were used for experimentation because it was not expected that effects of colonization would continue to harm the organisms once mussels were removed. Twelve of the 15 individuals were used for experimentation.

The 12 F-2 instars were placed individually in plastic shoeboxes (30 x 16.5 x 10.5 cm) filled with sand (2 cm deep) and lake water (2 cm above the level of the sand substrate). Containers were maintained at ambient temperature and light outside the Lakeside Lab under a roof overhang to protect them from rain. A screen was placed over the containers to prevent larvae from escaping or anything from entering.

To determine natural burying behavior, burial depth and time were measured between 8:00 – 11:30 daily for five days beginning 20 July 2009. At the start of each session, initial depth was determined using a 0-3 scale: 0 = uncovered, 1 = lightly covered with sand but the body still visible, 2 = completely covered with sand but the body outline still visible, and 3 = completely covered with sand and the body outline not visible. Each individual was uncovered and placed in the middle of its container. For the first two hours, body parts (head, legs, thorax, upper abdomen, lower abdomen) covered with sand were recorded every five minutes. After two hours, if individuals were still covering themselves, observations continued every 10 minutes until all individuals stopped moving. “Burial time” was calculated as the difference between the time when an individual began moving and the time when the individual was completely covered. Water temperature and weather was noted at the end of each timed trial. Instars were fed two amphipods daily in the late morning after each trial (O. Fincke, pers. comm.).

To determine whether burying behavior affected the probability that a zebra mussel would attach to a larvae, 50 zebra mussels ranging in size from 5-11 mm in length were added to each container on the evening of 24 July 2009. Fifty was chosen for the number of zebra mussels to allow for successful colonization. The zebra mussels were collected that afternoon along the same beach of Douglas Lake. Every morning and evening for five days, burial depth was measured. The length of each attached mussel was measured with calipers to the nearest 0.1 mm, and the location of each mussel on an instar was recorded. Zebra mussels that were attached to the

container walls or one another were detached and put back in the sand. Instars were fed five amphipods daily after the evening measurements were taken.

To determine whether mussel colonization affected instar burying behavior, timed burying trials were performed for three additional days beginning 30 July 2009. All instars were removed from their containers and placed in wax cups (11 cm diameter, 6 cm tall) filled with sand (2 cm deep) and lake water (2 cm above the level of the sand substrate). All mussels that had colonized instars were left attached, but no additional mussels were placed in the cups. The mussel-free instars were used as controls. Instars were fed 2 amphipods in the late morning after each trial. At the end of the third trial, all remaining zebra mussels were removed and instars were released back into Douglas Lake.

On 2 August 2009, a new set of 12 F-2 instars was collected from Douglas Lake, and the experiment was repeated to create a total sample size of 24 individuals. The timed burying trials began on the morning of 3 August 2009 and included one afternoon trial. Mussels were added on the evening of 6 August 2009. Post-mussel timed burying trials began 12 August 2009 and included one afternoon trial. Throughout the entire experiment, instars were fed five amphipods every evening for consistency. During the colonization trials, one uncolonized dragonfly larva was found dead. It was believed to have died due to causes unrelated to mussels, so a total of 23 individuals were used for analysis.

A variety of statistical tests were used to analyze the data. Pooled standard deviation ( $s_p$ ) was calculated for burial time and burial depth. A homogeneous groups test (Archie 1985) comparing two times  $s_p$  across means of individual burial times

and burial depths was used to test the null hypothesis that burial times and burial depths among individuals were homogeneous. Burial time was log transformed to normalize the data. Pairs of dependent and independent variables were analyzed using least squares regressions and one-way analysis of variance (ANOVA) using Statistica 8. A G-test using an R x C test of independence, calculated manually, was used to determine the relationship between burial depth and weather categories during the uncolonized timed burying trials (Sokal & Rohlf 1995). A G-test of independence, calculated manually, was used to determine the relationship between time of day and weather category during the colonization trials.

### 3. Results

#### 3.1 Burying Behavior

All individuals buried themselves in the same way. First, a larva backed its posterior end into the sand. After wiggling backwards enough to cover its entire abdomen and thorax, it used its front two legs to push sand over its head leaving its eyes uncovered. Lastly, it pushed each of its legs into the sand.

Individuals varied in burial time ranging from 5 to 120 minutes with a mean of 22.6 minutes. One individual took 120 minutes to bury; without this outlier, burial time ranged from 5 to 80 minutes with a mean of 21.6 minutes. Twenty-two individuals buried themselves more than once in the five trials. Based on these 22 individuals, we failed to reject the null hypothesis that their burial times comprised a single homogeneous group both with ( $n = 99$ ;  $s_p = 17.05$ ;  $p > 0.05$ ; Figure 3.1) and without ( $n = 98$ ;  $s_p = 15.25$ ;  $p > 0.05$ ) the 120 minute outlier, suggesting that variation in burial time formed a continuum.

Burial depth varied within and among individuals; no one individual was observed at the same burial depth category all five days. All burial depth categories were observed in the study (Figure 3.2), with category 3 (completely covered) observed most frequently (54%) and category 0 (uncovered) observed least frequently (2%). Based on the 23 larvae used for analysis, a homogeneous groups test found all individuals fell into a single homogeneous group ( $n = 115$ ;  $s_p = 0.7547$ ;  $p > 0.05$ ; Figure 3.3).

Water temperature in the containers was significantly affected by the weather, with warmer water temperatures on sunny days ( $df = 1,8$ ;  $F > 19$ ;  $p < 0.003$ ; Figure 3.4). Burial time was affected by both weather and water temperature, but burial depth was not. Burial time was significantly faster ( $df = 1,98$ ;  $F > 7.6$ ;  $p < 0.007$ ; Figure 3.5) on sunny days (mean = 18.04 min,  $n = 46$ ) than on cloudy days (mean = 26.48 min,  $n = 54$ ). Log water temperature explained 15% of the variation in log burial time ( $df = 1,98$ ;  $F > 17$ ;  $p < 0.001$ ; Figure 3.6). We failed to reject the null hypothesis that burial depth and weather are independent ( $n = 62$ ;  $G_{adj} = 1.86$ ;  $p > 0.5$ ; Figure 3.7), and burial depth was not affected by water temperature ( $df = 1,113$ ;  $F > 0.13$ ;  $p > 0.7$ ;  $r^2 = 0.0012$ ; Figure 3.8).

Head widths ranged from 4.9 mm to 5.8 mm with a mean of 5.2 mm; body area ranged from 167 mm<sup>2</sup> to 229 mm<sup>2</sup> with a mean of 192 mm<sup>2</sup> (Figures 3.9, 3.10). Head width and body area were positively correlated ( $n = 23$ ;  $r = 0.7422$ ;  $p < 0.05$ ). Neither burial time nor burial depth exhibited significant regressions ( $p > 0.05$ ) with morphological measurements (Figures 3.11-3.14).

### **3.2 Zebra Mussel Colonization**

Fifty zebra mussels were added to each dragonfly larva's container, and colonization rates were observed every morning and evening for five days. Of the 23 dragonfly larvae that survived the five days of zebra mussel colonization, all but two individuals (91%) were colonized by at least one zebra mussel during at least one observation period. A total of 67 zebra mussels were observed to colonize dragonfly larvae. Zebra mussels did not always remain attached throughout the five days; instead, 40 zebra mussels that had been seen attached to a dragonfly at one

observation period were not attached at the next. It is unknown whether the zebra mussels voluntarily detached themselves or whether the actions of the dragonfly larvae were responsible for detachment. It is also unknown whether the same zebra mussel ever reattached to a dragonfly. The total number of zebra mussels colonizing each individual at some observation period throughout the five days ranged from zero to nine, with a maximum of five zebra mussels seen on one individual simultaneously (Figure 3.15). Of those five mussels, two were attached to mussels that were attached to the dragonfly rather than directly attached to the dragonfly. One other dragonfly had two zebra mussels attached to mussels.

Individuals with slower uncolonized burial times were colonized by more zebra mussels. The trend was found to be marginally significant ( $df = 1,98$ ;  $F > 3.7$ ;  $p = 0.0542$ ; Figure 3.16), with uncolonized burial time explaining only 4% of the variation in the number of colonizing zebra mussels. To account for weather effects on burial time, the analysis was repeated separately for sunny and cloudy burial time data. A positive relationship was found between burial time during sunny observations and the total number of attached zebra mussels ( $df = 1,44$ ;  $F > 5$ ;  $p < 0.02$ ;  $r^2 = 0.1184$ ; Figure 3.17). No relationship was found between burial time during cloudy observations and the total number of attached zebra mussels ( $df = 1,55$ ;  $F > 0.7$ ;  $p > 0.3$ ;  $r^2 = 0.0143$ ; Figure 3.18). The total number of colonizing zebra mussels was not significantly associated with uncolonized burial depth ( $df = 1,113$ ;  $F > 0.6$ ;  $p > 0.4$ ;  $r = 0.0053$ ; Figure 3.19).

A G-test of independence found weather and time of day were correlated, with more morning observation periods cloudy and evening observation periods sunny (n

= 20;  $p < 0.05$ ). Weather affected colonization rates, with the 11 sunny observation periods showing, on average, more newly attached zebra mussels than the nine cloudy observation periods ( $df = 1,228$ ;  $F > 4.6$ ;  $p < 0.033$ ; Figure 3.20). There seemed to be an effect between time of day and colonization rate, with more zebra mussels colonizing dragonflies during the daytime, but it was not significant ( $df = 1,228$ ;  $F > 0.75$ ;  $p > 0.3$ ; Figure 3.21). Weather was, thus, found to affect colonization rates more than time of day.

Not enough water temperature data was collected to analyze the effects of water temperature on colonization, but it is hypothesized that warmer water temperatures would increase colonization because water temperature was again found to be positively associated with weather ( $df = 1,8$ ;  $F > 29$ ;  $p < 0.001$ ; Figure 3.22).

Individuals with wider heads and greater body areas were colonized by a significantly greater number of total zebra mussels throughout the five days ( $df = 1,228$ ;  $F > 9$ ;  $p < 0.005$ ;  $r^2 = 0.0389$ ; Figure 3.23;  $df = 1,228$ ;  $F > 96$ ;  $p < 0.001$ ;  $r^2 = 0.2971$ ; Figure 3.24). Colonization rate (the number of new zebra mussels per observation period) was not associated with head width ( $df = 1,228$ ;  $F > 0.8$ ;  $p > 0.3$ ;  $r^2 = 0.0038$ ; Figure 3.25). Body area explained 3% of the variation in colonization rate ( $df = 1,228$ ;  $F > 6$ ;  $p < 0.01$ ), but when four outliers were removed, the regression became insignificant (Figure 3.26).

### **3.3 Burying Behavior After Zebra Mussel Colonization**

At the end of the five days of zebra mussel colonization, only 12 of the 23 individuals were colonized by zebra mussels due to detachment of mussels during the colonization experiment. Of the 12 individuals with zebra mussels attached, six had

one mussel attached, four had two mussels attached, and two had four mussels attached (Figure 3.27). During the post-colonization timed trials, three individuals lost one or two attached zebra mussels, but each still had at least one zebra mussel attached.

Because burial time was associated with weather, weather was accounted for when analyzing post-colonization timed burial trials. When the data were analyzed separately for each weather category, significant relationships were not found between the number of attached zebra mussels and burial time ( $df = 1,35$ ;  $F > 1$ ;  $p > 0.2$ ;  $r^2 = 0.0407$ ; Figure 3.28;  $df = 1,7$ ;  $F > 1$ ;  $p > 0.3$ ;  $r^2 = 0.1507$ ; Figure 3.29). An apparent relationship was found between the means of the burial time on sunny days before colonization (mean = 18.04 minutes,  $n = 46$ ), after colonization with zebra mussels attached (mean = 23.33 minutes,  $n = 15$ ), and after colonization without zebra mussels attached (mean = 15.68,  $n = 22$ ). Individuals with attached mussels buried more slowly after colonization than individuals before colonization or individuals without zebra mussels after colonization, but the variation within each group was great enough that no treatment effect was identified ( $df = 2,80$ ;  $F > 1$ ;  $p > 0.2$ ; Figure 3.30). There were not sufficient data to repeat the analysis for cloudy days.

Weather was not included as a factor when analyzing post-colonization burial depth data because uncolonized burial depth was not affected by weather. Burial depth negatively related to the number of attached zebra mussels; individuals carrying more mussels buried more shallowly ( $df = 1,67$ ;  $F > 5$ ;  $p < 0.02$ ;  $r^2 = 0.0697$ ; Figure 3.31). An apparent relationship was found between means of burial depth before

colonization (mean = 2.33, n = 115), after colonization with zebra mussels attached (mean = 1.91, n = 35), and after colonization without zebra mussels attached (mean = 2.29, n = 34). Individuals with mussels attached after colonization buried marginally significantly more shallowly than the individuals before or after without zebra mussels (df = 2,181;  $F > 2$ ;  $p = 0.0654$ ; Figure 3.32). The marginal significant difference was between individuals before colonization and after colonization with zebra mussels attached (Post-Hoc Tukey Test:  $p = 0.0521$ ). The pair-wise relationship was found further significant by a T-test (df = 148;  $p = 0.0153$ ). A T-test of zebra mussels after colonization with zebra mussels attached compared to individuals after colonization without attached zebra mussels was not significant (df = 67;  $p = 0.1479$ ).

### 3.4 Graphs

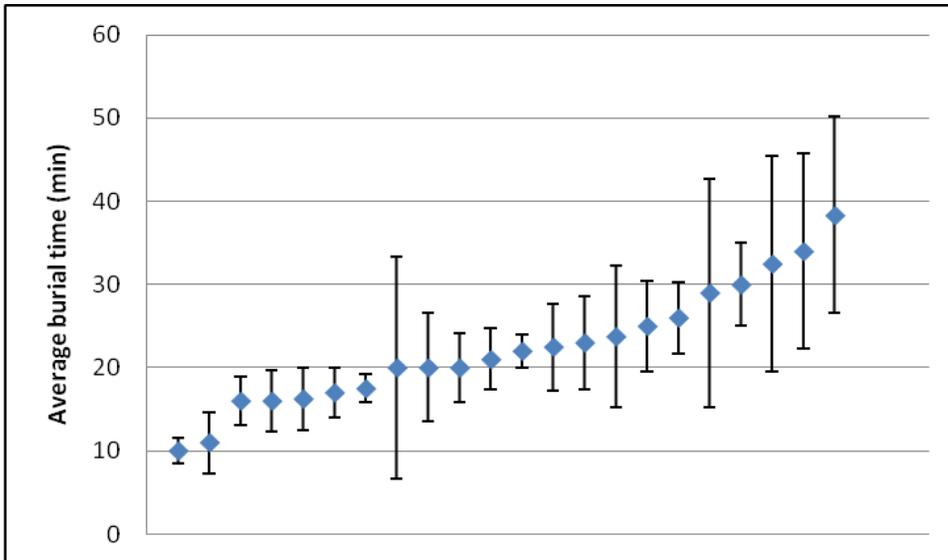


Figure 3.1: Variation in burial time among individuals ordered from fastest to slowest ( $n = 98$ ). Each diamond represents the mean burial time for one individual. Error bars indicate one standard error.

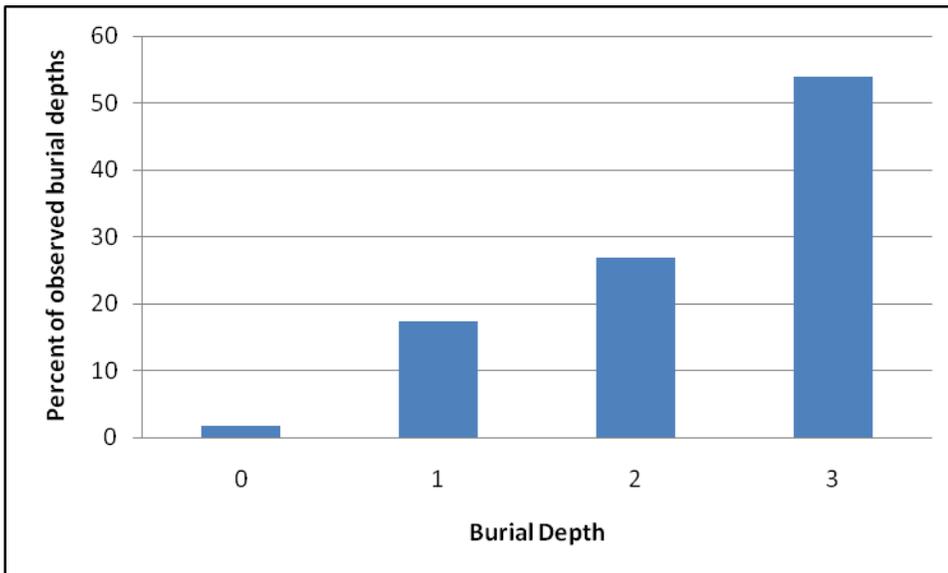


Figure 3.2: Percent of observations of each burial depth category without zebra mussels ( $n = 115$ ). Depth categories defined as: 0 = uncovered; 1 = lightly covered with sand but the body still visible; 2 = completely covered with sand but the body outline still visible; 3 = completely covered with sand and the body outline not visible.

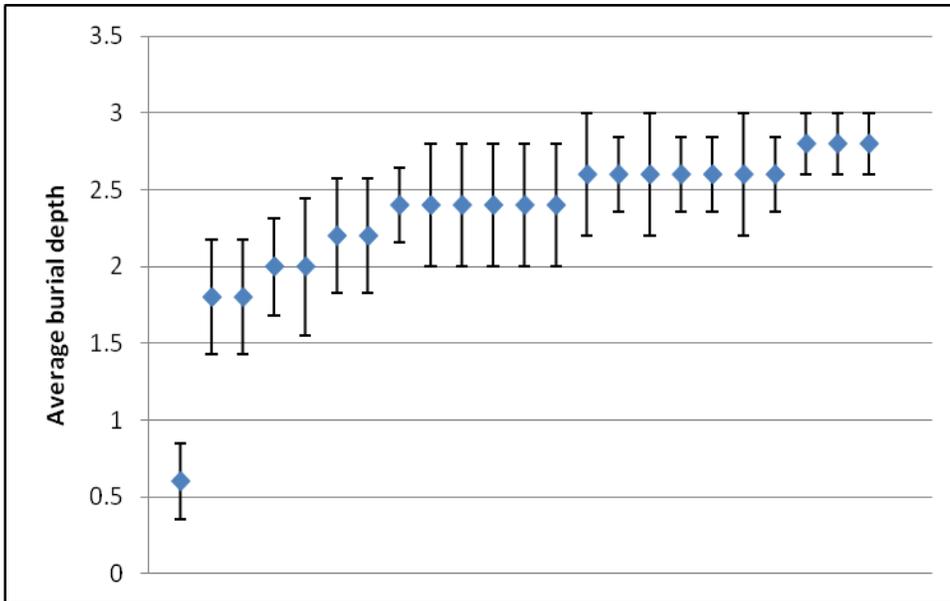


Figure 3.3: Variation in burial depth among all individuals ordered from shallowest to deepest (n = 115). Each diamond represents the mean burial depth of one individual. Error bars indicate one standard error.

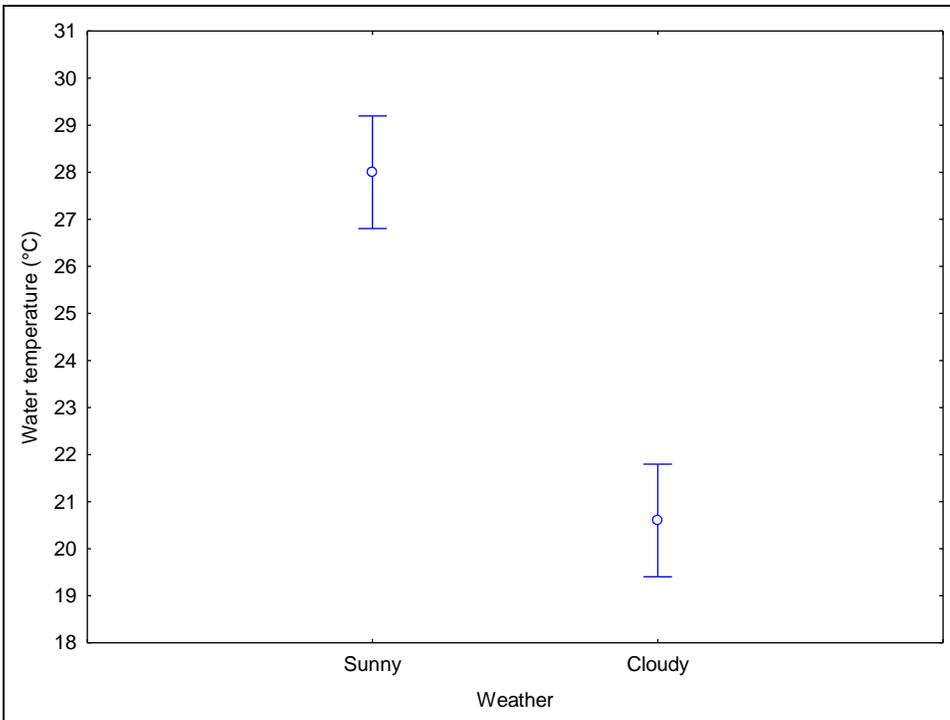


Figure 3.4: Mean water temperature on sunny (n = 5) and cloudy days (n = 5) during burying behavior period. Circles represent the mean water temperature for sunny and cloudy trials. Error bars indicate one standard error.

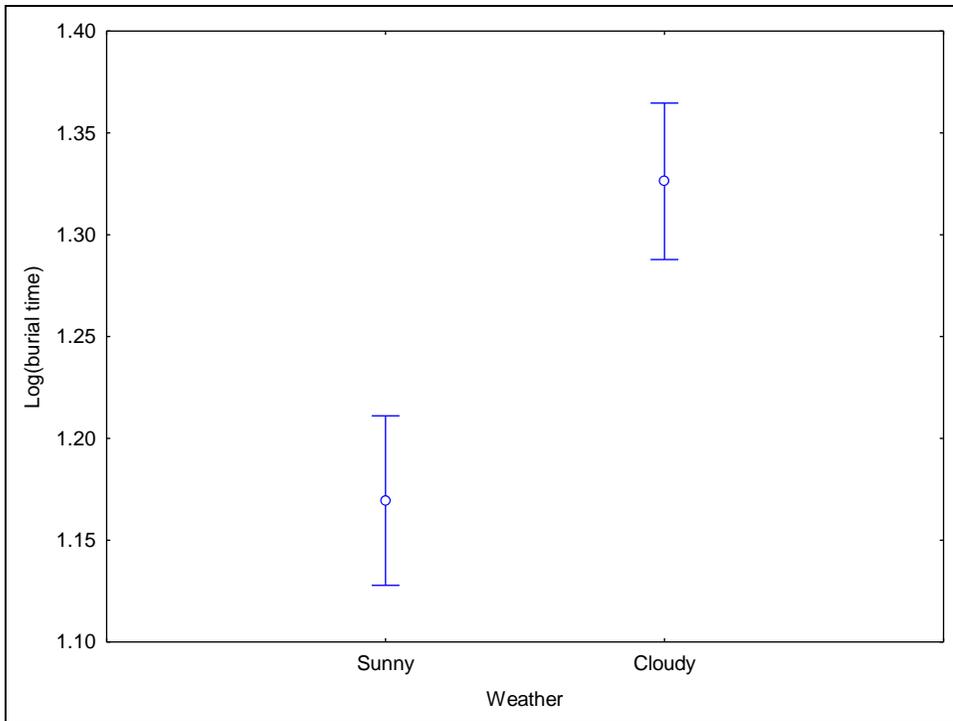


Figure 3.5: Log of mean burial time of all individuals on sunny (n = 46) and cloudy days (n = 54). Circles represent the log mean burial time for sunny and cloudy trials. Error bars indicate one standard error.

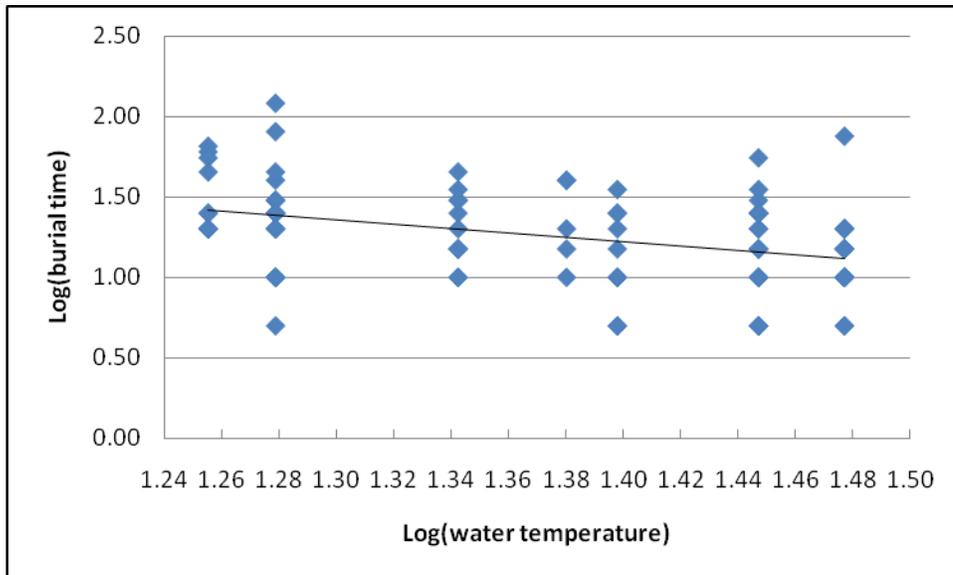


Figure 3.6: Log burial time of all individuals as a function of log water temperature (n = 100). Each diamond represents at least one observation.

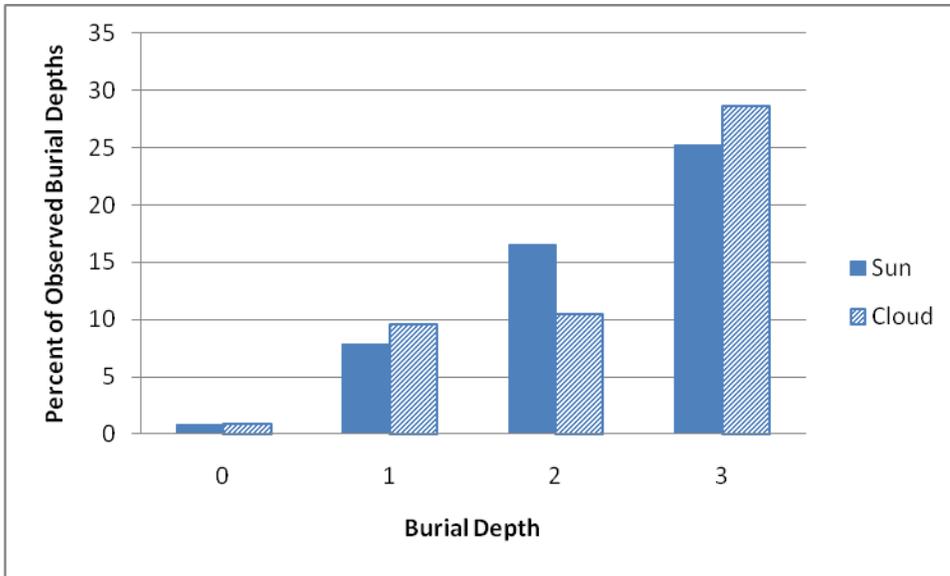


Figure 3.7: Percent of observations of each burial depth category by weather category (n = 115).

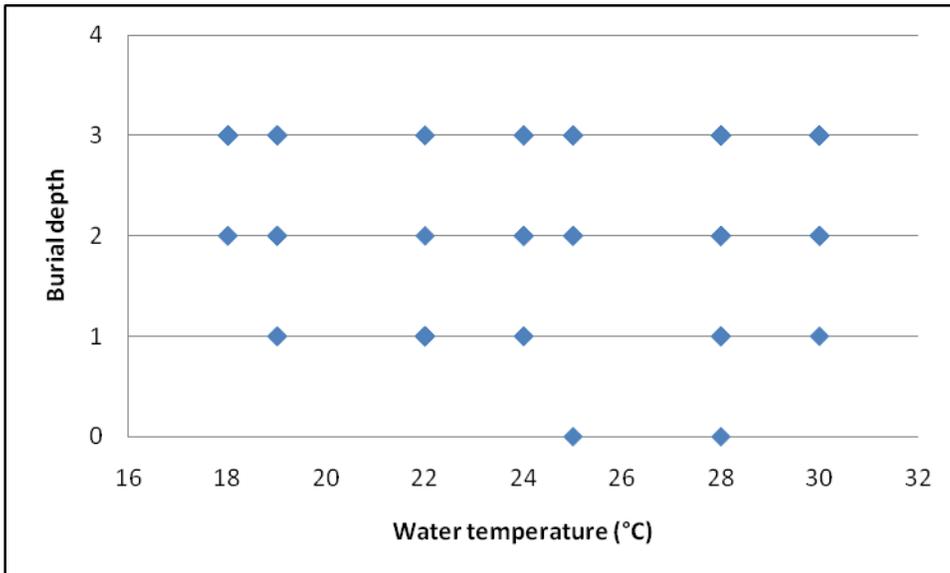


Figure 3.8: Burial depths of all individuals as a function of water temperature (n = 115). Each diamond represents at least one observation.

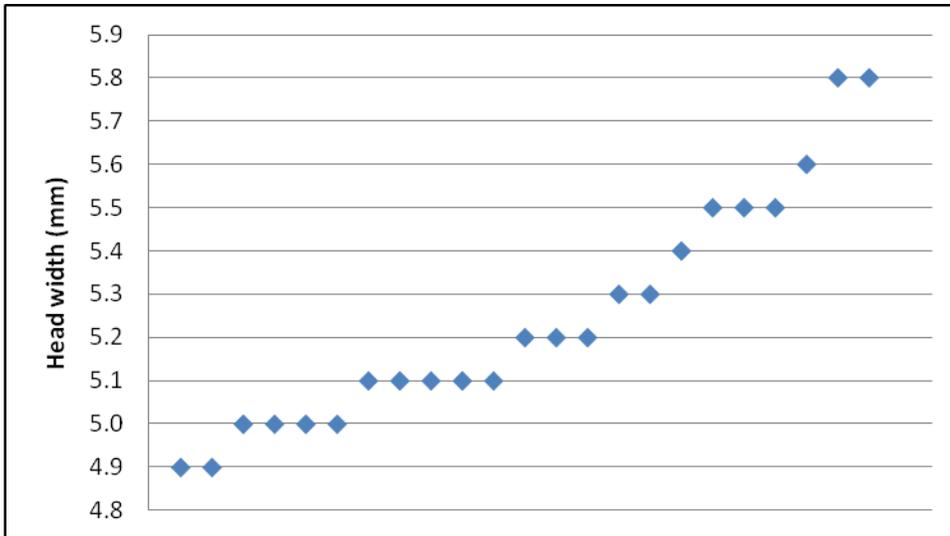


Figure 3.9: Head widths of the 23 dragonfly larvae measured ordered from narrowest to widest. Each diamond represents one individual.

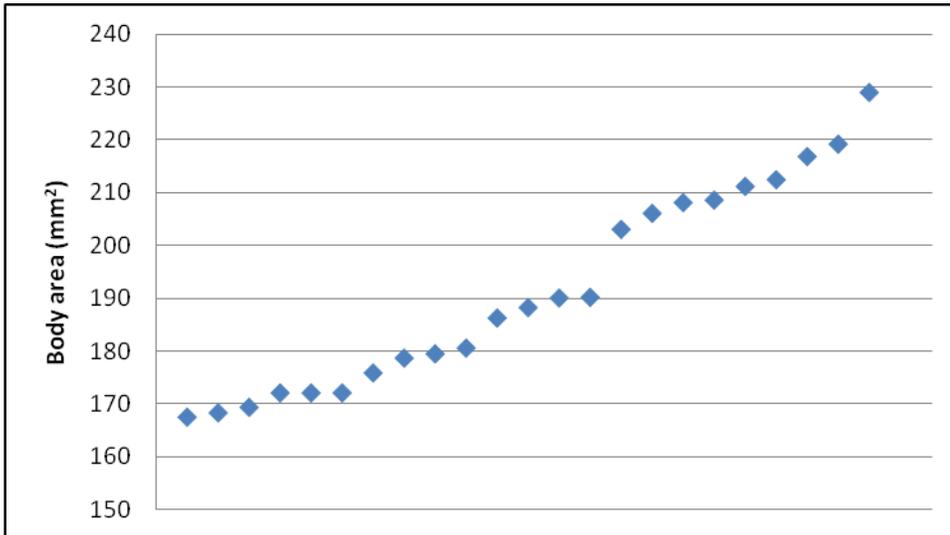


Figure 3.10: Body area of the 23 dragonfly larvae measured ordered from least to greatest. Each diamond represents one individual.

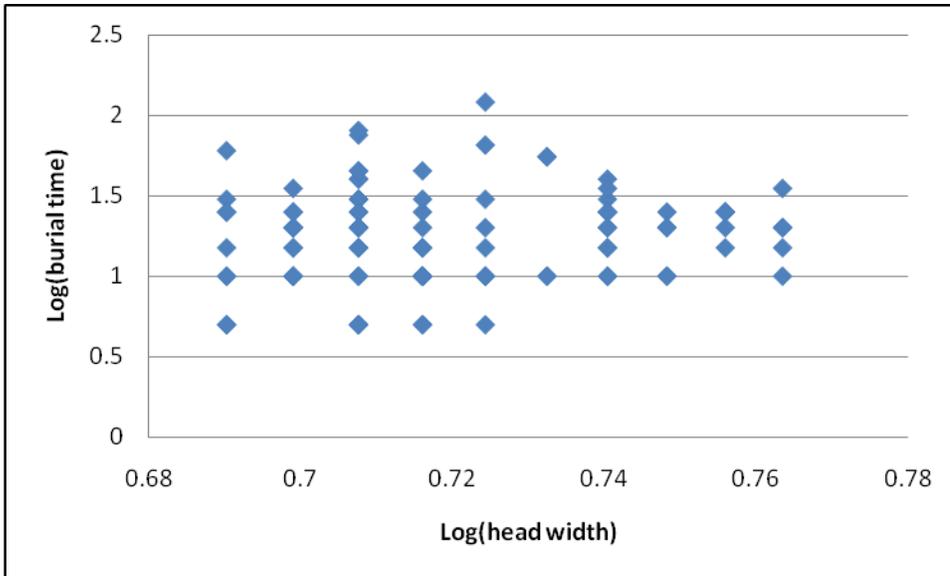


Figure 3.11: Log burial time of all individuals as a function of log head width (df = 1,98;  $F > 0.6$ ;  $p > 0.4$ ;  $r^2 = 0.0065$ ). Each diamond represents at least one observation.

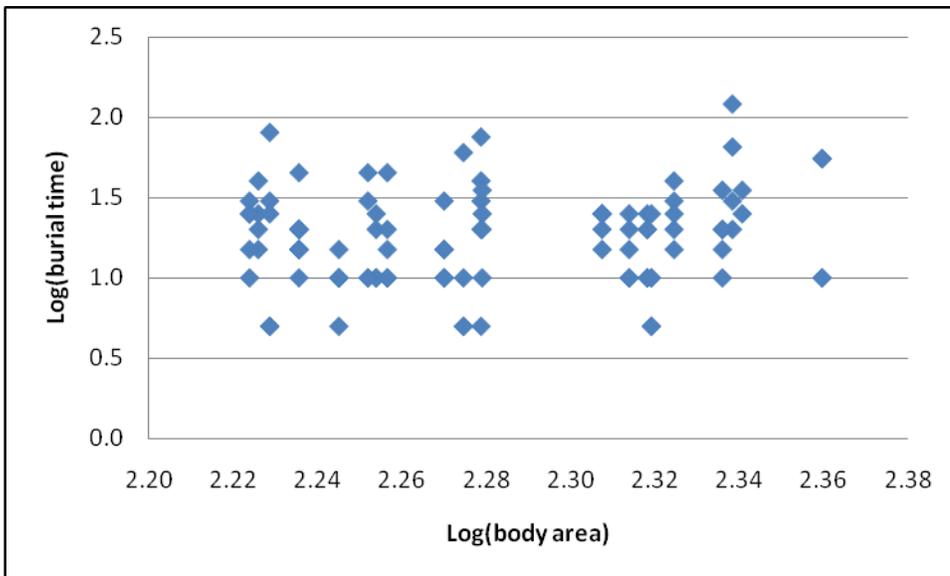


Figure 3.12: Log burial time of all individuals as a function of log body area (df = 1,98;  $F > 1.6$ ;  $p > 0.19$ ;  $r^2 = 0.0170$ ). Each diamond represents at least one observation.

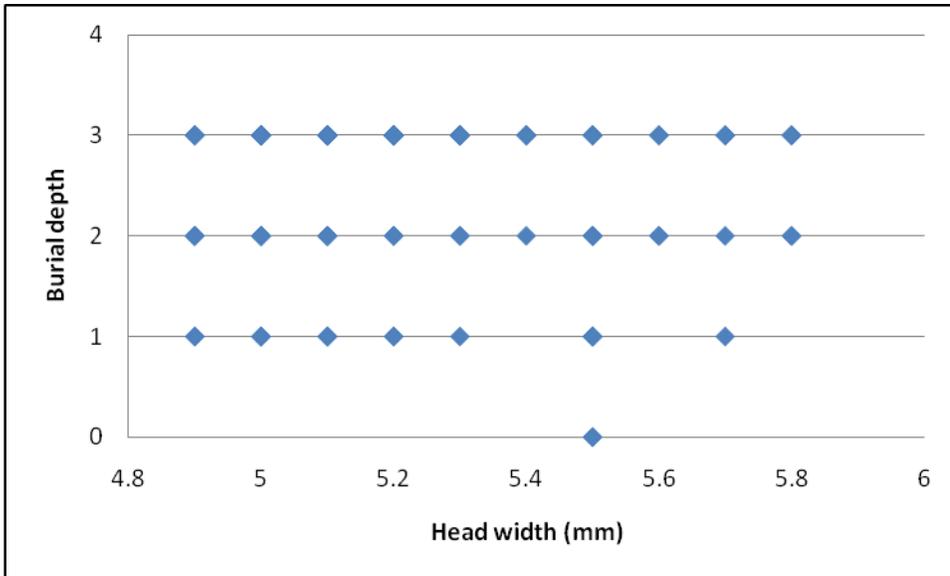


Figure 3.13: Burial depths of all individuals as a function of head width ( $df = 1,113$ ;  $F > 0.01$ ;  $p > 0.8$ ;  $r^2 = 0.0001$ ). Each diamond represents at least one observation.

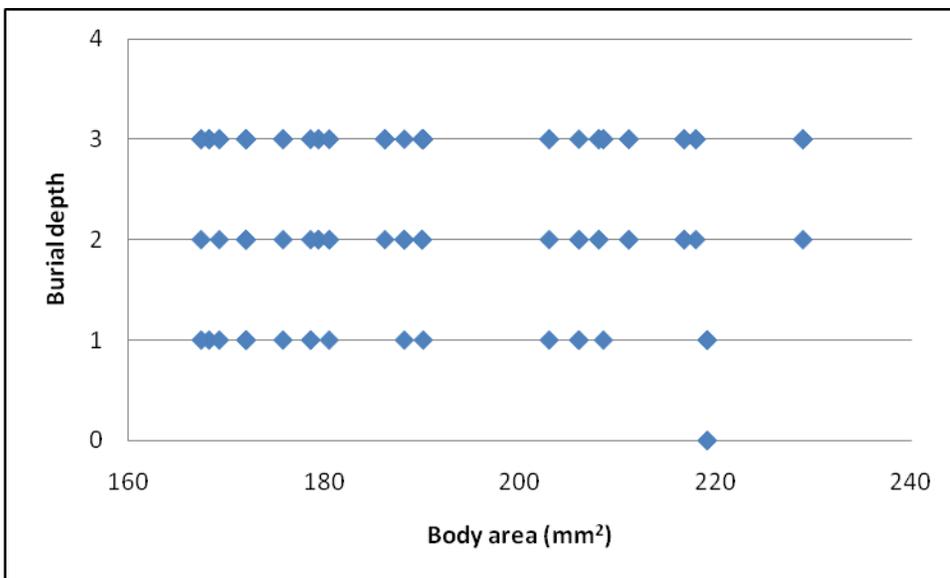


Figure 3.14: Burial depths of all individuals as a function of body area ( $df = 1,113$ ;  $F > 0.001$ ;  $p > 0.9$ ;  $r < 0,0001$ ). Each diamond represents at least one observation.

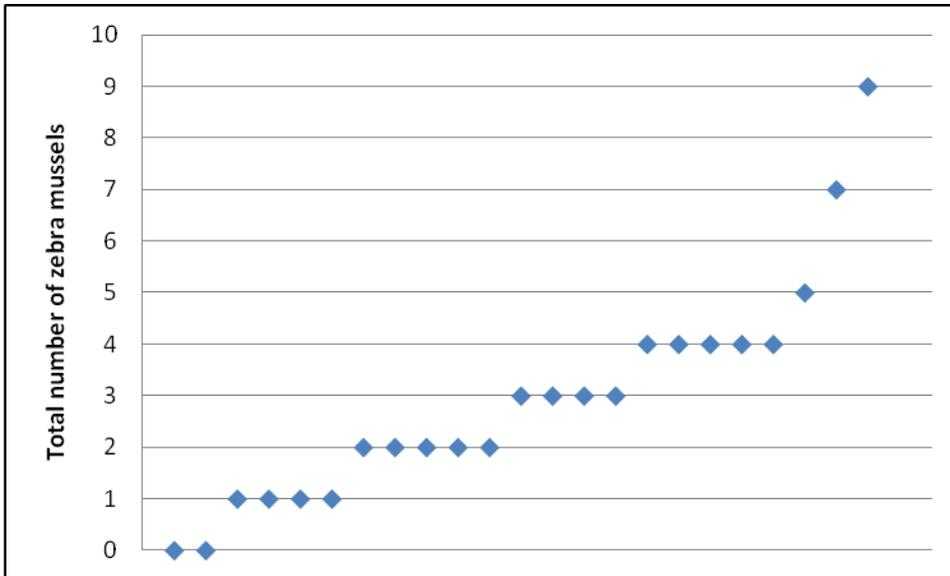


Figure 3.15: The total number of zebra mussels that attached to each individual throughout five observation days ordered from fewest to greatest ( $n = 23$ ). Each diamond represents one individual.

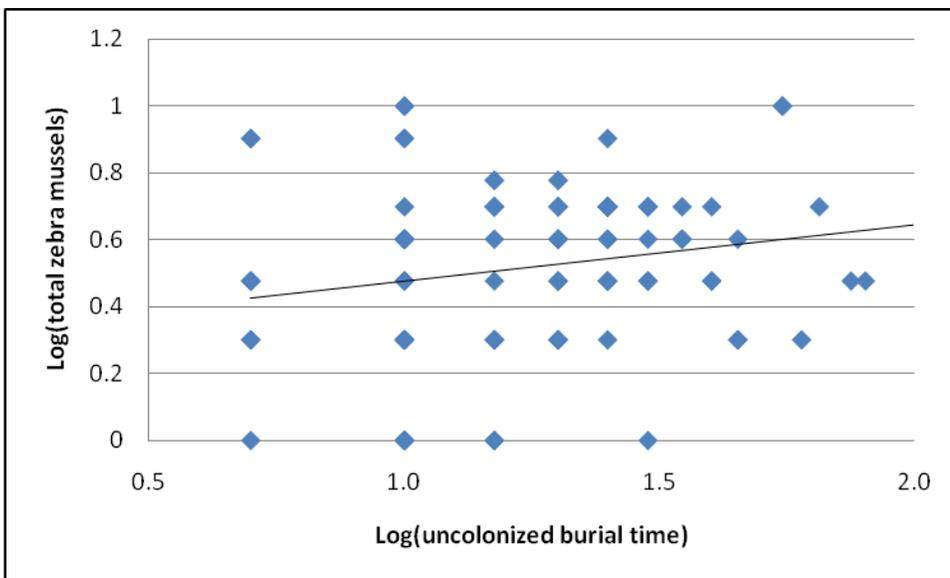


Figure 3.16: Log total number of zebra mussels that colonized each individual as a function of log uncolonized burial times ( $n = 100$ ). Each diamond represents at least one observation.

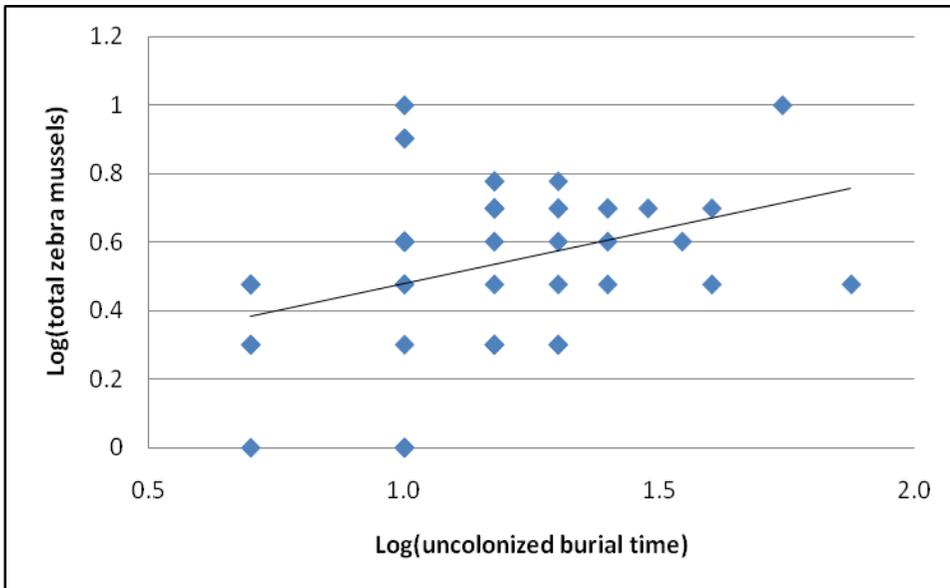


Figure 3.17: Log total number of zebra mussels that colonized each individual as a function of log uncolonized burial times on sunny days ( $n = 46$ ). Each diamond represents at least one observation.

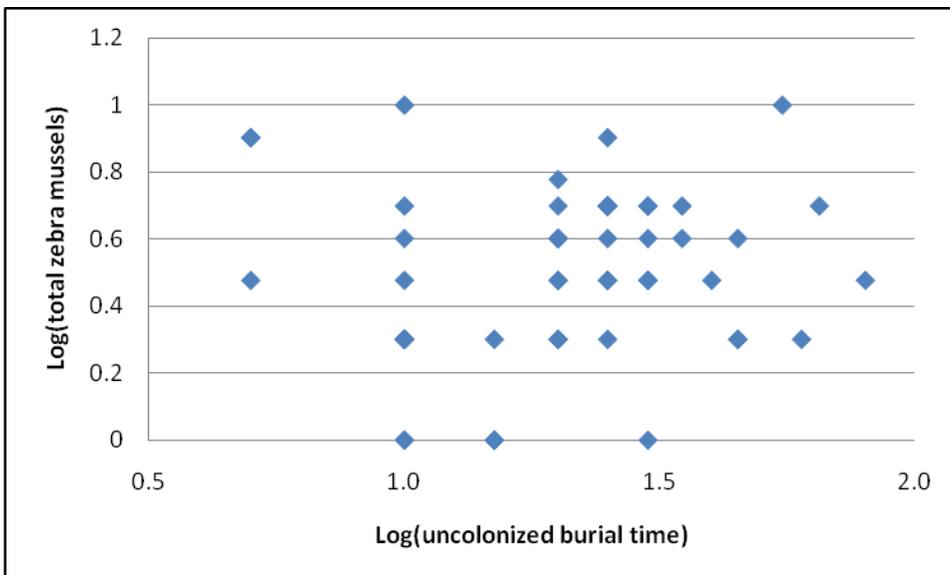


Figure 3.18: Log total number of zebra mussels that colonized each individual as a function of log uncolonized burial times on cloudy days ( $n = 54$ ). Each diamond represents at least one observation.

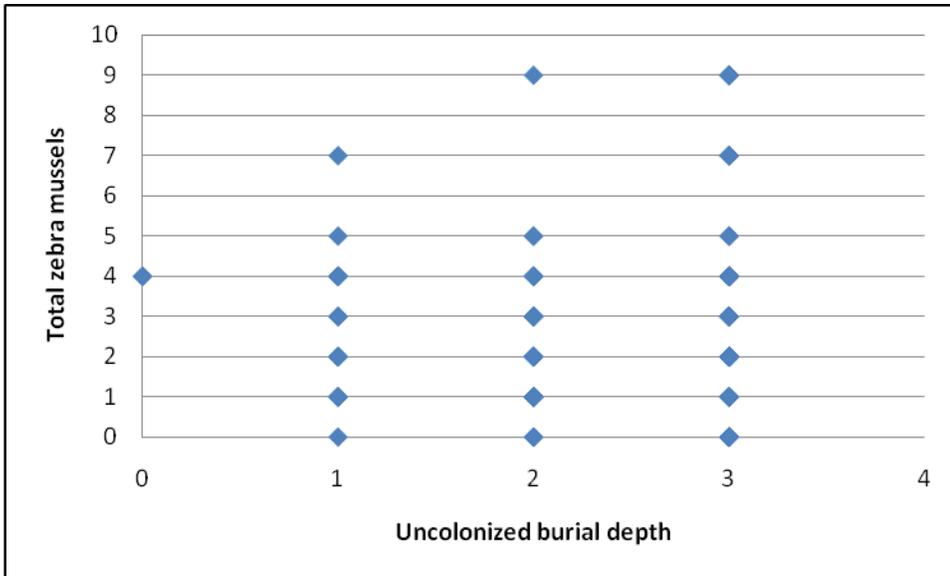


Figure 3.19: Total number of zebra mussels that colonized each individual as a function of uncolonized burial depths ( $n = 115$ ). Each diamond represents at least one observation.

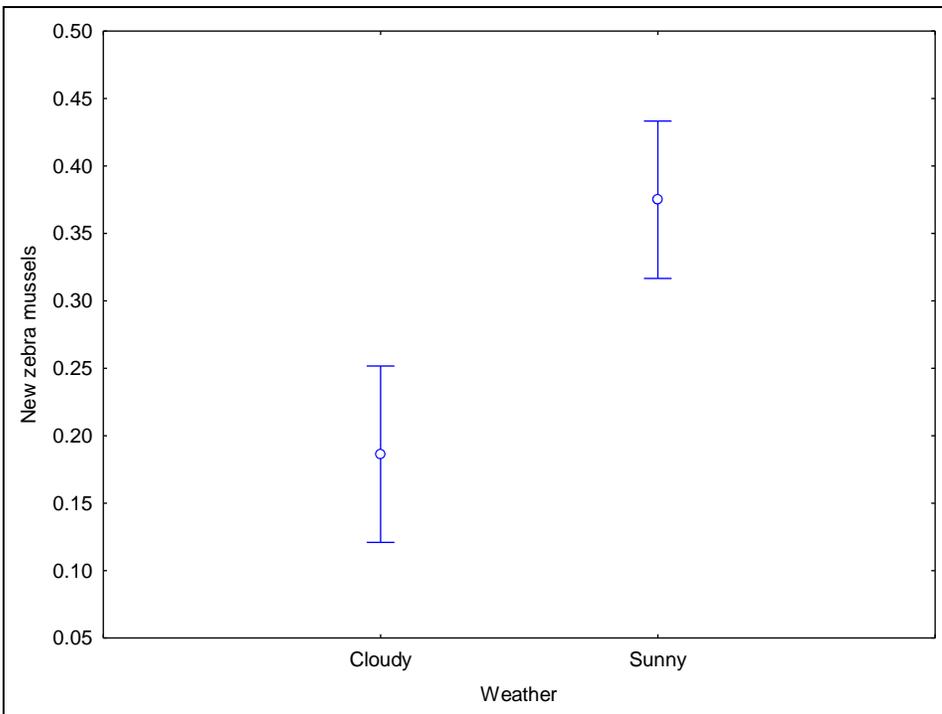


Figure 3.20: Mean number of newly attached zebra mussels at sunny observation periods ( $n = 128$ ) versus cloudy observation periods ( $n = 102$ ). Circles represent the mean new zebra mussels for sunny and cloudy trials. Error bars indicate one standard error.

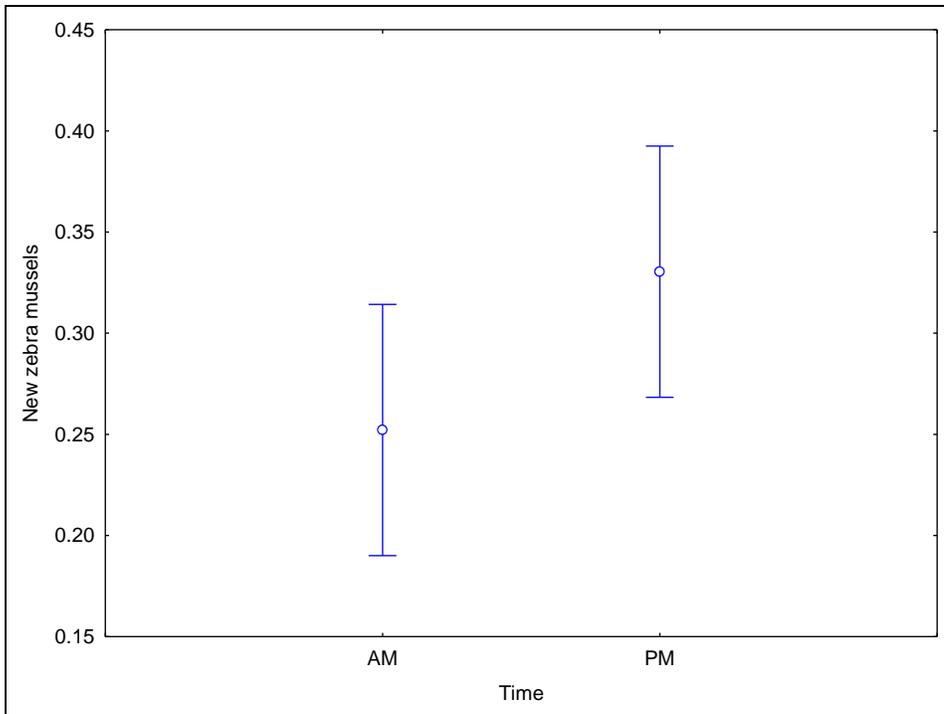


Figure 3.21: Mean newly attached zebra mussels at morning observation periods (nighttime colonization,  $n = 115$ ) and evening observation periods (daytime colonization,  $n = 115$ ). Circles represent the mean new zebra mussels for morning and evening observation periods. Error bars indicate one standard error.

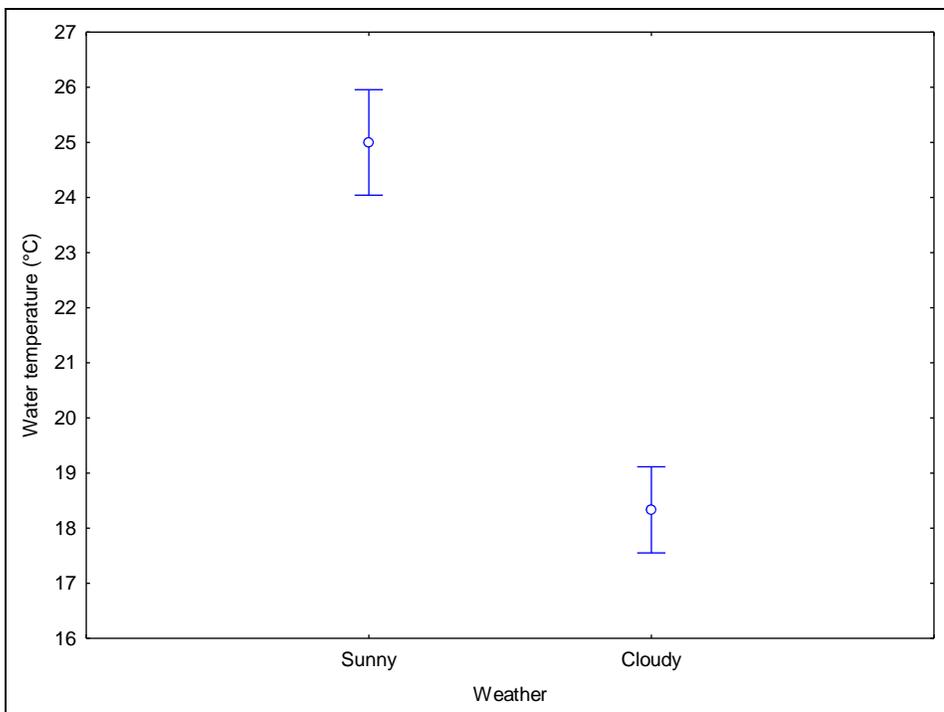


Figure 3.22: Mean water temperature on sunny ( $n = 4$ ) and cloudy ( $n = 6$ ) days during colonization period. Circles represent the mean water temperature for sunny and cloudy trials. Error bars indicate one standard error.

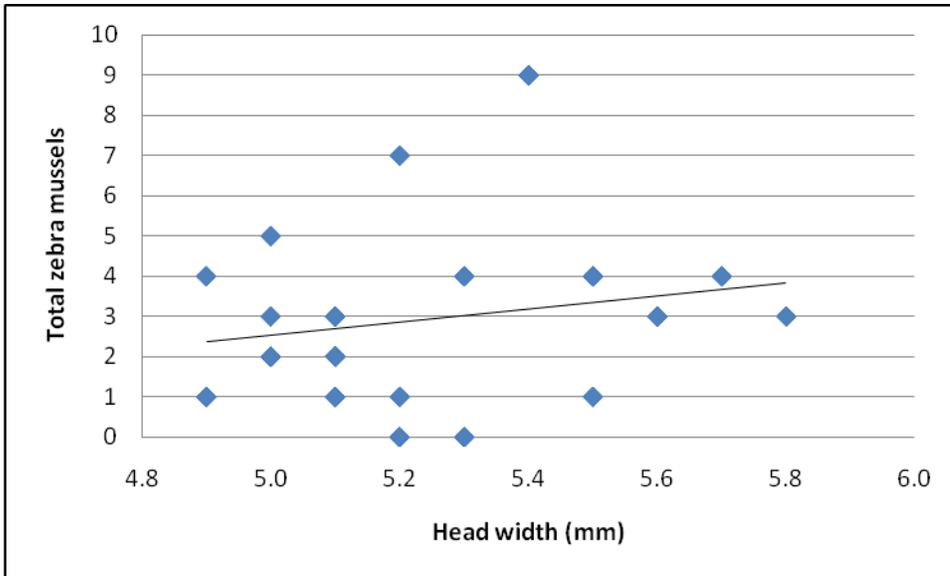


Figure 3.23: The total number of zebra mussels that colonized each individual as a function of head width ( $n = 230$ ). Each diamond represents at least one observation.

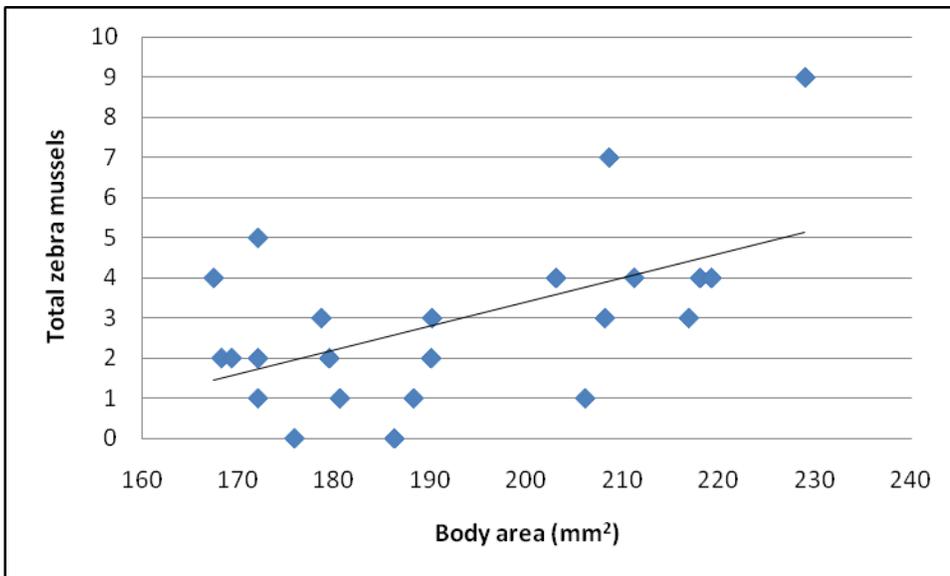


Figure 3.24: The total number of zebra mussels that colonized each individual as a function of body area ( $n = 230$ ). Each diamond represents at least one observation.

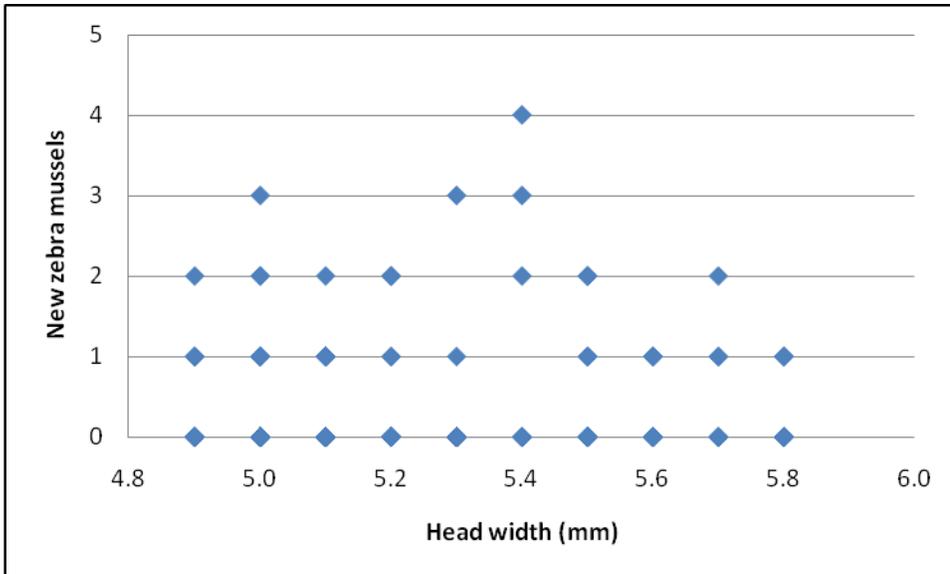


Figure 3.25: The number of new zebra mussels per individual every 12 hours (colonization rate) as a function of head width ( $n = 230$ ). Each diamond represents at least one observation.

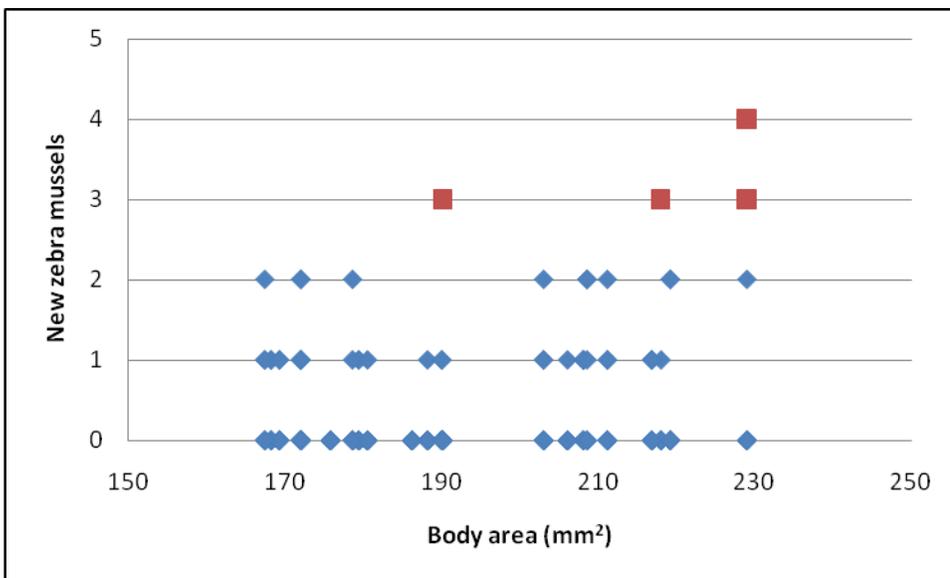


Figure 3.26: The number of new zebra mussels per individual every 12 hours (colonization rate) as a function of body area ( $n = 230$ ). Diamonds represent at least one observation. Squares represent the four outliers.



Figure 3.27: Number of zebra mussels attached to each of the 23 individuals at the end of the colonization period. Note that this reflects some losing mussels during the experiment.

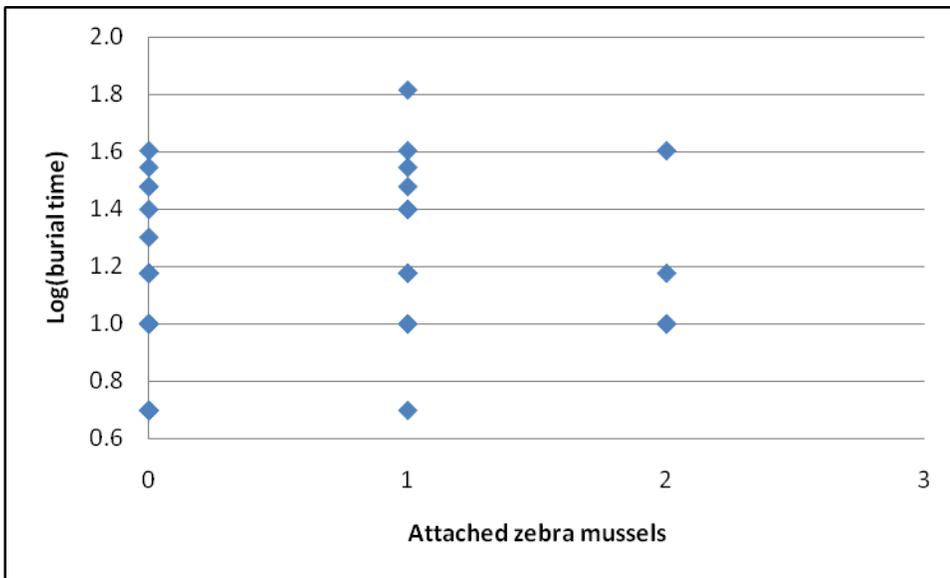


Figure 3.28: Burial time of each individual on sunny days as a function of the number of zebra mussels attached to each individual (n = 37). Each diamond represents at least one observation.

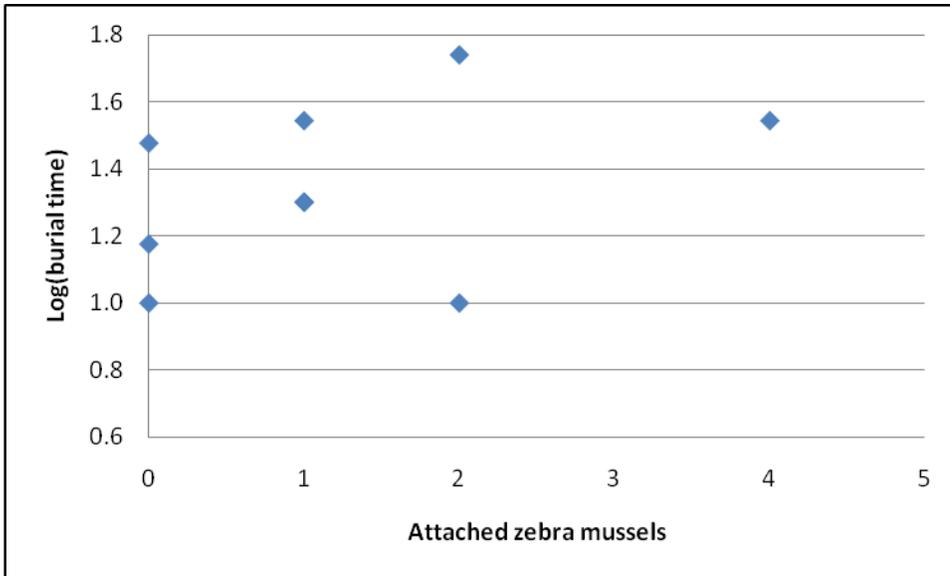


Figure 3.29: Burial time of each individual on cloudy days as a function of the number of zebra mussels attached to each individual ( $n = 9$ ). Each diamond represents at least one observation.

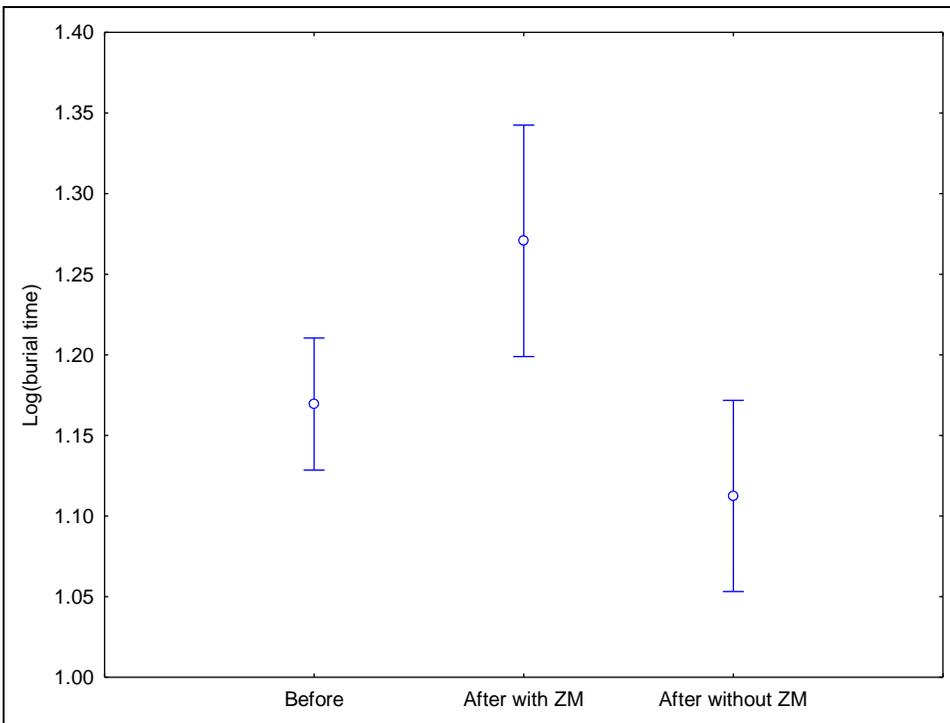


Figure 3.30: Mean burial time on sunny days of individuals before colonization ( $n = 46$ ), individuals after colonization with zebra mussels attached ( $n = 15$ ), and individuals after colonization without zebra mussels attached ( $n = 22$ ). Circles represent the mean log burial time for each category. Error bars indicate one standard error.

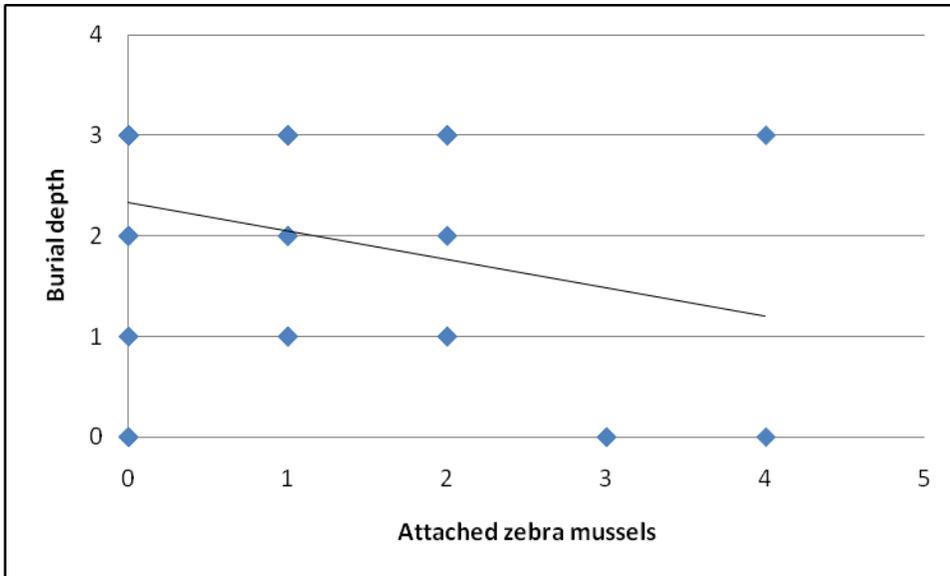


Figure 3.31: Burial depth of each individual as a function of the number of zebra mussels attached to each individual ( $n = 69$ ). Each diamond represents at least one observation.

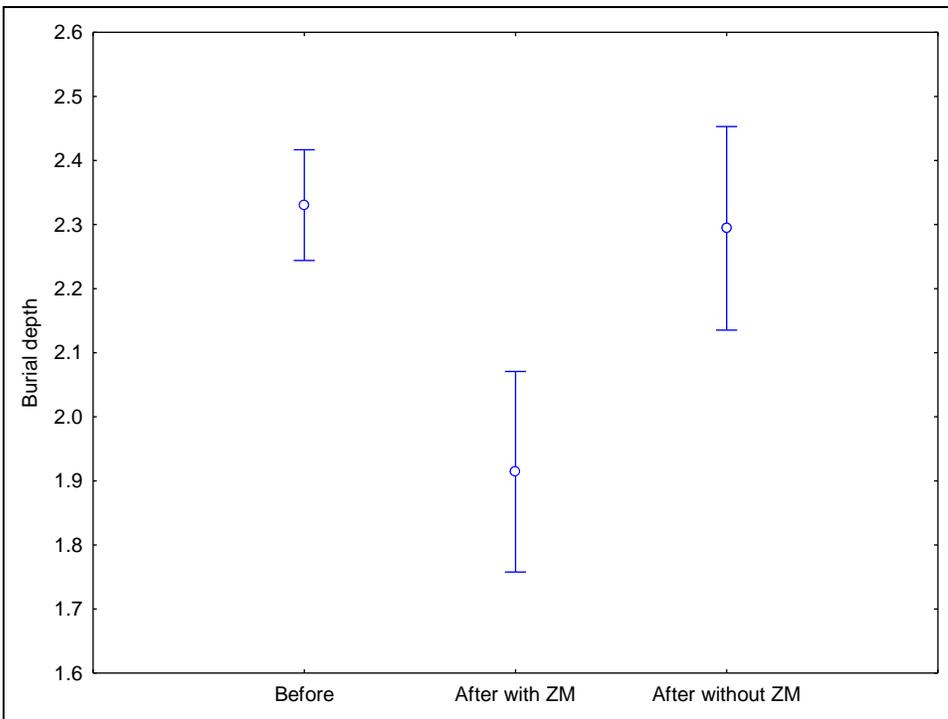


Figure 3.32: Mean burial depth of individuals before colonization ( $n = 115$ ), individuals after colonization with zebra mussels attached ( $n = 35$ ), and individuals after colonization without zebra mussels attached ( $n = 34$ ). Circles represent the mean burial depth for each category. Error bars indicate one standard error. Letters denote a significant difference between burial depths before and after with zebra mussels (Tukey:  $p = 0.052$ ).

## 4. Discussion and Conclusions

### 4.1 Burying Behavior

While *M. illinoensis* was characterized by Corbet (1999) as a sprawler species, this study provides evidence that *M. illinoensis* bury themselves under a layer of sand when living in a sandy environment. This burying behavior, however, was only seen during the day; at night, individuals were found sprawled above the sand. This diel cycle was also observed by Tylezak (2009 unpublished), who found *M. illinoensis* fed at night, suggesting the species is nocturnal. *M. illinoensis* were thus found to be a mixture of Corbet's sprawler and burrower classifications. Another dragonfly species, *Didymops transversa*, was characterized by Corbet as a sprawler species but individuals have been observed burying themselves on occasion (O. Fincke, pers. comm.), supporting the idea that Corbet's classifications are not mutually exclusive.

Although individuals varied in burial time and burial depth, both variables fell along a statistical continuum rather than in discrete categories, suggesting that all individuals came from a single population. All individuals also shared the same burial style – pushing their posterior end down into the sand and pushing sand over their heads with their legs. Such common behavior across the 23 observed individuals suggests that all members of the population use the same burying strategy. It is necessary to note that the use of categories to measure burial depth may have limited the true variation in burial depth among individuals. The chosen categories, however, were functional categories. For example, category 3 meant that an individual was

completely covered. Once covered, the measured depth does not matter because an individual is camouflaged regardless of how deep it buries.

While all four burial depth categories were observed at least once during the study, 54% of uncolonized dragonflies buried themselves until their body outlines were no longer visible (burial depth category 3). Such common final positions for all individuals suggest that *M. illinoensis* bury themselves for the same ultimate goal – camouflage. Because individuals were found sprawled above the sand at night waiting for prey, it is hypothesized that *M. illinoensis* use camouflage during the day to hide from predators as suggested by Corbet (1999) rather than to hide from prey as suggested by Needham et al. (2000).

As predicted, weather and water temperature reduced burial time ( $p < 0.007$  and  $p < 0.001$ , respectively); unexpectedly, however, weather and water temperature did not affect burial depth ( $p > 0.5$  and  $p > 0.7$ , respectively). Burial time was expected to be affected by weather and water temperature because dragonflies are poikilotherms, organisms whose body temperatures vary with their external environments. Their metabolism and other activity increase as temperature increases up to a certain threshold; for *M. illinoensis*, the lethal limit is 43°C (Garten & Gentry 1976). Weather and water temperature were also associated ( $p < 0.003$ ), so it is hypothesized that sunny weather indirectly decreased burial time by directly increasing water temperature. The lack of effect of weather and water temperature on burial depth, however, suggests that dragonflies are consistent in their ultimate goal of camouflage – individuals bury themselves until they are no longer visible regardless of the weather or water temperature.

Neither burial time nor burial depth was affected by head width or body area, which was unexpected. It was predicted that different morphological features would alter the ease or difficulty of burying quickly and completely, thereby changing the burial time and depth. There may, however, be unmeasured morphological features, such as leg length or leg shape, or a ratio between morphological features, such as leg length to body area, that do affect burial time and depth.

#### **4.2 Zebra Mussel Colonization**

Natural colonization rates were found to be much lower than they could potentially be based on high rates of colonization observed during experimentation. When collected from Douglas Lake, two of the 23 studied dragonfly larvae (9%) were each colonized by one zebra mussel. During the five-day colonization experiment, 21 of the 23 studied larvae (91%) were colonized by at least one mussel. Colonization was likely higher in laboratory experimentation because there was a lack of natural substrates, such as sticks, rocks, and other animals, onto which the zebra mussels could attach. When limited to dragonfly larvae as substrates, zebra mussels may be more likely to use them. Therefore, dragonflies living in environments with fewer natural substrates are more threatened. Studies have found that in a matter of years, zebra mussels can kill off entire unionid populations (Schloesser et al. 1996). In such environments, dragonflies will be more vulnerable to colonization by zebra mussels because unionids provided hard substrates.

Zebra mussels were found to detach from dragonflies, which is a novel finding (O. Fincke, pers. comm.). It is unknown, however, whether the mussels released themselves or whether the dragonflies removed them. Once in this study a

dragonfly was observed hitting a mussel with its front leg, but it did not successfully remove the mussel. Dragonflies, therefore, actively try to remove mussels, but it is unknown whether this strategy is successful. Twice, dragonflies became uncolonized by molting because zebra mussels remained attached to the molted exoskeleton. We, therefore, predict that colonization may be more harmful to final instars trying to emerge than younger instars because younger instars can molt off attached zebra mussels. Molting, however, does not explain the majority of the detachment observed during this study. Instead, another mechanism must account for the majority of the detachments, and it is still unclear whether that mechanism is controlled by the mussel or the dragonfly. In addition, it is unknown whether mussels are able to reattach themselves to dragonflies after being detached.

Total colonization was marginally associated with uncolonized burial time ( $p = 0.0542$ ) – the slower burying dragonflies were more likely to get colonized. When the data was separated into weather categories to account for effects of weather on burial time, the burial times on sunny days highly affected the likelihood of colonization ( $p < 0.02$ ), while the burial times on cloudy days were not associated with colonization ( $p > 0.3$ ). The sunny burial trials, therefore, are what caused the overall trend to be marginally significant. Individuals that remained uncovered longer on sunny days thus had a higher chance of being colonized. In addition, being covered with sand appears to reduce the likelihood of getting colonized. Finke et al. (2009) support this observation, finding that sprawler dragonfly species suffered from higher colonization than burrower dragonfly species. Colonization was not associated

with burial depth, possibly because there was less variation in burial depth because, supposedly, all individuals had the same ultimate goal of camouflage.

Colonization rate was significantly higher with sunny exposures ( $p < 0.04$ ), as expected because zebra mussels also are temperature-dependent (McMahon 1996). Not enough data on water temperature was collected in this study to directly test the effects of temperature on colonization rate. Since water temperature data that was collected was associated with weather, it is hypothesized that water temperature would affect colonization rates. In addition, byssal thread production has been found to increase with increasing water temperature (Clarke & McMahon 1996), which suggests colonization rate would be higher in warmer water. This finding helps explain the relationship between uncolonized burial time and likelihood of getting colonized. When it is sunny, zebra mussels are active and burial time affects the likelihood of colonization; when it is cloudy, zebra mussels are not active and therefore burial time does not affect the likelihood of colonization. Finally, zebra mussels have a thermal limit of 26-32°C (Mackie & Schloesser 1996), roughly 10°C lower than *M. illinoensis*. Therefore, while *M. illinoensis* may be increasingly threatened in warmer habitats due to increased zebra mussel activity, there is a possibility of some *M. illinoensis* living in habitats that zebra mussels cannot thrive in, thereby creating a refugium for *M. illinoensis*.

While time of day did not have a significant effect on zebra mussel colonization rate (measured by the number of new zebra mussels attached every 12 hours), there was an apparent relationship between colonization rate and time of day, with more new zebra mussels attaching themselves during the day. This may be

important because *M. illinoensis* tended to be buried during the day and sprawled above the sand while waiting for prey at night. Dragonflies thus tended to be buried when zebra mussels are potentially most likely to colonize them. The time it takes a dragonfly to bury itself becomes important: if a dragonfly gets uncovered during the day, it needs to re-bury itself quickly because that is potentially when zebra mussels are more likely to colonize it.

Total colonization was affected by both head width ( $p < 0.005$ ) and body area ( $p < 0.001$ ), which was predicted because bigger head widths and body areas provide greater surface area on which zebra mussels can attach. Body area had a greater effect on total colonization than head width, with body area explaining 30% and head width only 4% of the variation in total colonization. Body area presents a much larger potential substrate than head width. Based on the ratio of dragonfly body area to surface area of the containers, dragonflies only took up 3% – 4% of the available space. It is impressive that the zebra mussels found them at all. The effect of size on the likelihood of being colonized was predicted by McCauley and Wehrly (2007). In addition, Fincke et al. (2009) found that larger dragonfly species, specifically *Hagenius brevistylus* and *Didymops transversa*, were more highly colonized than smaller species, specifically *Epitheca princeps*, *Progomphus obscurus*, and *Dromogomphus spinosus*.

The rate of colonization of zebra mussels, however, was not affected by head width ( $p > 0.3$ ) or, with outliers removed, body area ( $p > 0.2$ ). The four body area data points designated outliers were removed because head width and body area were so highly correlated that it was expected that both head width and body area would

have the same effects on colonization rate. Colonization rate may have been unaffected by morphology due to the overpowering effects of variation in weather. Sample sizes were not large enough to test for a morphological effect independent of weather.

### **4.3 Burying Behavior After Zebra Mussel Colonization**

As predicted by McCauley and Wehrly (2007), attachment of zebra mussels on dragonfly larvae affected burying behavior. Colonization was found to affect dragonfly burial depth more than burial time. This may partially be due to the smaller sample sizes of burial time data, especially after dividing the data into weather categories for analysis.

No significant relationship was found between the number of attached zebra mussels and burial time. Although not significant, an apparent relationship was found with dragonflies after colonization with attached zebra mussels burying themselves more slowly than those before colonization and those after colonization without attached zebra mussels. This apparent relationship may become significant with a larger sample size. If so, this would be a direct effect of zebra mussel colonization slowing dragonfly time for burial. As previously discussed, slower burial time increases the likelihood of colonization; hence, a potential positive feedback loop could be created between increased colonization by zebra mussels and decreased burial time.

More importantly, larger numbers of attached zebra mussels significantly decreased burial depth ( $p < 0.02$ ). In addition, dragonflies with attached zebra mussels buried themselves significantly more shallowly than those before

colonization ( $p < 0.02$ ). They also appeared to bury significantly more shallowly than individuals after colonization without attached zebra mussels, but this relationship was not significant ( $p > 0.1$ ), possibly due to small sample sizes. These results demonstrate that zebra mussels directly impair dragonfly burial depth. While more effects may become significant with larger sample sizes, there is clearly an effect of zebra mussel attachment reducing dragonfly burial depth. By burying less deeply, *M. illinoensis* become more visible predators, so the effects of zebra mussel colonization could be detrimental to their ability to hide from predators. Other burrower dragonfly species that are sit-and-wait predators during the day could be additionally affected by impairing their ability to be camouflaged from prey. The effects of zebra mussel colonization on dragonfly larvae, thus, could be fatal.

#### **4.4 Burying Behavior and Colonization: Putting It All Together**

Zebra mussels are continuing to invade new habitats throughout North America (Benson & Raikow 2009). This study supports several studies that document zebra mussel colonization of dragonfly larvae (Tucker & Camerer 1994; Weihrauch & Borcharding 2002; McCauley & Wehrly 2007; Fincke et al. 2009). In particular, Fincke et al. (2009) found that both sprawler and burrower species are colonized by zebra mussels, with sprawler species showing higher colonization rates. This study found that a species with both sprawling and burying behavior, *M. illinoensis*, gets colonized, and that colonization lowers their fitness. Burial time was found to affect the likelihood of colonization, and colonization was found to affect burial depth. Specifically, weather and water temperature affect uncolonized burial time, which, along with morphology, affects total colonization. In turn, total colonization affects

burial depth that leads to early mortality. In other words, slower burying *M. illinoensis* larvae are more likely to get colonized by zebra mussels, which in turn decreases their burial depth, impairing their camouflage and increasing their vulnerability to predation.

While zebra mussels threaten burrower species by impairing their burying behavior, zebra mussels threaten all dragonfly species by impairing their ability to climb out of their aquatic habitats and emerge successfully as adults (McCauley & Wehrly 2007; Fincke et al. 2009). Because dragonflies link trophic levels within and across ecosystems (Knight et al. 2005; Wesner unpublished), zebra mussels may have detrimental indirect effects on other components of communities or ecosystems. For example, based on Knight et al. (2005), if zebra mussels reduce the number of successfully emergent dragonflies, the lack of adult dragonflies would result in top-down cascades in terrestrial ecosystems, with fewer adult dragonflies preying on pollinating insects. In turn, more plants would be pollinated, which may lead to overpopulation of plants and alterations of entire terrestrial ecosystem communities. Similarly, if insectivorous fish cannot consume zebra mussels, colonization by zebra mussels on dragonfly larvae may cause bottom-up cascades, limiting food resources for insectivorous fish and altering entire aquatic ecosystem communities.

#### **4.5 Areas for Further Investigation**

Many pieces of the story of zebra mussel colonization of dragonfly larvae remain unknown. Although studies have shown that zebra mussels affect sprawler and burrower dragonfly species, it remains unknown whether and to what degree zebra mussels colonize and affect climber and hider species. Ecologically, the effects

of zebra mussels on dragonflies may alter trophic cascades, which could be tested by comparing trophic levels at invaded and non-invaded lakes. Evolutionarily, zebra mussels may apply selective pressures favoring faster buriers. This effect could be tested by comparing uncolonized burying behavior of dragonflies from invaded and non-invaded lakes. Therefore, while the negative impacts of zebra mussels on aquatic organisms and systems have long been known, zebra mussels may indirectly harm terrestrial organisms and systems. Ways to stop this invasive species must be investigated.

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