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Effects of zebra mussel attachment on the foraging behaviour of a larval dragonfly, *Macromia illinoiensis*

OLA M. FINCKE[1] and LESLEY A. TYLCZAK[2] [1]Ecology and Evolutionary Biology Program, Department of Zoology, University of Oklahoma, Norman, Oklahoma, U.S.A. and [2]University of Michigan Biological Station, Pellston, Michigan, U.S.A.

Abstract. 1. Larvae of *Macromia illinoiensis* Walsh are often colonised by the zebra mussel, *Dreissena polymorpha* Pallas, a recent invader to North America. To determine how mussel attachment affects an individual's foraging behaviour, we quantified capture of *Hexagenia limbata* Hexes mayfly prey and the distance moved by newly-molted final instars before and after an individual's colonisation with zebra mussels.

2. In night trials, larvae sprawled above the sand, and caught more mayflies than individuals in daytime trials, but the estimated distance travelled did not differ. When resting under a layer of sand with only its eyes exposed during the day, an individual could capture a mayfly prey using a sit-and-wait ambush strategy. When sprawled above the sand, some larvae caught prey that rested on their legs.

3. When mussel-free, individuals captured more prey than they did when carrying zebra mussels, although mussel attachment *per se* did not affect the estimated distance that a larva moved.

4. During day trials, but not night ones, the increasing mussel load of colonised individuals decreased prey capture and the distance moved in an apparent step-wise function. Although the number of mussels carried did not differ, night foragers carried a heavier load. Independent of time of the day, the distance an individual travelled when mussel-free was predictive of the number of prey it caught when colonised, suggesting that the greater general activity of some individuals helped mitigate negative effects that mussel attachment had on prey capture.

5. Our results add to a growing number of negative effects of zebra mussel colonisation on sprawling and hiding dragonfly larvae. Although the impact of these costs on dragonfly populations remains to be determined, a decrease in this guild of predators whose life cycle spans aquatic and terrestrial habitats might have cascading effects across ecosystems.

Key words. Anisoptera, *Dreissena polymorpha*, *Hexagenia*, invasive species, predation, trophic cascades.

Introduction

Since its introduction into North America several decades ago, the invasive freshwater zebra mussel, *Dreissena polymorpha* Pallas, has caused declines in native populations of unionid mussels and diatoms, resulting in dramatic changes in the structure of aquatic communities (e.g., Haag *et al*., 1993; Schloesser & Nalepa, 1994; Lowe & Pillsbury, 1995; MacIsaac, 1996; McNickle *et al*., 2006). Zebra mussels also attach to hosts other than bivalves, such as gastropods (Van Appledorn *et al*., 2007), crayfish (Brazner & Jensen, 2000; Ďuriš *et al*., 2007) and dragonfly larvae (Weihrauch & Borcherding, 2002; McCauley & Wehrly, 2007; Fincke *et al*., 2009).

Because as larva and as adults, odonates serve as both predators and prey, this insect order plays an important role in the community structure of aquatic and terrestrial habitats (e.g. Benke & Benke, 1975; Wissinger, 1988; Smith & Smock, 1992). For example, dragonfly larvae are often the largest predators in fishless lakes (McPeek, 1998); more generally, dragonfly larvae are important prey for fish, birds, and other odonates (Crowley & Johnson, 1982; Corbet, 1999). Similarly, as adults, odonates are generalist predators on a wide variety

Correspondence: Ola M. Fincke, Department of Zoology, University of Oklahoma, Norman, OK 73019, U.S.A. E-mail: fincke@ou.edu

of insects (e.g. Pritchard, 1964; Folsom & Collins, 1984; Blois, 1985) while also being major prey for vertebrates, especially birds (reviewed by Corbet, 1999). Thus, a reduction in odonate numbers as the consequence of zebra mussel colonisation could have far-reaching effects on the ecosystems of which they are a part (e.g. Knight *et al*., 2005; Wesner, 2010).

The odonates that are most vulnerable to attachment by zebra mussels are the final instars of large, sprawling or hiding species whose larvae rest on the surface of the benthos. Although some sprawling dragonfly larvae bury themselves under a thin layer of sand (e.g., *Didymops*, *Epitheca*, and *Macromia*) whereas others hide under debris (e.g. *Hagenius*), most remain susceptible to attachment by *Dreissena* mussels (Fincke *et al*., 2009; O. M. Fincke, unpublished). In contrast, species whose larvae burrow more deeply under the sand rarely carry attached mussels. Colonised larvae can lose mussels in the first days after attachment and an instar sheds its entire mussel load when it molts, either into another larval stage or into an adult (Hughes & Fincke, in review). Zebra mussels can directly impede feeding and emergence of dragonfly larvae if they attach to the labium or the area of the thorax from whence the adult emerges, respectively. Indirect negative effects of zebra mussels on odonates are more common, and include (i) impeding their burying behaviour, thereby increasing the risk of colonisation by multiple mussels, and probably making larvae more conspicuous to visual predators (Hughes & Fincke, in review), (ii) difficulty in righting themselves when overturned, and (iii) decreased survivorship as a result of the inability of heavily colonised individuals to move out of the water prior to emergence as adults (Fincke *et al*., 2009).

Nevertheless, we know little about the effect of attached mussels on a larva's foraging behaviour. Indeed, there is a paucity of studies on larval foraging in odonates more generally (Corbet, 1999). The mass that a final instar acquires by its foraging success determines its body size as an adult, which is fixed at emergence. In contrast, adult dragonflies gain most of their adult body mass by foraging during the teneral stage (Anholt *et al*., 1991). Fincke *et al*. (2009) suggested that zebra mussels might have minimal effects on foraging of species such as *Didymops transversa* Say, because their larvae are thought to use a sit-and-wait foraging tactic to ambush prey that come within reach of a larva's extendable labium (Needham *et al*., 2000). However, both *D. transversa* and *Macromia illinoisensis* Walsh, the only two North American members of the sub-family Macromiinae, sprawl above the sand at night, suggesting that they may not always use a purely stationary mode of prey capture. To the extent that such larvae move around to catch prey, then carrying a load of mussels might decrease their foraging success more than previously thought.

The aim of the present study was to document how attached mussels affect the foraging behaviour of larvae of the Illinois River Cruiser, *Macromia illinoiensis,* a dragonfly common in sandy bottomed lakes and shallow areas of broad rivers (Worthen, 2002). Using an individual as its own control, we predicted that attached zebra mussels would reduce an individual's prey capture compared with its foraging success when mussel-free. Based on its relatively small eyes, *M. illinoiensis* is considered to be nocturnal (Corbet, 1999). Thus, we predicted that colonisation would have less of an effect on prey capture during the day when larvae are thought to rest, than at night when they should be most active. Finally, we predicted that when colonised, individuals that carried heavier loads relative to their own weight would move less and/or capture fewer prey.

Materials and methods

The present study was conducted from late June to early August, 2009 at the University of Michigan Biological Station on Douglas Lake (43°35′N, 84°42′W), which was invaded by the zebra mussel, *Dreissena polymorpha*, *c*. 2001. Periodically, from 29 June to 25 July, the dragonfly predator, *Macromia illinoiensis*, the common burrowing mayfly, *Hexagenia limbata* Hexes (Hunt, 1953; Schloesser & Hiltunen, 1984), which was the only prey used in this study, and the colonising zebra mussel *D. polymorpha* were collected live from Douglas Lake, along the shore near Pine Point. The dragonfly larvae were collected by pulling a long-handled D-net through sandy sediment at a water depth of 0.5–1.4 m on sunny days with low wind. *Hexagenia limbata*, which burrows beneath the sand, was found in the same area as the dragonfly larvae.

We controlled for the developmental stage of *Macromia illinoiensis* by keeping only mussel-free, penultimate instars from the collections. Larvae were maintained individually in waxed cups (11.5 cm diameter, 7.5 cm deep) filled up to 2 cm with sand and to 4 cm with lake water. Each instar was assigned an ID; head width, abdomen width at the widest point, and body length were measured using electronic calipers. After towelling a larva dry, its wet weight was taken with an electronic balance. Penultimate instars were fed *ad libitum* mayfly larvae until they molted into final instars. After molting, a final instar was measured and weighed as above before being used in the foraging experiment. Each larva was paired with another individual of similar size and weight (hereafter referred to as 'an instar pair'). Two of the penultimate instars failed to molt; these were assigned to the same instar pair and used in the day trials.

All *M. illinoiensis* larvae were starved for 2 days before being used in two foraging treatments (i.e. with and without attached zebra mussels), each with two trials. To control for the possible effects of treatment order, one member of each instar pair was colonised for the first foraging treatment, whereas its partner was mussel-free for the first treatment. The treatments were then reversed for each member of the pair.

Dragonfly larvae were colonised with zebra mussels by piling several small-to-medium-sized mussels (i.e., 4–14 mm in length) atop a larva and leaving them overnight. The process was repeated until two to three mussels remained attached for a day. The length of each attached mussel was measured and its wet weight was estimated using the power function: mass (g) = length$^{3.035}$(mm) × 10^{-4}(Hughes & Fincke, in review). For colonised individuals, the 'mussel load ratio', the proportion of a larva's own weight that it additionally had to carry, was calculated as the summed wet weights of all attached mussels divided by the larva's wet weight.

Foraging trials were run on single individuals each placed in a shallow metal pan (28 × 40 cm × 6 cm in depth) containing 1.5-cm-deep sand, and covered with lake water to a depth of 3 cm. Five toothpicks were placed at regular intervals at the end of the pan to provide a clinging surface for the larval mayfly prey. The pans were placed on a table below a bank of south facing windows in the boat well of the Lakeside Lab, an area protected from rain and direct sun.

Daytime foraging trials, which involved 14 unique individuals, began at 08:30. Five mayfly larvae of a standardised length (7–12 mm) were added to the toothpick end of each pan and allowed to acclimate for several minutes. Then, one colonised or mussel-free *M. illinoiensis* larva was placed in a righted position at the opposite end of the pan. The larva's position was noted relative to sections of a string-gridded frame that was placed above the pan. The number of mayfly captured and the estimated distance that a dragonfly larva moved (i.e. the shortest straight-line distance between its initial and subsequent position) were recorded at 2-h intervals over a span of 12 hours. At each check, additional mayflies were added as necessary to make a total of five prey. The entire above procedure was repeated on the following day using the same individuals, resulting in a total of 12 daytime checks per individual. Then the other larva in a pair was colonised overnight, whereas the attached mussels were removed from its partner, after which a second treatment set was conducted as above. Zebra mussels were removed by gently twisting a mussel in a clockwise direction to break its byssus threads, a method that resulted in no apparent injury to any of the dragonfly larvae that were subsequently used in feeding trials as un-colonised individuals.

Nighttime trials used a different set of 14 unique dragonfly larvae. These trials were identical to those above except that the trials began at 21.00, were checked only once after 12 hours (i.e. at 09:00 the following day), and were initially stocked with a total of 10 mayfly larva that were not replenished until the beginning of the second night trial of the two-trial set.

Three or four instar pairs were run at any given time. Two types of controls for non-predatory mortality of mayflies were used. These were identical to the experimental pans except that each lacked a dragonfly larva. Whereas both controls contained 10 mayfly larvae, the second type additionally contained 10 small-to-medium-sized zebra mussels, which controlled for any negative effect of zebra mussels on mayfly survivorship in the trials with colonised dragonfly larvae. After being used in the foraging experiment, dragonfly larvae were retained for several days in the wax cups to ensure there were no unforeseen after effects, and to more closely observe prey capture. Then, after removing any attached mussels, all *M. illinoiensis* and any remaining *Hexagenia* larvae were returned live to Douglas Lake.

Shapiro–Wilk tests were used to insure that all data were normally distributed (univariate procedure, SAS 8.1; SAS Inc., Cary, North Carolina). In our experimental design, each individual was tested with and without mussels, serving as its own control. Two repeated measures ANOVAS (one for each dependent variable), with the time of day and treatment order (i.e. mussel-free during an individual's first trial or its second trial) as independent variables, were used to test for an effect of mussel state (i.e. mussels absent and mussels present, factors repeated within individuals) and interaction effects on the dependent variables, number of prey captured, and the estimated distance moved a larva moved. ANCOVAS, with time of day as an independent variable, were used to determine whether the number of prey captured co-varied with the distance a larva moved (continuous variable). Head width, which is highly correlated with body length and abdomen width, (Hughes & Fincke, in review) was used to control for body size in partial correlations, which determined the relationship between an individual's mussel load ratio, the number of prey it captured, and the distance moved. Throughout, means are presented ± 1 SE; all *t*-tests are two-tailed.

Results

The 26 penultimate larvae that molted to final instars did so within 11 days of capture ($\overline{x} = 4.17 \pm 0.78$ days). The median day of molting to final instar in our sample was 8 July. Both individuals in the sole penultimate instar pair used in the experiment failed to molt before being returned to the lake. A few final instars kept for observation after the experiment emerged as adults in the boat well.

Some penultimate instars were nearly twice as heavy as others. Table 1 summarises the changes in body size and mass after penultimate instars molted into final instars. On average, *M. illinoiensis* gained 0.308 ± 0.014 g ($n = 23$) after molting to a final instar. After 1–2 days with zebra mussels piled on them, dragonfly larvae were colonised by two to three mussels (Table 1). All mussels were attached to the larvae on the abdomen; none of these spontaneously detached during the subsequent foraging trial.

In the foraging trials, the *Hexagenia* mayfly prey were found both above and below the sand, which had to be disturbed to find all of them during a check. No mayfly larvae disappeared in either of the controls (i.e. mayflies alone or mayflies with zebra mussels), which were pooled below. In contrast, during all of the checks with dragonfly larvae, one or more mayfly larvae were missing ($X^2 = 64.0$, 1 d.f., $P < 0.0001$), indicating that the disappearance of mayfly larvae from a foraging trial could be attributed to the presence of the *M. illinoiensis* dragonfly larva.

Macromia illinoiensis were sometimes found eating prey. Additionally, we observed three cases of prey capture. One larva was observed catching a mayfly during the day when only the dragonfly's eyes were exposed. It rested covered with sand, until the mayfly larva approached within striking distance of extendable labium of the dragonfly larva (see Figure S1). At night larvae were unburied, sprawling above the sand. Twice, a sprawling individual was observed with a mayfly larva resting on its hind leg. One *M. illinoiensis* moved its leg as if to try to remove the mayfly, which persisted in holding on. In each case, the *M. illinoiensis* turned quickly, dislodging the mayfly, then grabbed it by the head and consumed the prey in a matter of minutes. During daytime checks, the maximum number of prey captured by a dragonfly during a

Table 1. Mean (mm) morphological characteristics of penultimate instars (F-1) before and after molting to the final instars of *Macromia illinoiensis* used in our experiment.

Instar stage	Head width (mm)	Body length	Abdomen width	Weight (g)	Mean mussel load when colonised: Number	Mean mussel load when colonised: Total mass (g)
Penultimate	5.58 ± 0.04* (26)	19.86 ± 0.14* (26)	9.86 ± 0.10* (26)	0.467 ± 0.01* (26)	—	—
Final	7.29 ± 0.04 (26)	23.88 ± 0.14 (26)	11.45 ± 0.09 (26)	0.778 ± 0.01 (26)	2.39 ± 0.09 (28)	0.254 ± 0.02 (28)

The two F-1 larva that did not molt while in captivity are not included in the morphological data above. *$P < 0.05$ (t-tests, comparing F-1 and final instars). Mean mussel loads of final instars are those carried during the foraging experiment. Sample sizes are in parentheses.

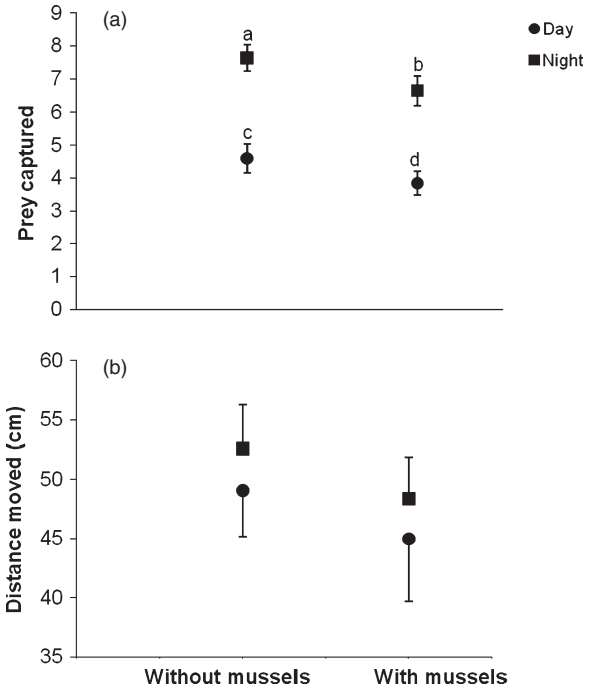

Fig. 1. Mean ± SE performance of 28 unique *Macromia illinioensis* larvae during day ($n = 14$) and night trials ($n = 14$): (a) foraging success of dragonfly larvae on *Hexagenia* mayfly prey during 24 h of the day and night, (b) estimated distance moved by individuals during 24 h of the day and night. Within each time period, each individual was tested when it was mussel-free, and when it carried two to three zebra mussels. Letters indicate significantly different means (paired t-tests, $P < 0.05$.)

2 h period was three, or 60% of those available. The maximum number caught during an entire 12-h daytime trial was six (i.e. 75% of those available). The maximum number of prey captured during a 12-h night trial was five, or 50% of those available.

As shown in Fig. 1a, time of the day had a significant effect on prey capture; individuals foraging during the day captured fewer mayfly larvae than those foraging at night ($F_{1,24} = 52.49$, $P < 0.001$). In contrast, there was no difference in the estimated distance moved between day- and nighttime foragers ($F_{1,24} = 0.54$, $P = 0.47$, Fig. 1b).

Independent of time of the day, relative to their performance when mussel-free, individuals captured fewer prey when colonised by mussels ($F_{1,24} = 5.99$, $P = 0.02$, Fig. 1a) but did not move less ($F_{1,24} = 1.33$, $P = 0.26$, Fig. 1b). There was no interaction between colonisation state (i.e. with or without mussels) and time of the day on prey capture ($F_{1,24} = 0.09$, $P = 0.77$) nor between colonisation state and treatment order on prey capture ($F_{1,24} = 2.16$, $P = 0.15$). However, there was a three-way interaction effect of colonisation state, time of day, and treatment order on prey capture ($F_{1,24} = 10.44$ $P = 0.004$). For the night trials only, *M. illinoiensis* individuals that first carried mussels had a greater difference in their performance (i.e. prey captured when mussel-free minus prey captured when colonised, $\overline{x} = 2.71 \pm 0.60$) than their partners whose first trials were mussel-free ($\overline{x}$ difference = 0.71 ± 0.78, paired $t = -3.48$, d.f. = 12, $P = 0.005$). There was no treatment order effect during the daytime trials (paired $t = 1.72$, d . f . = 12, $P = 0.14$). Nor was there an interaction effect between colonisation state and time of the day ($F_{1,24} = 0.00$, $P = 0.98$) on the distance moved, or an interaction between colonisation state and treatment order ($F_{1,24} = 3.65$, $P = 0.07$). Finally, there was no three-way interaction with respect to distance moved ($F_{1,24} = 0.33$, $P = 0.57$).

As shown in Fig. 2a, controlling for time of the day, when mussel-free, the estimated distance moved by individuals did not predict the number of prey they captured ($F_{1,25} = 0.02$, $P = 0.89$). Similarly, when colonised, the estimated distance individuals moved did not predict the number of prey captured ($F_{1,25} = 1.78$, $P = 0.19$). However, the estimated distance moved when mussel-free was a good predictor of the number of prey captured when an individual was colonised by mussels ($F_{1,25} = 34.43$, $P < 0.001$), explaining 76% of the variation in prey capture when colonised (Fig. 2b).

Among colonised *M. illinoiensis* foraging during the day, the number of prey captured (Fig. 3a) and the distance an individual moved (Fig. 3b) both decreased as a function of a larva's mussel load ratio. In contrast, for individuals foraging at night, neither prey capture (Fig. 3c), nor distance moved (Fig. 3d) was correlated with an individual's mussel load ratio. Unfortunately, and not by design, although colonised individuals used in the night trials did not differ in the number of attached mussels ($\overline{x} = 2.4 \pm 0.10$) compared with those in the day trials ($\overline{x} = 2.2 \pm 0.08$), the nighttime foragers carried on average, 0.328 ± 0.03 g of mussels, making their mussel load ratio higher ($\overline{x} = 0.416 \pm 0.27$) than that of larvae used in the day trials (0.279 ± 0.03, $t = -2.05$, $d.f. = 26$, $P = 0.05$), which on average carried 0.289 ± 0.56 g of mussels.

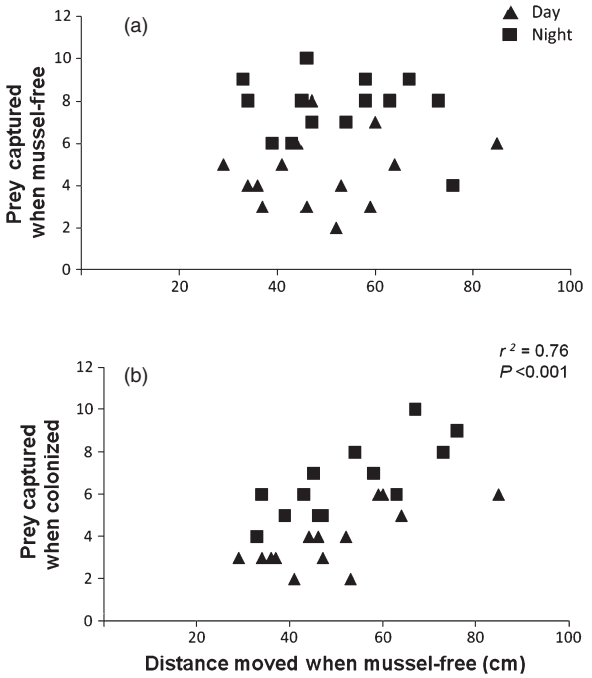

Fig. 2. Number of prey captured (a) when mussel free and (b) when colonised by zebra mussels, as a function of the distance it moved when it was mussel-free. $n = 14$ unique individuals in day trials, $n = 14$ unique individuals in night trials.

Discussion

Our results indicated that *M. illinoiensis* was primarily nocturnal. At night, when they caught the most prey, individuals were typically found exposed, sprawled above the sand. However, even during the day when checks on larval movement were taken every 2 h, *M. illinoiensis* moved roughly 8 cm per check, with an individual capturing on average four mayflies over 24 daytime hours. These results counter the suggestion that members of the subfamily *Macromiinae* are 'of strictly sedentary habits' (Needham *et al*., 2000, p. 473). During the day, larvae typically covered themselves with a light layer of sand, a behaviour that probably makes them more cryptic to visually-orienting predators (Hughes & Fincke, in review). They sometimes left their eyes exposed, a behaviour that makes sense if they forage diurnally, at least occasionally. The lower daytime activity of the dragonfly larvae in our experiment was consistent with an anti-predator tactic. Corbet (1999) speculated that most dragonfly larvae, even small-eyed ones like *M. illinoiensis*, use both an ambush tactic as well as a hunting mode, depending on, among other things, hunger levels. If so, then because we starved larvae for 48 h, we may have inadvertently increased the amount they moved during the first foraging trials. Nevertheless because the initial trials were equally balanced between mussel treatments (i.e. present or absent), any effects of hunger should not have biased our results. Additionally, the absence of fish predators in our experiment may have increased the daytime activity of the dragonfly larvae, which would naturally encounter potential predators such as catfish in the sandy, shallow areas of Douglas Lake.

Relative to a larva's performance without mussels, carrying attached mussels decreased the foraging rate of individual *M. illinoiensis,* independent of time of the day (Fig. 1a). Although a mussel attached to the labium would have impeded feeding, in our experiment no mussel was attached where it could have interfered with prey capture *per se*. Hence, any impediment to prey capture probably resulted from carrying the increased weight of mussels. Although the trends were in the expected direction, carrying two to three mussels did not decrease the estimated distance a larva moved in either the day or night trials (Fig. 1b). Among colonised larvae, increasing mussel loads decreased both prey capture and the estimated distance moved during the day, but not in the night trials, as discussed below.

With one exception, a larva's behaviour in its second treatment (i.e. with or without mussels) was not influenced by its experience in its first treatment. During the night trials, there was a significant treatment order effect on prey capture, but it was in the opposite direction expected if colonisation by zebra mussels during an individual's first trial negatively affected its performance during its second trial when it was mussel-free. Rather, there appeared to be a compensatory effect such that colonised individuals performed better at prey capture after being freed from their mussel load. Importantly, this result also suggested that the dragonfly larvae were not harmed during the process of removing the attached mussels.

The sit-and-wait ambush tactic was observed to be used during both day and night foraging, but a larva probably increased its prey capture by moving. Mechanoreceptors, located on the legs and possibly in the unique horn of *Macromia* larvae, enable them to sense vibrations in water (reviewed by Corbet, 1999). Thus, by moving towards vibrations arising from its *Hexagenia* prey, *M. illinoiensis* might have increased its capture success while still using an ambush tactic at a close distance. Interestingly, general activity as estimated by distance moved when mussel-free was an important predictor of foraging success of colonised larvae, independent of time of day. The distance travelled may thus have been a surrogate of the general activity level, which could have helped a colonised larva compensate for the additional weight it carried.

We expected that carrying mussels would be most detrimental to larvae that moved around while capturing prey. Surprisingly, we found no effect of increasing mussel load on prey capture or on the estimated distance moved by individuals in the night trials when larvae were typically sprawled above the sand. This result is probably explained by design problems of the night trials. First, it was unfortunate that larvae used in the night trails had been colonised by a similar number, but heavier mussels than those used in the daytime trials. Whereas 43% of the larvae in daytime trials carried loads of 20% or less of their own weight, none of the individuals in the night trials had such light load ratios (Fig. 3c,d). Moreover, it was the group of individuals with 20% or less load ratios in the daytime trials that contributed to the apparent step-wise relationship found between the mussel load ratio and

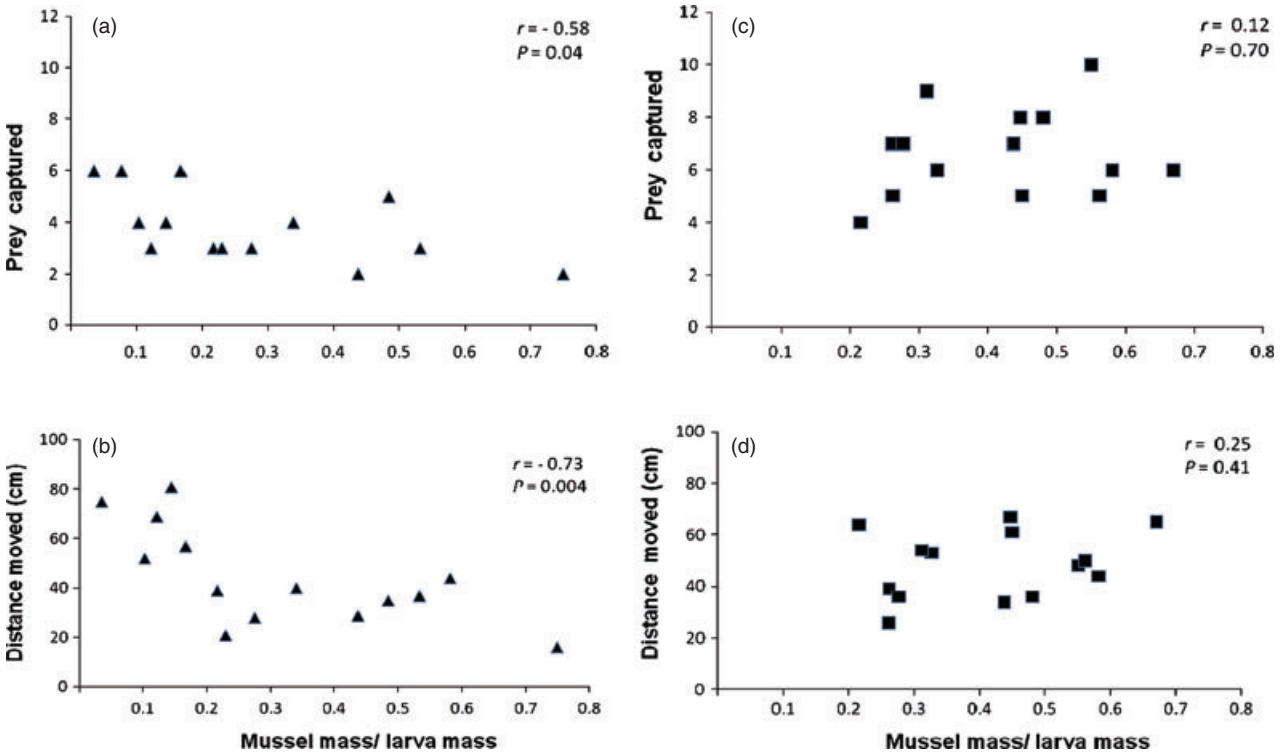

Fig. 3. Effects of mussel load ratio on the foraging behaviour of colonised *Macromia illinoiensis*. Day trials: (a) total *Hexagenia* captured during two, 12-h daytime periods in which prey were replenished to five mayfly larvae every 2 h, (b) total distance moved by the colonised dragonfly larvae during the same time period, $n = 14$ individuals. Night trials: (c) total prey captured by a different set of 14 individuals over two, 12-h night periods during which the total available prey per run was 10, and (d) distance moved over same time frame. r values are partial correlations, controlling for larval head width.

prey capture and distance moved (Fig. 3a,b). It appeared that once a threshold of a mussel load ratio of 20% was crossed, foraging activity was suppressed uniformly across load ratios to 70%, after which there was another decrease in movement to only 20 cm in a 24-h period.

Second, there seemed to be a ceiling effect of prey availability in the night trials. The two-fold greater prey density at the beginning of the night trials compared with the daytime trials may not have compensated for the lack of prey replenishment during the 12-h night trials. Finally, the distance a larva moved was certainly underestimated, especially at night when travel distance was calculated from only two positions, one at the beginning and one at the end of each 12-h trial. Indeed, larvae often left tracks in the sand that indicated they had travelled much greater distances than were detected using the grid method. Underestimating the distance travelled might also explain why we found that colonisation *per se* did not decrease the estimated distance a larva moved either at night or during the day (Fig. 1b). Under natural conditions, increasing mussel loads decrease not only the probability that a final instar reaches the shore (Fincke *et al*., 2009), but they decrease the horizontal distance a larva moves from the shore, as well as the vertical distance it crawls up a bank and/or up a tree before emerging as an adult (O. M. Fincke, unpublished).

Hexagenia limbata, which is active at night and during the day, is known to bury up to 10 cm deep under natural conditions (Charbonneau & Hare, 1998). In the present experiment, although this prey often buried under sand, that behaviour did not prevent *H. limbata* from being captured by both colonised and uncolonised dragonfly larvae. *Hexagenia* was often collected in areas with a complex substrate. Zebra mussels increase substrate complexity (Beeky *et al*., 2004) and *H. limbata* prefer areas with high zebra mussel density (DeVanna *et al*., 2011). Nevertheless, possible gains in prey capture as a result of the attraction of prey to colonised larvae was apparently insufficient to counter the negative effects of the mussel load ratio on prey capture by *M. illinoiensis*. Indeed, the mayfly larvae seemed to treat even uncolonised dragonfly larvae as a resting site. Interestingly, larvae of smaller mayfly species are also attracted to both zebra mussels on colonised *M. illinoiensis*, as well as to the bodies of mussel-free larvae of *D. transversa* and *Hagenius brevistylus* Selys (O. M. Fincke, pers. obs.).

Colonised final instars in our experiment carried two to three mussels, a similar number found on exuviae (i.e. naturally colonised final instars that successfully emerged) of *M. illinoiensis* at our study site in the same year (2.26 ± 0.33 mussels). However, the mean weight of total mussels carried by colonised larvae in our experiment (Table 1) was about

1.5 times the weight of mussels found on the exuviae ($\bar{x} = 0.168 \pm 0.032$ g, Hughes & Fincke, in review). Our finding that zebra mussel attachment impeded foraging success in a predator that uses a sit-and-wait foraging mode is consistent with earlier data on final instars of the closely related *D. transversa*. Fincke *et al*. (2009) found no difference in the body size of exuviae of colonised and mussel-free *D. transversa*. Lacking data on body mass, they prematurely suggested that attached mussels might not affect the larval foraging behavior. However, the body size of a final instar must reflect foraging success during its penultimate (F-1) stadium, because body size is fixed when a larva molts. Nevertheless, a final instar larva continues to add mass, and it is mass, rather than body size, that should reflect the rate of prey capture during a current stadium. Under natural conditions, final instars have a higher probability of being colonised than penultimate instars (Fincke *et al*., 2009; Hughes & Fincke, in review). Hence, the maximum effect of zebra mussels on foraging success of colonised larvae probably occurs during the final stadium. The decreased foraging success of colonised final instars that we found would probably translate into smaller emerging adults. Alternatively, if colonised individuals actually move less (contrary to our result, Fig. 1b), they may consume less energy, which might mitigate the negative effect of mussel attachment on larval mass. Colonised individuals might also compensate for a lower growth rate by delaying emergence. However, that would expose them to aquatic predators for a longer time, and delaying emergence would increase the risk of colonisation by multiple zebra mussels, as a result of a positive feed-back effect of colonisation on burying behaviour (Hughes & Fincke, in review). Measuring foraging success and weight gain of colonised and mussel-free larvae throughout the final stadium until the adults emerge could determine whether attached zebra mussels increase development time and/or reduce adult body size.

Lipid analysis on natural final instars with and without zebra mussels would shed light on the extent to which decreased foraging success translates into significant decrease in fat content of newly emerged adults. In 2010, although lipid analysis was conducted on final instars of *M. illinoiensis*, no significant difference was found in the fat content of colonised and uncolonised final instars (J. C. Osborn, unpublished). However, in 2010, zebra mussels appeared less frequently than in previous years (see Strayer & Malcom, 2006), and colonisation rates of *D. polymorpha* on *M. illinoiensis* and other sprawling species were much lower than in 2009 (O. M. Fincke, unpublished). For example, in 2010, the maximum number of mussels found on *M. illinoiensis* was 2, much less than the 17 mussels found on one final instar during our 2009 collection. Gut analysis on natural final instars with a varying number of attached mussels might indicate if colonisation affects the type of prey taken.

Conclusions

Our finding that zebra mussels decreased foraging success of *M. illinoiensis* adds to a growing number of negative effects of this invasive mussel on sprawling and hiding dragonfly larvae: (i) decreased mobility and ability to right themselves after being overturned, (ii) a lower probability of moving out of the lake to emerge (Fincke *et al*., 2009), and (iii) interference in burying behaviour, resulting in a greater exposure and decreased crypsis, and an increased risk of further colonisation (Hughes & Fincke, in review). Although the population impact of these combined costs has yet to be measured, persistent colonisation caused by zebra mussels should decrease the abundance of its dragonfly hosts, which could have cascading effects across both aquatic and terrestrial ecosystems. A decrease in sprawling dragonflies should increase the abundance of *Hexagenia* and other mayfly prey, which are detritivores in aquatic ecosystems (Rutter *et al*., 1975; Zimmerman & Wissing, 1978; Heise, 1985). Furthermore, a decrease in adult *M. illinoiensis* and similar sprawlers could in turn affect its terrestrial predators and/or an increase in its terrestrial prey, as was documented for libellulid dragonflies (Knight *et al*., 2005) and experimentally, for the dragonfly *Pantala flavescens* Fabricus (Wesner, 2010). Thus, a better understanding of the degree to which decreased foraging success caused by mussel attachments affects the fitness of emerging adults is critical to assessing the effect of this invasive species on the guild of sprawling dragonflies that are important predators in freshwater habitats.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/j.1365-2311.2011.01329.x

Figure S1. A semi-buried *Macromia illinioensis* dragonfly larva eating a larval mayfly, *Hexagenia limbata* (arrow), that it captured during the day.

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