

The effect of water depth on predation frequency by diving beetles on mosquito larvae prey

Shin-ya OHBA^{1,2} and Masayuki USHIO^{1,†}

¹*Center for Ecological Research, Kyoto University, Otsu, Japan and* ²*Biological Laboratory, Faculty of Education, Nagasaki University, Nagasaki, Japan*

Correspondence: Biological Laboratory, Faculty of Education, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan.

Email: ooba@nagasaki-u.ac.jp

[†] *Present address:* Department of Environmental Solution Technology, Faculty of Science and Technology, Ryukoku University, Otsu, Japan.

Abstract

Diving behavior and its frequency may differ between species of mosquito larvae owing to differences in predation pressure. The present study aimed to investigate the relationship between water depth and predation frequency on two mosquito species, *Culex tritaeniorhynchus* (wetland breeder) and *Aedes albopictus* (container breeder), by the diving beetle *Eretes griseus*. *Culex tritaeniorhynchus* spends more time at the surface than *A. albopictus*, which spends more time thrashing underwater. When intact mosquito larvae of both species were present, the diving beetles consumed almost *A. albopictus* larvae (98.3%). After all the *A. albopictus* larvae had been consumed, the diving beetles began to prey upon *C. tritaeniorhynchus*. In order to compare the effect of position on the predation preference of the diving beetles, equal numbers of both species were heat-killed and allowed to settle on the bottom of the container. When all the dead mosquito larvae had sunk to the bottom of a plastic container, the diving beetles caught both mosquito species at random. These results indicate that mosquito larvae near the surface were eaten less frequently by diving beetles than those at the bottom. The low diving frequency of *C. tritaeniorhynchus* is regarded as a form of anti-predatory behavior.

Key words: anti-predatory behavior, Culicidae, Dytiscidae, predator–prey relationship.

In species inhabiting temporary waters, the populations are strongly influenced by physicochemical conditions and predators (Williams 2006). Distributions of species inhabiting temporary ponds are often constrained because adaptations that enhance the developmental rate and competitive ability also tend to increase susceptibility to predation (Wellborn *et al.* 1996; Williams 2006). Generally, predation pressure

increases along with the hydroperiod (Williams 2006). Very active and rapidly developing prey species occur in predator-free temporary habitats, whereas moderately active prey animals occur in more permanent habitats that include invertebrate predators (Williams 2006).

The aquatic stages of mosquitoes are found in a variety of habitats ranging in size from small containers (e.g. tree holes, phytotelmata, jars and tyres) and water-filled animal footprints to the edges of large water bodies such as rice fields and ponds. The pattern and frequency of larval diving behavior differ between mosquito species (Sih 1986; Yee 2008; Ohba *et al.* 2012). For example, the wetland breeder *Culex tritaeniorhynchus* spends more time at the surface of the water than the container breeder *Aedes albopictus*, which spends more time thrashing underwater (Ohba *et al.* 2012). In a previous study, *C. tritaeniorhynchus*, which commonly lives in association with the diving beetle *Eretes griseus*, displayed far stronger responses to a diving beetle cue, whereas *A. albopictus*, which shares no evolutionary history with this predator, did not respond to such cues (Ohba *et al.* 2012). In a study of the mosquito *Aedes triseriatus* and its predator, *Toxorhynchites rutilus*, thrashing was associated with the highest risk of predation, while resting was associated with the lowest (Juliano & Reminger 1992). Similarly, inhabitation at the bottom of the water body of microbeakers posed a greater risk than inhabitation at the surface, which posed the lowest risk (Juliano & Reminger 1992).

The present study aimed to investigate the relationship between water depth and predation frequency on two mosquito species, *C. tritaeniorhynchus* and *A. albopictus*, by the diving beetle *E. griseus*. A previous study (Juliano & Reminger 1992) showed that activity and position were correlated: larvae at the surface rarely

thrashed and frequently filtered. This study focused on the position of the mosquitoes within the water column. We hypothesize that motionless behavior resulting in increased time spent at the water surface by mosquito larva is a form of anti-predator behavior.

Eggs of the two mosquito species, *C. tritaeniorhynchus* and *A. albopictus*, which were collected from Nagasaki, Japan, and used in this study were established at the Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan. These strains are the same as those used in our previous studies (Ohba *et al.* 2012). About 200 hatched larvae were reared in plastic trays (30 × 21 cm or 35 × 24.5 cm, 4 cm water depth), filled with dechlorinated tap water (ca. 2.5 L), under standard laboratory conditions (25°C, LD 16:8, ca. 70% humidity) and fed an artificial diet *ad libitum*. The artificial diet was composed of crushed mouse pellets (Tagawa Jikken-Dobutsu Co. Ltd., Nagasaki, Japan) mixed with an equal amount of dried yeast (Asahi Food and Health Care Co. Ltd., Tokyo, Japan). Mature fourth instar larvae were used in this study and were fasted for 24 h before the experiment. The predacious diving beetle *E. griseus*, which is a widely distributed species in Japan, were collected from rice fields and swimming pools in Nagasaki and rice fields in Hyogo, western Japan. All beetles were maintained in individual plastic cups (10 cm diameter, 4.5 cm in height) filled with 100 mL dechlorinated tap water to a depth of 1.5 cm. All beetles were fed *ad libitum* with boiled dried fish on a daily basis. Each beetle was kept without food for a day before the experiment. All subsequent experiments were conducted under the conditions detailed above. All statistical tests mentioned below were conducted using the R statistical package, v3.1.0 (R Core Team 2014).

To compare the spatial locations within the plastic cups between two mosquito

species, single larva from each mosquito was observed in a plastic cup (12 cm diameter, 10 cm in height, 8 cm water depth). After 5 min of acclimation time, the water depth of each larva was recorded at 1 cm intervals every 30 s for 5 min. We obtained data for 15 larvae (replication) from both *C. tritaeniorhynchus* and *A. albopictus*. To compare the spatial distribution between the two species, we performed a generalized linear mixed model (GLMM) with a Poisson distribution. “Species”, “time” and their interaction term were incorporated into the GLMM. Individual was considered to be a random effect in order to avoid pseudoreplication in this model. In a similar way, single *E. griseus* adults were individually observed in the same plastic cup in order to confirm the diving beetle’s position in a plastic cup. After 5 min of acclimation time, the water depth of each beetle was recorded at 1 cm intervals every 30 s for 5 min ($n = 15$).

To observe the preferred order of predation by a diving beetle, five *C. tritaeniorhynchus* and five *A. albopictus* larvae were placed in a plastic cup, as described. After 5 min, one beetle was added to the plastic cup and predation behavior of the beetle was observed for 30 min. When the diving beetle caught a mosquito larva, the mosquito species and the order of predation were recorded. The experiment was repeated 12 times.

Diving beetles frequently consumed *A. albopictus* larvae first. This may have been caused by differences in spatial distribution between the two mosquito species. To examine the prey selectivity of the diving beetle when the mosquito larvae of both species had been forced to sink to the bottom of a plastic cup, dead mosquito larvae were used. In preliminary tests, we confirmed that all mosquito larvae killed by hot water had sunk to the bottom of the plastic cup. Mosquito larvae were placed into hot water (approximately 80°C) for 10 s to kill them. Five dead larvae from both species as

well as a diving beetle were added to the plastic cup, and the order of predation by the beetle was observed for 30 min. When the diving beetle caught a dead mosquito larva, the mosquito species was recorded. The experiment was repeated 12 times.

To test the difference in the order of predation among the experimental treatments, we performed a randomized simulation (i.e. Monte Carlo method). In the randomized simulation analysis, we assumed that the diving beetle randomly consumed the mosquito larvae (the diving beetle showed no preference for the prey species or behavior). Briefly, the simulation analysis was performed on ten prey larvae (five *C. tritaeniorhynchus* larvae and five *A. albopictus* larvae), and the order of the species, which was randomly selected in the simulation, was recorded. In the real experiment, some of the ten mosquito larvae were not consumed within 30 min, which was also taken into account in the simulation analysis (i.e. the number of larvae consumed was not always ten). We repeated the simulation analysis 9999 times, and a 95% confidence interval was determined. The proportion of each species consumed was considered significant when the value was not within the 95% confidence interval. In addition to the randomized simulation analysis, data regarding the proportion of each species eaten by a diving beetle (predation rate) within 30 min was analyzed using GLMM with a binomial distribution for each species. The predation rate was used as the response variable; treatment (intact and killed mosquito) and species were used as the explanatory variables. Each individual diving beetle was considered to be a random effect. Interactions that were not found to be significant in the full GLMM model were removed from the final model. The GLMM models were performed separately for each treatment.

The GLMM revealed that the species, time, and species \times time interaction were

significant (Table S1). Although the species \times time interaction was significant, there was no opposite trend of spatial distribution along time between the two species (Fig. 1). The mean water depth occupied over 5 min was 0.35 cm for *C. tritaeniorhynchus* and 3.25 cm for *A. albopictus* (Fig. 1). The mean water depth *E. griseus* occupied over 5 min was 5.76 cm. Therefore, we regarded the spatial distribution (water depth) of mosquito larvae as a more important factor that influences the predation event than mosquito behavior.

When five mosquito larvae of each species were present, the diving beetles normally consumed *A. albopictus* larvae until they had consumed the first five larvae (Fig. 2). Once the *A. albopictus* larvae had been consumed, the beetles began to prey upon *C. tritaeniorhynchus*. Thus, the probability of predation on *A. albopictus* was approximately 90–100% until the first five larvae had been eaten, which was significantly high compared with a 95% confidence interval of the randomized simulation model (i.e. the simulation model assumes that the diving beetle has no preference; dashed lines in Fig. 2). When all mosquito larvae had been forced to sink to the bottom of the plastic container, all predation events occurred at the bottom of the container. The diving beetles randomly caught both mosquito species, being within the 95% confidence interval of the simulation model indicated by the dashed lines in Figure 2. This result indicates that the diving beetles evenly attack both species when present at the water bottom. Regarding the predation rate during the total 30 min, the species \times treatment interaction was removed from the final GLMM model because it was not significant ($z = 0.02$, $P = 0.98$). The final model revealed that the effects of species, and treatment were significant (Table S2). The predation rate against *A. albopictus* of intact mosquito larva treatment was significantly higher than that against *C. tritaeniorhynchus*

(98.3% for *A. albopictus* and 53.3% for *C. tritaeniorhynchus*; GLMM: $z = 3.828$, $P = 0.0001$). However, the predation rate against killed mosquito larvae by a diving beetle was not statistically different between the two species (100% for *A. albopictus* and 96.7% for *C. tritaeniorhynchus*; GLMM: $z = 0.002$, $P = 0.998$).

Culex tritaeniorhynchus spend more time at the surface than *A. albopictus*.

When *C. tritaeniorhynchus* detect predator cues, they reduce their movement and foraging activity as a form of anti-predatory behavior (Ohba *et al.* 2012). This behavior is also observed in other aquatic animals (Kolar & Rahel 1993; Skelly & Kiesecker 2001; Van Buskirk 2002; McIntyre *et al.* 2004), including mosquitoes (Sih 1986; Kesavaraju *et al.* 2007). *Culex pipiens* larvae become motionless at the water surface in the presence of backswimmers (Sih 1986). Similarly, *A. triseriatus* larvae that spend three generations with predacious mosquitoes tend to remain near the water surface for longer periods (Juliano & Gravel 2002). These results indicate that an effective way to escape from aquatic predators is by ceasing diving behavior. In contrast, diving behavior allows *Anopheles gambiae* to escape predation by the wolf spider *Pardosa messingerae*, which is often observed on the water surface (Futami *et al.* 2008). Anophelines are surface feeder and are more vulnerable to terrestrial (spider) and aerial (shore fly) predators. Therefore, diving behavior may have evolved in response to this predation pressure (Minakawa *et al.* 2007; Futami *et al.* 2008).

We conclude that motionless behavior at the water surface by *C. tritaeniorhynchus* is a form of predator-avoidance behavior against diving beetles. Consistent with this, we found that larvae near the water surface were eaten less frequently by diving beetles than those at the bottom. On the contrary, *A. albopictus* breed in small containers that are likely to dry out, and the larvae usually forage on

organic matter found at the bottom. Although the motionless behavior at the water surface was revealed to be a more effective way to avoid predators in the small containers used in this study, further studies should evaluate the relationship between predation frequencies on mosquito larvae by diving beetles and water depth in their natural habitats.

ACKNOWLEDGMENTS

We would like to thank C. Tsurukawa and K. Ohashi for providing the mosquitoes, and R. Murakami for collecting the diving beetles. We also wish to thank Y. Ohba for her assistance during this study. This study was supported by JSPS KAKENHI Grant Number 25830152.

REFERENCES

- Futami K, Sonye G, Akweywa P, Kaneko S, Minakawa N (2008) Diving behavior in *Anopheles gambiae* (Diptera: Culicidae): avoidance of a predacious wolf spider (Araneae: Lycosidae) in relation to life stage and water depth. *Journal of Medical Entomology* **45**, 1050–1056.
- Juliano SA, Gravel ME (2002) Predation and the evolution of prey behavior: an experiment with tree hole mosquitoes. *Behavioral Ecology* **13**, 301–311.
- Juliano SA, Reminger L (1992) The relationship between vulnerability to predation and behavior: geographic and ontogenetic differences in larval treehole mosquitoes. *Oikos* **63**, 465–476.
- Kesavaraju B, Alto BW, Lounibos LP, Juliano SA (2007) Behavioural responses of larval container mosquitoes to a size-selective predator. *Ecological Entomology* **32**, 262–272.
- Kolar C, Rahel F (1993) Interaction of a biotic factor (predator presence) and an abiotic factor (low oxygen) as an influence on benthic invertebrate communities. *Oecologia* **95**, 210–219.

- McIntyre P, Baldwin S, Flecker A (2004) Effects of behavioral and morphological plasticity on risk of predation in a Neotropical tadpole. *Oecologia* **141**, 130–138.
- Minakawa N, Futami K, Sonye G, Akweywa P, Kaneko S (2007) Predatory capacity of a shorefly, *Ochthera chalybescens*, on malaria vectors. *Malaria Journal* **6**, 104.
- Ohba S, Ohtsuka M, Sunahara T, Sonoda Y, Kawashima E, Takagi M (2012) Differential responses to predator cues between two mosquito species breeding in different habitats. *Ecological Entomology* **37**, 410–418.
- R Core Team (2014) *R: A Language and Environment for Statistical Computing, Version 3.1.0*. R Foundation for Statistical Computing, Vienna. Available from URL: <http://www.R-project.org>
- Sih A (1986) Antipredator responses and the perception of danger by mosquito larvae. *Ecology* **67**, 434–441.
- Skelly D, Kiesecker J (2001) Venue and outcome in ecological experiments: manipulations of larval anurans. *Oikos* **94**, 198–208.
- Van Buskirk J (2002) Phenotypic lability and the evolution of predator-induced plasticity in tadpoles. *Evolution* **56**, 361–370.
- Wellborn G, Skelly D, Werner E (1996) Mechanisms creating community structure across a freshwater habitat gradient. *Annual Review of Ecology and Systematics* **27**, 337–363.
- Williams D (2006) *The Biology of Temporary Waters*. Oxford University Press, New York.
- Yee DA (2008) Tires as habitats for mosquitoes: a review of studies within the eastern United States. *Journal of Medical Entomology* **45**, 581–593.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1 Generalized linear mixed model results for water depth.

Table S2 Generalized linear mixed model results for predation rate.

Figure Legends

Figure 1 Comparison of the water depth occupied by two mosquito species.

Figure 2 Predation order and proportion of mosquitoes eaten by the diving beetle. Solid circle, intact mosquito; open circle, killed mosquito. Dashed line and black solid line indicate upper and lower 95% confidence intervals and the average proportion, respectively (calculated by 9999 times randomisation).

Fig. 1

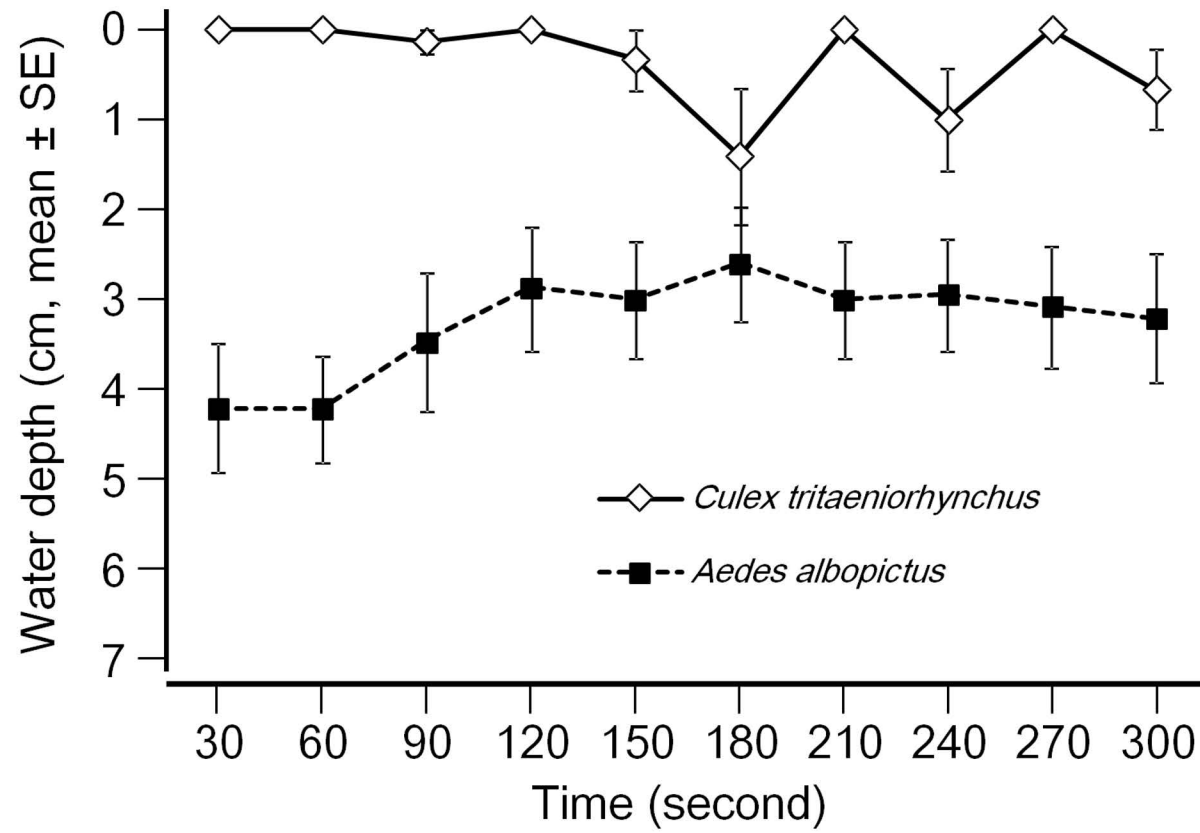


Fig. 2

