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Diving as an Anti-Predator Behavior in Mosquito Pupae

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Amit Kant Awasthi, Cheng-Han Wu, and Jiang-Shiou Hwang (2012) Diving as an anti-predator behavior in mosquito pupae. *Zoological Studies* **51**(8): 1225-1234. Diving is considered an anti-predator (escape) behavior in mosquito pupae. However, pupal diving has not yet been properly studied or characterized. Our videographic 2-dimensional observations in this study elucidate the pupal behavior of *Culex pipiens quinquefasciatus* in the presence and absence of the predatory fish *Poecilia reticulata*. Pupae exhibited significantly higher speeds and more-diverse escape responses in the presence of the fish compared to the control. These escape responses included shallow, medium, and deep diving, surface movements, floating up, and fish-following upward movement. The no-predator control showed merely shallow diving and surface movements. Pupae adjusted their self-righting behavior according to the fish-following speed and effectively shortened their self-righting time during a fast chase. Although diving pupae preferred smaller acute angles in the presence and absence of predators, the medium diving angles were significantly higher than the shallow diving angles in the presence of a predator. Pupae achieved complete escape success during diving down, but the escape success of a pupa depended on whether or not the predatory fish continued to pursue the pupa. Although 96% of pupae successfully escaped during the 1st fish attack, their ultimate escape success decreased when fish continued their pursuit. Pupae had more responses and significantly higher escape speeds in the presence of a predator than in the control. Mosquito pupae diving behaviors demonstrate their escape behavior during predator encounters. Diving tactics in *Culex* pupae that help them escape from aquatic predators lead to increased fitness. http://zoolstud.sinica.edu.tw/Journals/51.8/1225.pdf

Key words: *Culex*, Anti-predator behavior, Diving behavior, Diving angle, Escape success.

Mosquito pupae demonstrate 2 main behavioral states in their natural habitat: resting at the air-water interface or arbitrarily swimming in random directions in response to stimuli (e.g., passing shadows, predators, or hydrodynamic microturbulence) (Brackenbury 1999, Rodriguez-Preito et al. 2006). Mosquito pupae respond to such physical disturbances by diving down, and they can regulate their buoyancy state and diving behavior (Clements 1999, Romoser and Lucas 1999, Lucas and Romoser 2001, Rodriguez-Preito et al. 2006).

Non-consumptive and consumptive predator effects can alter prey behavior, induce predator avoidance, and elicit prey escape behavior (Hwang et al. 2009, Hwang and Martens 2011, Mahjoub et al. 2011a b 2012). Examples include the use of refuges and reduced feeding behaviors that may ultimately affect prey development (Lima and Dill 1990, Grostal and Dicke 1999, Brodin and Johansson 2004, Dmitriew and Rowe 2005, Preisser et al. 2005, Creel and Christianson 2008). Predator-induced behavior in prey is often associated with lower vulnerability to predation (Sih 1986 1987, Gilliam and Fraser 1987, Peckarsky and Wilcox 1989, Skelly and Werner 1990, Grill and Juliano 1996, Watkins 1996, Dhanker et al. 2012, Kumar et al. 2012). However, the persistent presence of a predator causes rapid divergence in prey plasticity which manifests through changes in prey traits (i.e., behavioral, chemical, or morphological traits) (Juliano and Gravel 2002).

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Potential predators of mosquito pupae include fish, carnivorous diving beetles and their larvae, and notonectid back swimmers (Heteroptera) (Brackenbury 1999, Juliano and Gravel 2002). Pupae use diving behavior to avoid both predation (Lucas and Romoser 2001, Rodriguez-Preito et al. 2006) and being washed out of their habitats during rainfall (Koenraadt and Harrington 2008).

Mosquito pupae do not feed during the pupal stage, and rely only on stored energy from earlier larval stages. Thus, the survival and disease transmission of a newly emerging adult mosquito depends upon its energy reserves (Lucas and Romoser 2001). Pupae take in oxygen by placing their air siphons above the water. Therefore, excessive diving increases energetic expenditure, which in turn affects adult survival (Timmermann and Briegel 1993, Lucas and Romoser 2001). Diving also increases larval mortality (Tuno et al. 2004) and reduces survival after eclosion (Lucas and Romoser 2001). Because pupae must balance the cost of oxygen consumption with the benefit of reduced predation risk, they should modulate their escape dive according to the perceived risk of predation (Lucas and Romoser 2001, Rodriguez-Preito et al. 2006).

A cost/benefit tradeoff may often constrain escape behavior, as it requires energy expenditure and time to avoid predators. Since movement expends energy, behaviors such as resting at the air-water interface or staying in a neutral (hovering) or negative buoyant state (including bottom sitting) conserve energy. Pupae respond to buoyancy variations in an energy-minimizing way in order to promote adult emergence and survival (Lucas and Romoser 2001, Koenraadt and Harrington 2008).

Although diving is considered to be an anti-predator behavior in mosquito pupae, very little is known about how predation risk affects pupal diving. According to Sih (1986), prey and predator co-occurring within an experimental system typically exhibit more-precise responses rather than to apply any artificial stimulation as a predator for prey-predator interactions study. In previous studies, diving was described as an anti-predator behavior without using any natural predator within the experimental system (Brackenbury 1999, Rodriguez-Preito et al. 2006). Thus, it is very difficult to identify specific behaviors as anti-predator responses without including a predator within the system. We used predatory fish to test the hypothesis that diving is an anti-predator behavior in mosquito pupae. We also wanted to determine the particular escape responses, patterns, and behavior of *Culex* pupae in the presence and absence of a predator. We conducted videographic observations to determine specific escape responses of *Culex pipiens quinquefasciatus* pupae in the presence of a predatory fish *Poecilia reticulata*, a widely used predator for mosquito biocontrol. We observed several kinds of escape responses and diving patterns of mosquito pupae in the presence of the predatory fish. We also measured, analyzed, and described their diving speeds, diving angles, and self-righting times in the presence and absence of predatory fish to investigate our research question.

MATERIALS AND METHODS

Culex pipiens quinquefasciatus mosquito larvae were collected from a stream near National Taiwan Ocean University during summer 2008 using a mosquito larvae collecting net with a 153-μm mesh and 0.5-m radius. Third and 4th instars of *Cx. p. quinquefasciatus* larvae were reared in a beef and yeast extract mixture (2: 1) at a temperature of 27 ± 1°C, at a relative humidity of 75%, and a photoperiod of 12: 12 h (light: dark) within an incubator to obtain pupae. These early pupae (0-6 h old) were used in the experiment due to their high energy content and to avoid the emergence of adults during the experiment. This study used *Poecilia reticulata*, a predatory fish which is widely used for mosquito control. Laboratory-reared female *P. reticulata* measuring approximately 5 cm in total length were used as an experimental predator. Mosquito pupae were provided as food 2 d prior to the experiment to acclimatize the fish. Fish were starved for 3 h prior to the experiment.

Video-recording

Pupal behavior was recorded in the presence and absence of predators at 30 frames/s with an HDD camera (Hard Disk Drive-Sony DCR-SR 100 camera, Japan) which faced the experimental tank horizontally. This same filming technique was used as described in many published papers from our laboratory (Strickler and Hwang 1999, Dahms and Hwang 2010, Vandromme et al. 2010, Chang et al. 2011, Awasthi et al. 2012, Dur et al. 2012). All experiments were performed in a dark room with a single fluorescent light (with a light intensity of 1400 lux) situated above the experimental aquarium to simulate being in the wild. The experimental aquarium (17 × 12.5 × 14 cm) contained 2.5 L of dechlorinated autoclaved tap water with 1 predator and 20 pupae as prey in each trial for the study. In total, 5 trials were applied in this study in which 100 pupae (prey) and 5 fish (predator) were used in the experiment, with similar numbers for the control (without a predator). Behavioral observations were recorded for 15 min for each trial with (treatment) and without (control) predators for the 5 trials.

Video analysis of trajectories and characterization of different escape behaviors

The technically best diving sequences were selected from the recorded video for further analysis. Image sequences for both pupae and fish were obtained and analyzed frame by frame using Track-It (Iguana Gurus, Milwaukee, WI, USA), a manual tracking software program, to digitize all trajectories. Trajectories were reconstructed numerically, and all reconstructed trajectories were further analyzed.

Acquisition of diving speed

The average diving speed was calculated as the movement of pupae breaking the water surface and beginning a downward journey until turning to come back up or stopping to self-right the body. The self-righting response in pupae occurs when swimming ceases and a pupa reorients its body within 1 s with its respiratory horns pointed directly upwards in order to float up. The time taken to reorient the body posture is called the self-righting time (SRT) (Brackenbury 1999). These diving/escape movements were further classified into shallow, medium, deep, and surface movements. The average speed of a mosquito pupa was determined by dividing the distance traveled by time.

The Pythagorean theorem $C^2 = A^2 + B^2$ was used to obtain distance data in pixels to calculate a distance scale for the video analysis. The distance (d) was calculated between 2 successive frames from the x and y coordinates as:

$$d = [(x_t - x_{t+1})^2 + (y_t - y_{t+1})^2]^{1/2}; \qquad (1)$$

where (x_t, y_t) and (x_{t+1}, y_{t+1}) are the positions of a mosquito pupa at the time interval t and t+1. The swimming speed v (mm/s) was estimated as follows:

$$v = df; \qquad (2)$$

where the frame rate (f) of the camera was 30 frames/s (Wu et al. 2010).

Diving depth

Diving depth was calculated as the deepest level the pupae reached before beginning to come up. To measure the diving depth, we used the y coordinates of the acquired numerical trajectories. Diving depth was measured as follows:

$$\text{Diving depth} = y_n - y_1;$$

where y_n and y_1 are the deepest and 1st coordinates of the dive. This study differentiated diving patterns according to the diving depth. Shallow diving (SD) represented a diving depth of > 5-20 mm, medium diving (MD) was > 20-50 mm, and fish-following deep diving (DD) was > 50 mm (Fig. 1).

Horizontal distance

The distance covered by the pupae on the water surface during an escape or in the control was considered the horizontal distance. The horizontal distances traveled by the pupae were further classified as surface movements (SMs) and fish-following surface movements (FFSMs). We used x coordinates to measure the horizontal distance covered by the pupae:

$$\text{Horizontal distance} = x_n - x_1;$$

where x_n and x_1 are the last and 1st coordinates of an SM.

Diving angle

Trajectories of each diving event were tracked to locate the turning point of each pupa. Diving angles were measured from the turning point relative to the horizontal axis. The success of a fish in capturing a pupa, the probability of the pupa escaping, and pupal movement patterns in the presence and absence of fish were observed. These parameters were described according to their respective diving depths and movement speeds.

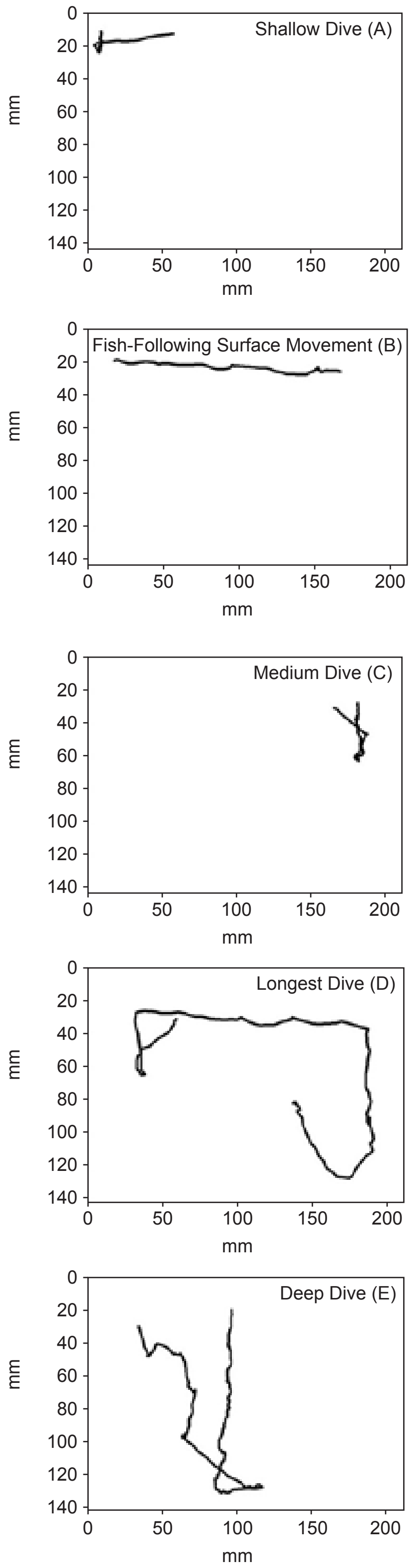


Fig. 1. Different types of movements recorded by *Culex p. quinquefasciatus* pupae in the presence of a predator. (A) Shallow dive; (B) fish-following surface movement; (C) medium dive; (D) longest dive; and (E) deep dive.

Statistical analysis

The null hypothesis for the Shapiro-Wilk test was based on a sample taken from a normal distribution. If $p < 0.05$ for the Shapiro-Wilk test rejected data normality, further statistical tests were conducted based on the results obtained from the Shapiro-Wilk test. The non-parametric Kruskal-Wallis test was conducted to evaluate differences among diving speeds, traveled distances, and diving angles of pupae in the presence and absence of predators. The Bonferroni correction was used to compute multiple comparisons for speeds, distances, and diving angles to control type I errors across tests. A one-way analysis of variance (ANOVA) was carried out for pupae captured during different escape movements while being pursued by a fish. The Wilcoxon-Mann-Whitney *U*-test was conducted to compare the self-righting times of a pupa when a fish was pursuing it with an approach speed or attack speed. The Wilcoxon-Mann-Whitney *U*-test was also used to compare the fish approach or attack speed. All statistical tests were conducted at the 95% confidence level.

RESULTS

Culex p. quinquefasciatus pupae demonstrated diverse diving patterns, including SD, MD, and DD along with SMs in response to a predator (Fig. 1). Pupal SMs were further described as SMs in the control and FFSM groups. There were differences between SMs and SD, in that mosquito pupae neither left the air-water interface nor demonstrated a floating-up (FUP) movement during SMs (Fig. 1A, B). Another critical characteristic of SMs in *Cx. p. quinquefasciatus* was a constant swimming speed from the beginning to the end of the journey without leaving the surface (Fig. 1B). Because *Cx. p. quinquefasciatus* pupae frequently preferred SD or SMs, this study considered a diving depth of > 5 cm as DD (Fig. 1, Table 1). Control pupae exhibited SD and SMs, and their respective traveled distances were 8.29 ± 2.05 and 18.59 ± 5.03 mm. The control group exhibited only 1 MD event with a diving depth of 20.73 mm, slightly greater than the SD (Table 1).

The Shapiro-Wilk test was significant for speeds. Thus, the Kruskal-Wallis test was performed on non-normally distributed data ($p < 0.05$). Pupae displayed diverse diving movements in the presence of a predator. All escape speeds in the presence of a predator (i.e., DD, MD, SD, and SMs) were significantly higher than the control (Kruskal-Wallis test, χ^2 (8, n = 250) = 182.39, p < 0.0001) (Fig. 2, Table 1). Multiple comparisons revealed non-significant differences among SD, MD, DD, FFSM, and fish FUP (FFUP) movements except for the FUP movement with a predator (Bonferroni-corrected p < 0.05). The presence of *P. reticulata* increased all escape speeds (Kruskal-Wallis test, χ^2 (8, n = 250) = 182.39, p < 0.0001) and the respective distances traveled by pupae (Kruskal-Wallis test, χ^2 (5, n = 129) = 102.98, p < 0.0001) compared to the control (Table 1). Pupae with FUP and without FFUP movements showed significant differences (Bonferroni-corrected p < 0.0001). FFUP movements did not significantly differ from SD (Bonferroni-corrected p = 0.084) or SMs of the control even when a fish was following a pupa. The DD depth with a following fish was significantly higher than SD and MD depths, with a depth of 72.31 ± 20.02 mm (p < 0 .0001) (Table 1).

Culex p. quinquefasciatus pupae performed 2 types of responses in the control of (i) SD (60.53%), and (ii) SMs (39.47%), but showed only 1 MD event. Pupae demonstrated highly diverse responses in the presence of fish: SD (42.47%), MD (32.19%), and DD (10.96%) with SMs (14.38%). Pupae demonstrated a preference toward SD instead of MD, DD, or SMs, even when escaping (Fig. 2).

Fish were able to capture pupae during various pupal movements (such as FUP and self-righting), but not during downward diving. The Shapiro-Wilk test was non-significant for capture, and the data were normally distributed (p > 0.05). The one-way ANOVA demonstrated differences in capture success for predators during diverse escape events (one-way ANOVA, $F_{3,16}$ = 23.77, p < 0.0001). Capture success was significantly higher when mosquito pupae were FUP to the surface than during self-righting (Tukey's honest significant difference (HSD) p > 0.0001). However, the pupae escape success rate did not significantly differ during FUP and resting at the air-water interface (Tukey's HSD p = 0.257). Eighty-two pupae were captured by fish in 5 trials with an average of 16.4 ± 4.6 pupae/trial within 15 min

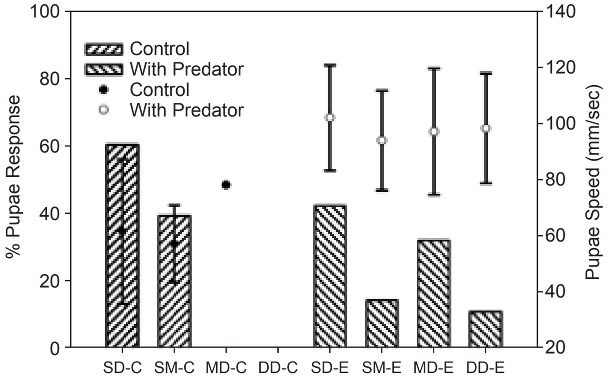


Fig. 2. *Culex* pupal responses are represented by a bar chart and their shallow diving (SD), medium diving (MD), deep diving (DD) and surface movement (SM) speeds by a scatter dot plot in the presence and absence of the fish predator *Poecilia reticulata*. Suffixes C and E respectively represent the control and experimental groups.

Table 1. *Culex pipiens quinquefasciatus* pupal speeds and distances traveled in the absence and presence of a fish predator

	Speed (mm/s)	No. of tracks observed for speed	Diving depth (mm)	Horizontal distance (mm)	No. of tracks observed for distance
In the absence of *Poecilia reticulata*					
Shallow diving	61.79 ± 25.77	16	8.29 ± 2.05	N/A	17
Medium diving	78.33	1	20.73	N/A	1
Surface movement	57.37 ± 13.72	9	N/A	18.59 ± 5.03	9
Floating up	9.073 ± 3.01	16	N/A	N/A	N/A
In the presence of *P. reticulata*					
Shallow diving	102.28 ± 18.78	29	11.27 ± 4.18	N/A	26
Medium diving	97.33 ± 22.50	32	33.32 ± 9.72	N/A	38
Deep diving	98.49 ± 19.62	44	72.31 ± 20.01	N/A	20
Fish-following surface movement	94.19 ± 17.77	35	N/A	64.86 ± 43.23	26
Fish-following up	81.71 ± 21.68	11	N/A	N/A	N/A
Without fish- following up	14.98 ± 4.12	58	N/A	N/A	N/A

(Table 2).

Fish frequently attacked pupae and were unable to capture pupae in the 1st attempt. A fish which initially failed to capture a pupa instantly followed the pupa and attacked it several times as it was diving. Pupal escape probabilities were very high, at 96% and 79%, respectively, for the 1st and 2nd attempts (Fig. 3). The average number of attacks in a single subsequent sequence was 5, but fish demonstrated a maximum of 13 attacks in all 5 trials (Fig. 1D). The average number of fish attacks successively decreased from 36.4 to 20.2, 12.4, 6.6, and finally to 3 for the 1st, 2nd, 3rd, 4th, and 5th consecutive fish attacks per trial in 15 min (Fig. 3). Although the average number of fish attacks gradually decreased, the fish capture success increased up to 33% when fish continued to follow the pupae (Fig. 3). This study observed 77 instances of fish following a diving pupa until the fish gave up following the same pupa (i.e., the pupa successfully escaped) or fish captured another pupa during the chasing event. Capture success (no. of attacks/no. of captures) gradually increased as the fish followed pupae in all 5 trials; however, pupae managed to escape 58.44% of the attacks (Table 3).

Poecilia reticulata attacked or followed pupae at a speed 68.60 ± 28.12 mm/s, and the attacking/following speed was significantly higher than the approach speed of 24.33 ± 7.33 mm/s (Wilcoxon-Mann-Whitney *U*-test, $p < 0.0001$). SRTs of pupae likewise changed with predator speeds; the pupal SRT was significantly longer when fish were pursuing pupae with an approach speed (24.33 ± 7.33 mm/s) than with an attack speed (68.60 ± 28.12 mm/s) (Wilcoxon-Mann-Whitney *U*-test, $p < 0.0001$), and SRTs for pupae were respectively observed to be 0.43 ± 0.11 and 0.2 ± 0.06 s.

Culex p. quinquefasciatus pupae displayed diverse diving angles (Fig. 4). Pupae diving angles significantly differed (Kruskal-Wallis test, χ^2 (2, n = 163) = 72.2, $p < 0.0001$). Pupae frequently took dives at shorter acute angles (10°-70°) in both the presence and absence of predators. SD angles in the presence of predators did not differ (Bonferroni-corrected $p = 1$) from those in the absence of predators. During MD with fish present, pupae showed significantly (Bonferroni-corrected $p < 0.0001$) larger acute diving angles than during SD with or without fish present which ranged 35°-80° (Fig. 4). Mosquito pupae usually preferred a shallow acute diving angle of around 45° in both the presence and absence of predators, but they occasionally demonstrated a straight dive at an angle of ~70°-90° when the fish mouth or body touched them or hindered their escape movement. Subsequently, they showed a direct (deep dive) DD instead of a sequential

Table 2. Number of pupae captured by fish in 15 min and their respective positions during capture. The number of pupae/trial was n = 20

	Captured pupae/trial
Total captured	16.4 ± 4.6
Air-water interface	6 ± 1.9
During diving	
Diving down	0
During self-righting	2.4 ± 0.55
Floating up	8 ± 2.65

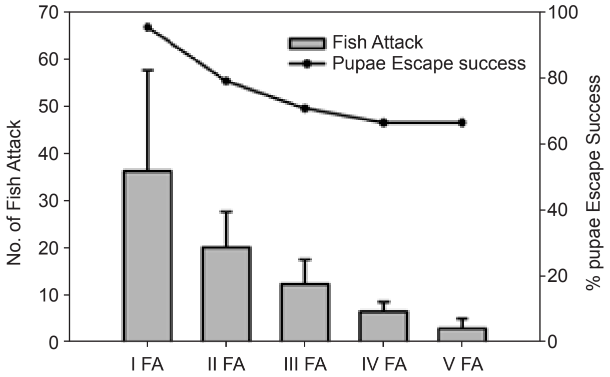


Fig. 3. Average number of attacks by a single *Poecilia reticulata* on *Culex* pupae in 5 trials shown as a bar chart, and a scatter line plot showing the successful escape percent of pupae during 5 successive fish attacks (FAs).

Table 3. Pupal escape success or failure when being pursued by fish

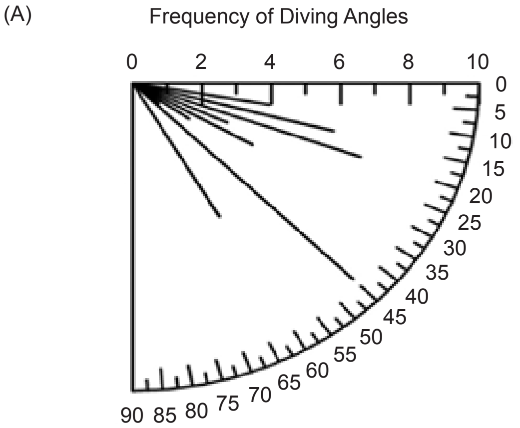
	Mean ± S.D.	Percent (%)	*n*
Fish followed when a pupa dove	15.4 ± 1.95	100	77
Pupa failed to escape	9 ± 1.41	58.44	45
Pupa succeeded in escaping	6.4 ± 3.05	41.56	32

DD. In the control group, pupae occasionally displayed straight diving angles of approximately 80°-90°, and these were also observed in the presence of a predator. These dives were too slow to be considered escape dives, and these were described as buoyancy correction methods in previous studies.

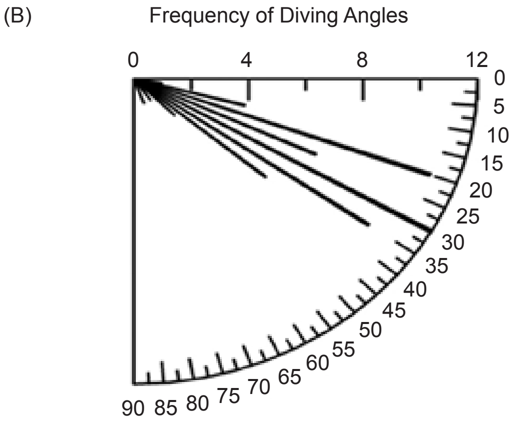
DISCUSSION

Mosquito pupae in calm water habitually prefer the air-water interface. Pupae detect threats in the environment through hydrodynamic disturbances and by visual stimuli (Brackenbury 1999, Rodriguez-Preito et al. 2006). This study confirms that *Culex p. quinquefasciatus* pupae respond to predatory fish by diving. Diving may be an excellent escape strategy for pupae because of their exceptionally high speed. The presence of alternative prey in naturally occurring aquatic ecosystems may also increase the probability of escape by *Culex* pupae. Effective anti-predator refuges may also improve the likelihood of pupal escape and survival.

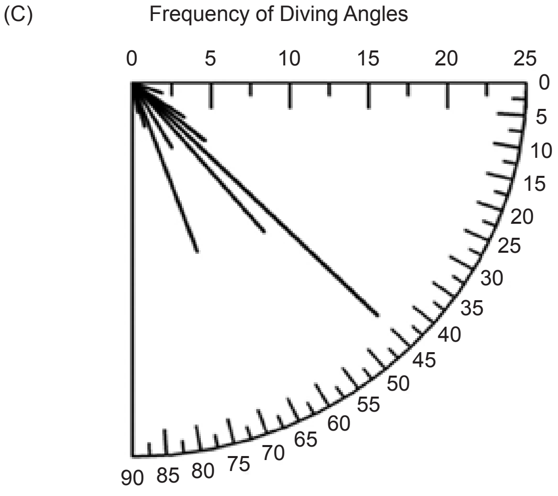
Mosquito pupae move quickly during their escape attempts, a prerequisite for prey escape (Walker et al. 2005, Wu et al. 2010, Awasthi et al. 2012). Fast escape speeds of culicid larvae and pupae were attributed to the fast-click mechanism of movable abdominal paddles (Nachtigall 1961 1974, Houlihan 1971, Romoser 1975, Wu 1977, Alexander 1988, Brackenbury 1999). Speeds of pupae engaged in behaviors such as SD, MD, DD, and FFSMs were significantly higher in the presence of a predator than in the control. Walker et al. (2005) demonstrated that faster starts increase the probability that prey successfully evade a predator. Irrespective of any diving/escape events, speeds of pupae remained nearly the same in the presence of *P. reticulata*. Since fish demonstrated their preferences towards slower pupae than rapidly diving pupae during the study (Table 2), fast pupal diving may be an excellent adaptive escape strategy to avoid capture by a predator. Because diving pupae lack an effective mechanism for quickly returning to the surface (Christopher 1960, Romoser 1978), they often engage in slow FUP movements. *Cx. p. quinquefasciatus* pupae revealed a very few fast-FUP (FFUP) movements during escapes in the presence of fish. These escape movements (fish-FUP) by pupae were faster than the fish attack speeds. The FFUP movement of pupae suggests



Shallow Diving Angles in Control



Shallow Diving Angles with fish



Medium Diving Angles with Fish

Fig. 4. Diving angles of *Culex* pupae in the control group and in the presence of *Poecilia reticulata*. (A) Shallow diving angles in the control; (B) shallow diving angles with fish present; and (C) medium diving angles with fish present.

either active escape from predators or pupae wanting to quickly achieve positive buoyancy lost during an escape. The loss of positive buoyancy might give pupae a more-rapid upward speed even in the absence of active up-movement skills (Christopher 1960, Romoser 1978). After a threatening stimulus is removed, pupae self-right (reorient) their body postures and slowly return to the surface (Brackebury 1999, Romoser and Lucas 1999). The presence of gas in the ventral air space (VAS) of the pupae renders them positively buoyant. Positive buoyancy allows a *Culex* pupa to passively rise by FUP after deeply diving during an escape attempt, without wasting much of its energy reserves, which is critical for its emergence as a healthy adult (Romoser 1978, Romoser and Lucas 1999, Lucas and Romoser 2001).

Fast speed often provides an effective response by prey to a threatening stimulus (Hwang et al. 1994, Hwang and Strickler 2001, Walker 2005, Awasthi et al. 2012). Fish were often unable to capture pupae due to their fast diving speeds. Fast diving speeds of pupae might be helpful for prey to effectively escape from a predator and simultaneously find a refuge (Srinivas et al. 2009). Most of the *Culex* pupae that were captured were either resting at the air-water interface, FUP, or self-righting their body posture between dives. Fish frequently failed to capture pupae in a single attack due to fast escape diving. In such cases, pupae were followed and attacked several times. The fish immediately modified their strategy and captured more slowly moving pupae than they did fast-diving ones (Table 2).

Prey typically displays strong responses to more-dangerous predators and weaker responses to less-dangerous predators (Sih 1980 1986 1987). Mosquito larvae can typically distinguish between predators and similar non-predators, and a strong stimulus can serve as a cue for escape behavior (Mayo and Mackie 1976, Sih 1980 1986 1987). Immature mosquito stages (i.e., larvae and pupae) exhibit diverse escape events in response to predators (Sih 1986, Rodriguez-Preito et al. 2006). Pupae in the predator treatment were more active and exhibited more-diverse escape responses than pupae in the control group (Fig. 2). However, greater pupal activity also decreases the odds of survival and decreases the time spent resting at the air-water interface (Brackenbury 1999). Because pupae must rely on stored energy reserves after the 4th larval stage, high pupal activity reduces adult emergence and survival after emergence (Brackenbury 1999, Lucas and Romoser 2001). There is thus a trade-off between avoiding predation, maintaining oxygen levels, and conserving energy reserves for adult emergence (Rodriguez-Preito et al. 2006).

Continuously followed pupae preferentially dove downwards instead of coming upwards. because they are incapable of active upward movements (Christopher 1960, Romoser 1978). It might also be possible that pupae could not self-right their body posture to float up. The SRT depends upon several attributes, such as how many times pupae dive and how long they are away from the air-water interface (Lucas and Romoser 2001). Mosquito pupae adjust their SRT according to the fish chasing speed and the number of dives they have just executed. The SRT was significantly lower when fish followed pupae at an attack speed. Strategies such as stopping for self-righting and making SDs might confuse predators because predators commonly follow moving prey rather than motionless prey, because they rely on prey movements as an attack cue (Walker et al. 2005). Mosquito pupae can regain the surface immediately after self righting their body posture or make another dive according to a threatening stimulus. Self-righting pupae might obtain sensory feedback from the predator and select an adaptive escape trajectory (Brackenbury 1999 2001).

Culex p. quinquefasciatus pupae typically preferred several SDs in succession rather than a single DD. Pupae typically make SDs at steep angles in order to remain positively buoyant (Romoser 1975) and conserve energy for survival. If a threat still exists after an SD, pupae performed an MD or DD. Pupal diving depths varied according to the diving events. According to Romoser and Lucas (1999), pupae can decrease their frequency of surfacing events to reduce the possibility of being captured at the water surface. However, pupae habitually do not prefer a straight DD (Lucas and Romoser 2001). When a fish blocked a pupa's escape path, the pupa was forced into a straight DD at an angle of approximately 70°-80° even though its preferential diving pattern is an SD or MD. The escape response cannot be completely random among animals (Brackenbury 1999 2001). Thus, pupae with straight DDs revealed that their escape responses were correlated to the threat direction and mechanical factors such as the body structure, shape, and symmetry of the predator. Escape paths may be biased or associated with available escape routes and less-risky environments during an escape (Brackenbury 1999 2001). In the control group, pupae occasionally conducted very slow and shallow straight dives. This might be a mechanism for pupae to correct their buoyancy state as they periodically change their buoyancy (Romoser 1978, Romoser and Lucas 1999).

These adaptive escape behaviors of mosquito pupae in the presence of predators are not restricted to fast movements (e.g., diving, FFSMs, and FFUP), but they can also manipulate their SRT according to the pursuing predator. While these adaptive fast escape diving events provide an advantage over predators, the SRT may help pupae obtain sensory cues to respond accordingly to predators. Mosquito pupae rapidly diving down bestows them complete escape success in the presence of fish. *Culex* pupae exhibited more-diverse escape responses in the presence of a predator, compared to the control, confirming that diving in *Culex* pupae is an anti-predator behavior. The behavioral adaptations of immature mosquito stages can provide critical information for mosquito management and control.

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