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Anti-Predator Behavioral Responses of Mosquito Pupae to Aerial Predation Risk

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Aquatic insects have two potential sources of predation risk: aquatic predators and aerial predators. Our goal was to assess anti-predator responses of Culex pipiens *to aerial predation. By simulating predator attacks, we assessed (a) the distance fled in relation to depth and group size, (b) the distribution of individuals at different depths, and (c) the duration of surfacing events to obtain air in scenarios with varying predation risk. Pupae located closer to the surface fled deeper into the water, and the number of conspecifics decreased the distance fled. When the risk of predation increased, more individuals were found deeper in the water column, and the interval between two consecutive surfacing events increased.* Culex pipiens *shows a trade-off between avoiding aerial predation and maintaining oxygen acquisition, which may be regulated by the need to conserve energy reserves.*

KEY WORDS: aerial predation risk; anti-predator behavior; *Culex* pupae; water depth; oxygen consumption; resource use.

INTRODUCTION

Nearly all organisms are preyed upon by many different species of predators. Most studies investigating antipredator behavior have only examined the responses of prey to one type of predator (Sih *et al.*, 1998). However,

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if predators vary in hunting tactics or locations (Lima, 2002), prey should develop specific behavioral responses for each type of predator (Matsuda *et al.*, 1993). Aquatic insects have two potential sources of predation risk: aquatic predators and aerial predators. Aquatic predators influence prey behavior by inducing temporal and or spatial shifts (e.g. Sih, 1986; Muotka *et al.*, 1999; Biro *et al.*, 2003) and reducing feeding activity (e.g. Koperski, 1997; Dmitriew and Rowe, 2005), eventually leading to growth and developmental costs (e.g. Tseng, 2003; Brodin and Johansson, 2004). Aerial predators could also affect aquatic insect behavior, and since the location of the potential source of predation is fixed (the air-water interface), aerial predation risk could produce qualitatively different effects from those of aquatic predation (see Templeton and Shriner, 2004 for an example in freshwater fishes), or even could interact with aquatic predation to generate emergent impacts on aquatic prey (Sih *et al.*, 1998).

Although the effects of aerial predation risk have been studied to some extent on freshwater fishes (e.g. Allouche and Gaudin, 2001; Johnsson *et al.*, 2004), to our knowledge little is known about the anti-predator behavioral responses of aquatic insects to aerial predators (but see Martín and López, 2004). In this study, we assessed such responses using *Culex pipiens* as our model organism taking into account the trade-off between oxygen acquisition and safety from predation.

Mosquito pupae do not feed, relying solely on the energy reserves stored from the larval stage that constitute a crucial component of body condition and the later survivorship of emerged adults (Lucas and Romoser, 2001). Pupae get aerial oxygen by sticking their air siphons above water. When undisturbed, pupae rest at water surface, but they react to physical and visual disturbance by swimming down (Sih, 1986; Lucas and Romoser, 2001). However, excessive diving increases energetic expenditure, which may affect survival (Timmermann and Briegel, 1993; Lucas and Romoser, 2001). Moreover, *Culex pipiens* pupae remain positively buoyant when diving (Romoser and Lucas, 1999), so they need to actively counteract buoyancy in order to remain at a safe depth. Thus, pupae should balance the need to avoid predation in relation to oxygen consumption and energy expenditure. It is expected that pupae have evolved an optimal anti-predator strategy by modulating the magnitude of their escape dive in relation to the perceived risk of predation (Lima and Dill, 1990).

We predicted that if the water surface is perceived as the riskiest place in an aerial attack, the magnitude of escape dive would decrease as the depth at which the pupa was situated increased due to greater distance to the source of predation, and as the number of surrounding conspecifics increased due to dilution effects (Foster and Treherne, 1981; Roberts, 1996). We also predicted that the perceived risk of predation would determine the distribution of pupae along a depth gradient, with decreasing numbers of individuals situated at shallow depths as aerial predation risk level increased. Finally, we predicted that pupae would reduce the duration of each surfacing event in which they obtain air, and would increase the time interval between two consecutive surfacing events as the perceived risk of aerial predation increased.

MATERIAL AND METHODS

The study was performed in two consecutive winters (2003/2004 and 2004/2005), in a cattle drinking trough located in a mixed open forest of *Quercus ilex*, *Q. pyrenaica* and *Fraxinus angustifolia* in Collado Villalba (40°37′N, 4°01′W), Central Spain, at 900 m elevation. The drinking trough was made of granite rock with a pale coloration and was composed of four interconnected subsections, each measuring 1.10 × 0.60 × 0.30 m. We attached a tape measure to one wall of each trough subsection in order to have a reference for our measurements. There was no aquatic vegetation in the trough, the water was very clear, and the pupae were easily visible against the background. At the time of the study, the trough held a population of around 500 *Culex pipiens* mosquito pupae each year. For the duration of the study, cattle were prevented from accessing the trough. There was no aquatic predator in the trough, so the effects we found could be ascribed mostly to aerial predation.

Distance Fled

We simulated attacks to stationary focal mosquito pupae when they were situated at two depth intervals (0–1 cm and 1–5 cm) by entering a straight stick (40 cm long, 1 cm diameter) into the water without touching the pupa but passing by at less than 3 cm from it. With this procedure, we intended to simulate an attack from a predator such as a bird. All pupae attacked ($n = 45$) readily escaped by diving into deep water. To avoid inter-observer bias, the same person (IRP) performed all attacks in a systematic way and at the same speed. Despite the high abundance of pupae in the trough, we changed the location of attacks and looked for undisturbed pupae to minimize the chances of attacking the same focal individuals. We measured the distance between the initial point were the focal pupa was and the point where it stopped the escape dive (distance fled) with a metered stick and the wall tape measure, to the nearest 0.5 cm. We also recorded the number of pupae present within a radius of 2 cm around the focal pupa just before the attack (number of conspecifics). We waited at least 8 minutes without visual contact with the trough before performing another attack. This time interval was enough to allow the pupae to return to pre-disturbance position within the depth gradient.

Distribution of Individuals at Different Depths

We simulated three scenarios of risk (sample size, control = 16, medium risk = 15, high risk = 15). In the ‘high risk’ scenario, we randomly “attacked” the water of a trough subsection by entering the stick into it every 30 s. Note that in this treatment, we attacked randomly different portions of the water, but not a focal pupa. In the ‘medium risk’ scenario, we performed every 30 s random menacing movements at 50 cm over the water with the stick, but without getting the stick into the water. In the ‘control’ scenario, we remained close to the water trough but neither attacks nor movements with the stick were performed. For each replicate in each scenario, we counted the number of pupae situated at each of three depth intervals (0–1 cm, 1–5 cm and >5 cm) inside the area of a quarter of the trough subsection. We began recording the proportion of pupae at each depth interval 4 min after the beginning of the first disturbance event to allow individuals to familiarize with the risk scenario.

Obtaining Air

We simulated two scenarios of risk in days different from those in which we studied the distribution of individuals at different depths. In the ‘high risk’ scenario, we randomly “attacked” the water with a stick every 30 s, as described in the previous section. In the ‘control’ scenario, neither attacks nor movements were performed, and the observer was not in visual contact with the trough. For each scenario, we recorded the time interval between two consecutive surfacing events for a given randomly chosen focal pupa (control, $n = 25$; high risk, $n = 25$) and the duration of each surfacing event (control, $n = 30$; high risk, $n = 26$). Time intervals and durations were recorded only once for each focal pupa in each attack. We varied the location of attacks to reduce the chances of attacking the same individuals. We recorded surfacing intervals and surfacing durations at least 4 minutes after the first disturbance event until the last disturbance event. We detected easily when mosquito pupae stuck their air siphons above water to get oxygen (a surfacing event).

Statistical Analysis

We checked for the normality and homogeneity of variance of variables and residuals. Inter-surfacing interval and surfacing duration were log-transformed. We did not find significant differences between both winters in distance fled ($t_{42} = 0.23, P = 0.82$), proportion of individuals at each depth interval (at 0–1 cm depth: $t_{44} = 0.14$, $P = 0.89$; at 1–5 cm depth: $t_{44} = 0.88$, $P = 0.38$; at more than 5 cm depth: $t_{44} = -0.68$, $P = 0.50$), inter-surfacing interval ($t_{48} = -0.04$, $P = 0.96$), and surfacing duration ($t_{54} = -1.14$, $P = 0.26$). Therefore, we pooled the data from both breeding seasons.

In analyzing the effects of initial depth interval on distance fled by attacked pupae, we ran an ANCOVA, with depth interval as the categorical independent factor, and the number of conspecifics as the covariate. We ran a MANOVA to analyze the effects of risk on the proportion of pupae present at three different depth intervals (three dependent variables). One-way ANOVAs were used to analyze the effect of predation risk on inter-surfacing interval and surfacing duration.

RESULTS

Distance Fled

The distance fled by a pupa after an attack was significantly affected by depth. Pupae located at less than 1 cm from the surface fled significantly farther than pupae situated 1–5 cm from the surface (0–1 cm: 8.7 ± 0.4, 1–5 cm: 4.3 ± 0.3; $F_{1,41} = 76.71, P < 0.001$). The number of conspecifics close to the focal individual was also significantly related to distance fled, with increasing number of conspecifics being associated with a lower distance fled ($F_{1,41} = 5.16, P = 0.028$). However, there was no significant interaction between depth and number of conspecifics ($F_{1,41} = 0.18, P = 0.674$). Furthermore, we did not find variation in the number of conspecifics among depths ($F_{1,41} = 2.04, P = 0.160$).

Distribution of Individuals at Different Depths

Risk treatments significantly affected the distribution of individuals along the depth gradient (Wilks = 0.11, $F_{4,84} = 41.29, P < 0.001$; Figure 1). When risk increased, the proportion of individuals at 0–1 cm depth ($F_{2,43} = 38.80$, $P < 0.001$) and at 1–5 cm depth decreased ($F_{2,43} = 90.98$,

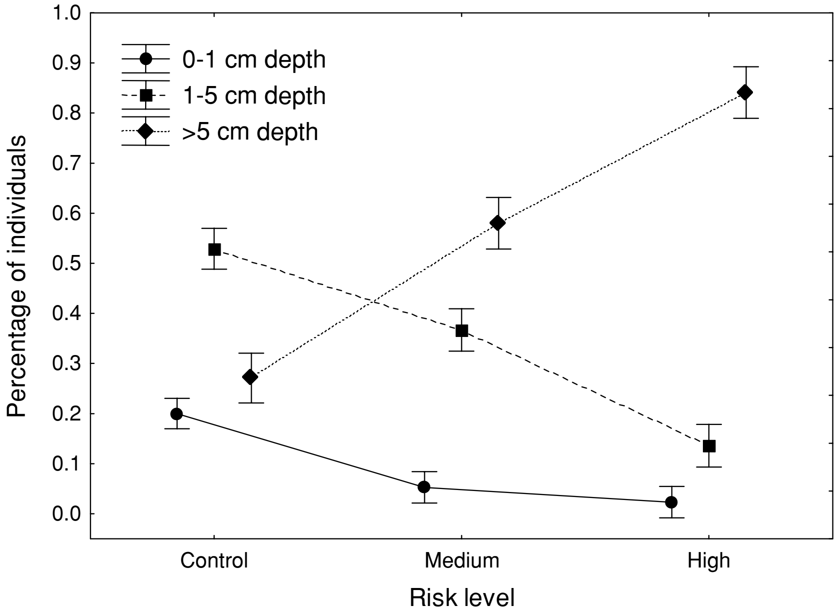

Fig. 1. Effect of risk treatment on the proportions of pupae present at each depth interval.

$P < 0.001$), whereas the proportion of individuals at >5 cm depth increased with risk ($F_{2,43} = 129.34$, $P < 0.001$).

Obtaining Air

The time interval between two consecutive surfacing events was significantly longer in the high risk scenario than in the control scenario (control: 25.2 ± 2.9 s; high risk: 268.3 ± 10.6 s; $F_{1,48} = 437.78$, $P < 0.001$). However, the duration of each surfacing event did not differ between the two predation risk levels (control: 7.4 ± 1.5 s; high risk: 6.0 ± 1.6 s; $F_{1,54} = 2.36$, $P = 0.13$)

DISCUSSION

Our results show that aerial predation risk level influenced pupae escape responses, depth selection, and behavior aimed at obtaining air, suggesting that in *Culex pipiens* pupae there exists a trade-off between avoiding aerial predation and maintaining oxygen acquisition, which may be modulated by the need to conserve energy reserves.

Pupae appear to optimize their escape responses under aerial attacks in a way consistent with anti-predator behavior optimization theory (Ydenberg and Dill, 1986). In the context of aerial predation, mosquitoes may have evolved an anti-predator strategy in which shallower depths are riskier, and consequently aversive responses are greater near the water-surface than at a deeper depth. With this strategy, mosquito pupae modulate their allocation of anti-predator effort in relation to the context of the attack (Martín and López, 2000; Papouchis *et al.*, 2001; Cooper *et al.*, 2003), thus, saving energy by avoiding superfluous diving. Olsson and Klowden (1998) found that mosquito larvae with low energy reserves incurred in less diving when alarmed than larvae with higher reserves, showing that conserving energy is a major factor governing diving behavior.

The perceived risk of predation also affects the distribution of pupae along the water column (see also Sih, 1986). Higher aerial predation risk leads to greater proportion of pupae situated at deeper locations, which are safer from this type of predation. Furthermore, the energy expenditure required in order to *stay* at a deeper location is lower than to *stay* at a shallower location due to increasing pressure with depth (Romoser and Lucas, 1999). However, pupae must travel to the water surface to obtain oxygen, and at higher depths travel time increases, which could result in more energy consumption. This could explain why pupae do not select the deepest locations at the first indication of risk, but adjust their depth to the perceived risk of predation, selecting deeper waters only as predation risk increases. Thus, at a given time, pupae are expected to be located at the depth where both types of costs balance each other. This optimal depth will vary as predation risk changes.

As risk increased, pupae showed a ten-fold decrease in their frequency of surfacing events, probably to reduce the likelihood of being caught at the risky water surface, and to decrease the energy expended in the increasingly longer travels to the water surface. Undergoing such high reduction in the number of events in which they obtain air suggests that pupae perceive a relatively high predation risk. Nevertheless, surfacing duration did not vary with risk levels. Wolf and Kramer (1987) also found that air-breathing fish increase their use of refuges and thus decrease their frequency of surfacing events in the presence of predatory fish. Similar reductions in the frequency of resource use with the frequency of predation events have been found in other taxa (White *et al.*, 1999; de la Torre *et al.*, 2000; Rodríguez-Prieto and Fernández-Juricic, 2005). Thus, in our system, high and continuous aerial predation risk could result in a reduction in the proportion of overall time devoted to oxygen acquisition. In other systems, anti-predator responses to aerial predation (fish being attacked by Little Egrets *Egretta garzetta*) also reduced access to oxygen-rich water surfaces, which has been suggested to affect growth rates (Kersten *et al.*, 1991).

Overall, the two main costs of the aerial anti-predator behavior in *Culex pipiens* are low oxygen acquisition rate and high energy expenditure associated with travel time. Future studies should asses the influence of these costs on fitness-related parameters, such as time to metamorphosis, pupa survival, adult survival, and mating success in populations subject to different levels of predation. Furthermore, it would be relevant to assess the combined effects of opposing predation sources (aquatic and aerial) to better understand anti-predator behavior in aquatic organisms. For instance, it is unclear whether prey would increase exposure to aquatic predators trying to avoid aerial predators or the risk of aquatic predation would be independent of depth and an increase in aerial predation would not affect the risk of aquatic predation.

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