BEHAVIOR OF LARVAL *CULICOIDES SONORENSIS* (DIPTERA: CERATOPOGONIDAE) IN RESPONSE TO AN INVERTEBRATE PREDATOR, *HYDRA LITTORALIS* (ANTHOMEDUSAE: HYDRIDAE)[1]

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Species of Ceratopogonidae are commonly known as biting midges, punkies, no-see-ums, or sand flies. Some are annoying pests and transmit a wide range of pathogens including arboviruses, protozoa, and filarial nematodes to humans, domestic animals, and wildlife (Borkent 2005). In North America, *Culicoides sonorensis* Wirth and Jones is economically important because it transmits orbiviruses to livestock and wildlife (Foster et al., 1963; Foster et al., 1977).

The basic behavior of larvae of *C. sonorensis* in response to predators has not been well studied. The larvae and pupae of *C. sonorensis* live in natural and man-made ponds and lakes and tend to dwell in the shallow mud along the margins or bottom. They can swim in the water column by writhing rapidly back and forth and superficially resemble nematodes. Larvae of *Culicoides* feed on a wide range of food items including algae, diatoms, certain bacteria, protozoa, and small invertebrates such as rotifers and nematodes (Mullens and Hribar, 1988). A wide range of predatory invertebrates, including insects, arachnids, and worms, parasitize, kill, and eat larvae of Ceratopogonidae including *Culicoides* spp. (Bacon, 1970). Predator avoidance behaviors have not been studied, however. Some other aquatic invertebrates respond to predators by avoiding habitats that experience predation (Rondelaud et al., 2002) or female flies can avoid ovipositing in water that harbors predators or parasites (Zahiri et al., 1997; Van Dam and Walton, 2008), but these behaviors are unknown in Ceratopogonidae.

Predators of aquatic Diptera include a wide range of organisms including vertebrates, worms, cnidarians, and other arthropods. One potentially overlooked group of predators are the *Hydra* spp. (Anthomedusae: Hydridae), which are nearly ubiquitous aquatic cnidarians found in most standing and slow flowing bodies of water. *Hydra* spp. can be cultured in the laboratory (Lenhoff and Brown, 1970). In the laboratory a single *Hydra littoralis* Hyman can kill and eat 2 to 7 larvae of *C. sonorensis* per day when hydra are maintained in open containers with *Culicoides* larvae (unpublished observations). *Hydra littoralis,* a brown hydra found throughout the Nearctic region, kills its prey with nematocysts on the feeding tentacles that inject a neurotoxic succinoxidase inhibitor

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(Kline and Waravdekar, 1960; Ruch and Cook, 1984). *Hydra* spp. are sessile predators that feed on a wide range of prey including immature insects. *Hydra littoralis* floats when starving or crowded and probably releases metabolic chemicals that can be detected by other *H. littoralis* when they are crowded (Hyman, 1938; Lomnicki and Slobodokin, 1966). During the spring and summer larvae of *C. sonorensis* primarily inhabit the surface of the mud layer of ponds and feed on phytoplankton, but larvae swim into the water column (Vaughn and Turner, 1987) where they could encounter predators such as *Hydra* spp. The goal of this experiment was to measure potential predator avoidance behaviors of larvae of *C. sonorensis* when placed in containers with *H. littoralis*.

METHODS

Colonies of *C. sonorensis* were continuously maintained at the USDA-Arthropod-Borne Animal Diseases Research Laboratory as described by Hunt (1994). Larvae from the VanRhyan colony, originating from California, USA were used for this experiment. Second and third instar larvae were collected from rearing pans and individually transferred with plastic pipettes into 3.5 ml (7 cm tall by 1.5 cm wide) glass tubes with 3 ml of deionized water. They were allowed to acclimate for approximately 5 minutes. An ethogram of discrete behaviors was constructed by observing 10 individual larvae for 5 min intervals. Discrete behaviors included active swimming, lying on bottom unmoving, sinking in the water column, looping to touch the head to anus, eating debris on the bottom, and hanging from the meniscus. New tubes were divided into three equal sections (top, middle, bottom). Time budgets were constructed for 10 control larvae measuring the time spent by individual larvae in each discrete behavior over 5 minutes. Fresh tubes were used for each observation. The laboratory was lit with white light and ranged from 25-27ºC.

Budding *H. littoralis* were purchased from Carolina Biological Supply (Burlington, NC). A single adult *H. littoralis* was released into unoccupied tubes and allowed to settle to the bottom and acclimate for approximately 1 h. The hydra always remained at the bottom of the tube. After acclimation individual 2nd or 3rd instar larvae were released into the tube and allowed to acclimate for approximately 5 min. A time budget was constructed for larvae as previously described. In most situations *H. littoralis* would capture and kill the larvae in less than 5 min. This procedure was replicated for 37 larvae.

RESULTS

The individual time spent in each behavior was analyzed using a Monte Carlo simulation (Resampling Statistics©, University of Vermont, Burlington) with 100000 bootstrap replicates, randomization, and replacement and a cut off P value of 5%. Swimming, sinking in the water column, or lying on the bottom accounted for 99% of the observed behaviors. Observations that accounted for less than 1% of the total time were not analyzed.

Larvae in the control tubes swam for an average of 5.8 sec with intermittent sinking periods lasting an average 5.9 sec. The average swimming time for larvae in tubes with *H. littoralis* was 7.1 sec and sinking for 3.5 sec. Both the sinking time and the swimming activity were significantly different (P= 0.001) between controls and treatment tubes. The time spent in the top 1/3 of the tubes averaged 15 sec and bottom 1/3 of the tube averaged 41 sec and larvae spent an average of 5 sec transitioning between the top and bottom. There were no significant differences (P> 0.05) between the controls and treatments.

DISCUSSION

Larvae of *C. sonorensis* have different behaviors when in the presence of *H. littoralis*. There were differences in the swimming and sinking behavior of larvae but predator avoidance has not been determined. *Hydra* spp. are some of the most ubiquitous aquatic predators of small invertebrates and larvae of *C. sonorensis* co-occur with them in many habitats. An increase in swimming behavior while in the proximity to *Hydra* spp. could benefit the insect larvae if they are able to swim away from the predator. In contrast the agitation of the larvae might benefit a sessile predator by increasing the movement of potential prey. Further tests of larval behavior and adult oviposition behavior could indicate a preference for hydra free zones of a container or hydra free water.

The taxonomy of the *Hydra* is controversial and voucher specimens of *H. littoralis* were deposited in the Georgia Museum of Natural History, Athens, Georgia, USA. Voucher specimens of *C. sonorensis* were previously deposited at the University of Wyoming Insect Collection, Laramie, Wyoming USA.

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