

# Mosquito larvae can detect water vibration patterns from a nearby predator

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## Abstract

Mosquito larvae have been shown to respond to water-borne kairomones from nearby predators by reducing their activity, and thus visibility. If they can identify the predator, they can then alter their response depending upon the associated predation risk. No studies have shown that mosquito larva may also detect water-borne vibrations from the predator. Final instar larvae of three mosquitoes: *Culiseta longiareolata*, *Culex perexiguus* and *C. quinquefasciatus*, were exposed to recorded vibrations from feeding dragonfly nymphs, to dragonfly kairomones and the combined effect of both. Predator vibrations caused *C. longiareolata* to significantly reduce bottom feeding and instead increased the more passive surface filter feeding. The larvae also significantly increased escape swimming activity. These behavioural changes were not significantly different from the effect of dragonfly kairomones, and there was no synergistic or additional effect of the two. *C. perexiguus* gave a smaller (but still significant) response to both dragonfly vibrations and to kairomones, probably due to a different feeding behaviour: when lying on the bottom, it was an inactive filter feeder. *C. quinquefasciatus* did not respond to either vibrations or kairomones and during these experiments was entirely an inactive surface filter feeder. Both *C. longiareolata* and *C. perexiguus* were thus able to detect and identify vibrations from feeding dragonfly nymphs as an anti-predator strategy. The lack of response in *C. quinquefasciatus* is probably a result of living in water that is highly polluted with organic material, where few predators can survive.

**Keywords:** *Culiseta longiareolata*, *Culex perexiguus*, *Culex quinquefasciatus*, mosquito larva, predator vibrations, predator kairomone, filter feeding, predator avoidance

(Accepted 17 November 2016; First published online 9 January 2017)

## Introduction

If a predator appears in their pool of water, mosquito larvae have the disadvantages of being poor swimmers and unable to escape to another pool of water. It is thus not surprising that the larvae of many mosquito develop anti-predator strategies by reducing their activity to make themselves less visible, or by other behavioural changes (see review by Ferrari *et al.*, 2010). Many mosquito larvae normally combine two feeding methods: filter feeding at the surface, and actively swimming over the bottom, where they scrape biofilms from surfaces. However, in the presence of a predator such as dragonfly nymphs, some species reduce bottom feeding and

instead increasing the safer surface filter feeding (Stav *et al.*, 2000). This is both less active (so less detectable to a predator) and more distant from bottom-feeding predators such as dragonfly nymphs (Roberts, 2014a). Other aquatic insects have been shown to use further behavioural defences. Thus Odonata avoid the location of predators (Pierce, 1988) and increase nocturnal feeding (Koperski, 1997), when they will be less visible to predators such as fish.

However, these phenotypic responses to the presence of a predator are traded off with other life history traits and present metabolic costs that are likely to result in a slowed physiological development (Stoks *et al.*, 2005). This will result in the trait compensation of producing smaller less-competitive adults. This strategy of reducing activity, but retarding growth, has been demonstrated in mosquito larvae in response to predators such as dragonfly nymphs (Roberts, 2012), notonectids (Beketov & Liess, 2007) and fish (Bond *et al.*, 2005; Van Uitregt *et al.*, 2012). Since these behavioural defences have

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metabolic costs, it would be advantageous for the mosquito larva to alter its response depending upon the predation risk. This would require them to be able to detect and recognise different predator species. For example, *Culiseta longiareolata* Macquart responded differently to kairomones from dragonfly nymphs, damselfly nymphs and to fish, but did not respond to final-instar nepids (Roberts, 2014a). A similar phenotypic plasticity to different predators has been shown in damselfly nymphs (Chivers *et al.*, 1996) and dragonfly nymphs (Hopper, 2001; Stoks *et al.*, 2003). Some mosquito species have no response to particular predators, probably because their specialised habitats have a low predation risk (Roberts, 2014a) or their species has little previous exposure to that predator (Sih, 1986; Kesavaraju & Juliano, 2004).

Most aquatic insects do not primarily rely on vision to detect and identify a predator. Vision among vegetation and through disturbed or polluted water may be limited and unreliable (Dodds & Whiles, 2010), while many aquatic larval insects, such as mosquitoes, have few ommatidia in their eyes and thus poor visual ability. In contrast, chemicals dispersing through the water can be more reliable for detecting a nearby predator and thus may allow the prey to detect the predator before the predator detects the prey (Takahara *et al.*, 2012). Many studies have shown that mosquito larvae use predator kairomones in the water to identify the predator (Ferrari *et al.*, 2010) and this response is often enhanced by alarm pheromones from damaged conspecifics (Ferrari *et al.*, 2007; Roberts, 2014a). Water vibrations could also be useful in detecting nearby predation, if the prey had the ability to detect water vibrations and to recognise specific patterns. Thus, Peckarsky (1987) showed that mayfly nymphs probably used water vibrations to detect and avoid predatory stoneflies. Among terrestrial insects, substrate vibrations are extensively used for communication (Cocroft & Rodríguez, 2005) and there are a number of studies showing the ability of insect prey to detect and respond to vibrations from predators. Examples are flying moths detecting echolocation by hunting bats (Miller & Surlykke, 2001), aphids detecting running coccinellid beetles on their leaf (Losey & Denno, 1998), and caterpillars detecting predatory wasps and stinkbugs that walked over their leaf (Castellanos & Barbosa, 2006).

These responses by the prey require a specific identifiable vibration pattern produced by the predator. Odonata nymphs have an unusual prey capture in which their labial mask is suddenly projected in front of the head to grasp the prey in a pair of claws. It is probable that this shock wave produces a characteristic vibration pattern that could be used by other potential prey for identification of a nearby Odonata predator.

In this present study, three species of mosquito larvae were examined: *C. longiareolata* is known to respond strongly to dragonfly kairomones (Roberts, 2014a) and lives in microhabitats with a high predation risk; *Culex quinquefasciatus* Say shows a much lower response to dragonfly kairomones and lives in microhabitats with only a low predation risk; *Culex perexiguus* Theobald, although a man-biting vector of West Nile virus (Harbach, 1988), has been little studied.

As a threat-sensitive response to a situation where the mosquito larva may be exposed to a very high predation risk, having two different species-specific detection systems may allow more subtle behavioural responses. Thus the hypotheses tested were:

- The dragonfly prey-capture behaviour produces a characteristic vibration pattern that is detected and specifically identified by some mosquito larvae.
- *C. longiareolata* and *C. perexiguus* respond to these vibrations by altering their feeding behaviour, but *C. quinquefasciatus* does not respond.

## Methods

### Source of mosquitoes and predators

Blood-fed *C. quinquefasciatus* were collected in the Sultan Qaboos University campus using large black peat bags as 'resting sites' (Roberts, 2010). The collected mosquitoes were kept in bucket containers, which had cotton pads soaked in sugar solution as food and a 280 ml container of water containing some yeast powder for the females to lay their eggs. *C. longiareolata* and *C. perexiguus* egg rafts were collected weekly from rain-filled rock pools in Wadi Qurai near the town of Sumail on the edge of the Jebel Akhdar mountains, 60 km from the university campus. Egg rafts and hatched larvae were kept at a laboratory temperature of 24°C.

All the hatched mosquito larvae were continually fed with yeast powder at a daily dosage of 0.09 mg/larva until they became 4th instar, and so ready for use in the experiments.

Dragonfly nymphs (*Crocothemis erythraea* Brullé) were collected from very small (<2 m<sup>2</sup>) fish-free pools in Wadi Al-Khod, about 5 km from the university. Only final instar dragonfly nymphs were used in the experiments. They were kept in containers of 200 ml water and fed on final (4th) instars of the mosquito species being tested.

### Stimuli preparation

#### Vibrations

Polystyrene containers (8 cm diam × 10 cm high) containing 200 ml of water were used in the experiments. Water-borne vibrations were detected by an extremely sensitive geo-phone sensor ('America' brand) touching the outside of the polystyrene container. The sensor was connected through an amplifier (Radioshack 40w pre amplifier) to a computer running Audacity software ver 2.1.0 of 2015 (<http://www.webaudacityteam.org>). A series of 1 min recordings were made and then joined together (using the Audacity software) with 30 s gaps between each segment. Thus, the sequence of recordings was fixed, but the starting point varied in different replicates. This was played back as a continuous loop through two netbooks (Acer Aspire V5-171) to six (three pairs) of headphones (each connected using Y-connectors) underneath the mosquito containers. Since each headphone loudspeaker was in physical contact with its polystyrene container, it vibrated the water containing the mosquito larva. The geo-phone sensor was used to check that the vibrations produced were the same as the original and at the same intensity. In pre-trials, underwater ear buds (Pyle PWP25B Waterproof Aqua Sport Headphones), which were immersed in the water of the mosquito container, worked just as well, but appeared to attract the mosquito larva, possibly because they became highly contaminated with the yeast.

The vibrations recorded were:

- (i) *Swimming mosquito larvae*. Fifty mosquito larvae were kept together in the polystyrene containers, so that crowding interference between the larvae resulted in frequent swimming behaviours, which were then recorded for playback.
- (ii) *Dragonfly prey-capture vibrations*. Individual dragonflies kept in the polystyrene container were fed mosquito

larvae and the vibrations produced when the nymph captured a mosquito larva using its labium were recorded. Eight different dragonfly nymphs were recorded and the recordings put together using Audacity software with 30 s intervals between feeding to produce a recording that was played back as a continuous loop.

- (iii) *Clicks from a light switch*. These were used in pre-trials at the same intensity as the dragonfly vibrations, to see whether the response to dragonfly prey-capture vibrations was specific and not just a factor of vibration intensity.

#### Water preparation

The day before each set of replicates, the following were prepared:

- (i) *Preparation of water for the mosquito larvae*. 12 polystyrene containers (described above) each contained 200 ml of conditioned tap water (water stored for at least 1 week) and approximately 0.09 mg of yeast ('Healthlife' brand of brewer's yeast) added, to give the yeast time (24 h) to become established. (ii) *Preparation of kairomone water*. Eight dragonfly nymphs were each fed with four mosquito larvae, then as soon as these were consumed (within 15 min), each nymph with its 200 ml of water was tipped into a container of 4 litres of conditioned tap water, to give time for predator kairomones to accumulate. At the start of the experiments on the next day, each dragonfly was fed with a further four mosquito larvae, so that the water contained chemicals from both the dragonfly and from the eaten mosquito larvae. Just before the predator kairomone experiment was due to start, the dragonflies were all removed into holding containers and the water from their containers was mixed together to give predator conformity. It was this mixed water that was used in the fourth and fifth experiments.

### Behavioural experiments

#### Experimental design

The 120 replicates were run in sets of 12 containers. At the start of each set of experiments, each mosquito larva was transferred into its polystyrene container (dimensions above) containing 200 ml of water + yeast and left for 30 min to adjust. Each container sat on top of a 6 cm diameter headphone (Danyin Dt-301) encased in polythene foam (the plastic band normally connecting a pair of headphones had been removed, so each of the two headphones were separate). The pad of polythene foam underneath the headphone (and thus mosquito container) isolated the water from external vibrations. As each experiment ran, each mosquito larva was observed every 5 min to determine over a 30 s period whether it was predominantly feeding or swimming (the frequent change of location during filter-feeding was thus not recorded, because each only lasted a few seconds; 'swimming' larva were ones that kept swimming for the 30 s period and afterwards always settled at the surface). If it was feeding, then its dominant location (at the surface, filter feeding or on the bottom or sides, scraping biofilms) was recorded. Eight measurements of 'feeding type' and of 'swimming or not' were simultaneously made for each larvae, giving a total of 4 min recording over the 40 min study period.

#### Exposure to stimuli

The experiments were carried out at a laboratory temperature of 24°C, starting with *C. longiareolata*. 120 replicates each consisted of one final instar (4th) larva in an 8 cm diameter polystyrene container containing 200 ml of conditioned tap water.

Each mosquito larva underwent a series of five experiments, with 2 h gaps between the experiments: (a) negative control (no vibrations or chemicals, so larva just in conditioned tap water); (b) exposed to swimming mosquito vibrations; (c) exposed to vibrations from feeding dragonfly nymphs; (d) exposed to kairomones from feeding dragonfly nymphs; (e) simultaneously exposed to both vibrations and kairomones of feeding dragonfly nymphs.

- (a) *Negative control*. In this experiment, each headphone was inactive (so there were no vibrations) and there were no kairomones present.
- (b) *Exposed to vibrations from swimming mosquito larvae* (referred to as 'mosquito vibrations' in the results). The headphones played back the continuous loop of vibrations from swimming larvae.
- (c) *Exposure to dragonfly capture vibrations* (referred to as 'dragonfly vibrations' in the results). The headphones played the continuous loop of dragonfly feeding vibrations.
- (d) *Exposure to dragonfly kairomones*. The day before the experiment, the 200 ml of water containing each dragonfly nymph was diluted by pouring into 4 litres of water. Then after removal of the nymphs, the waters in the eight dragonfly containers were mixed. From this, 100 ml of dragonfly water was put into each of the 200 ml mosquito containers and the larvae were left for 20 min, before the first readings were taken.
- (e) *Exposure to both dragonfly kairomones and vibrations* (referred to as 'both dragonfly stimuli' in the results). The mosquito larvae, already exposed to dragonfly kairomones, were now additionally exposed to the dragonfly feeding vibrations.

These experiments were repeated for *C. perexiguus* and for *C. quinquefasciatus*.

#### Data analysis

The data were tested for normality and being proportions, were given arcsine transformations. Analysis was by a general linear model with nested analysis of variance (ANOVA), since each set of five experiments was for the same larva. Thus the variables tested were for the experiments and for the nesting of larval cohorts. The ANOVA was followed by Tukey's *post-hoc* analysis using SPSS software (SPSS for Windows 10.0.0, 1999) for repeated measures. The data were then back-transformed to percentages for plotting the graphs.

### Results

#### Vibration patterns used in the experiments

The vibration patterns produced by eight swimming mosquito larvae were used as a background noise (three typical examples are shown in [fig. 1a](#)). The vibrations produced by dragonflies when capturing their mosquito larval prey gave a very distinctive pattern, resulting from the shockwave produced by the suddenly extending labium. Three typical examples of the eight recordings used are shown in [fig. 1b](#). To

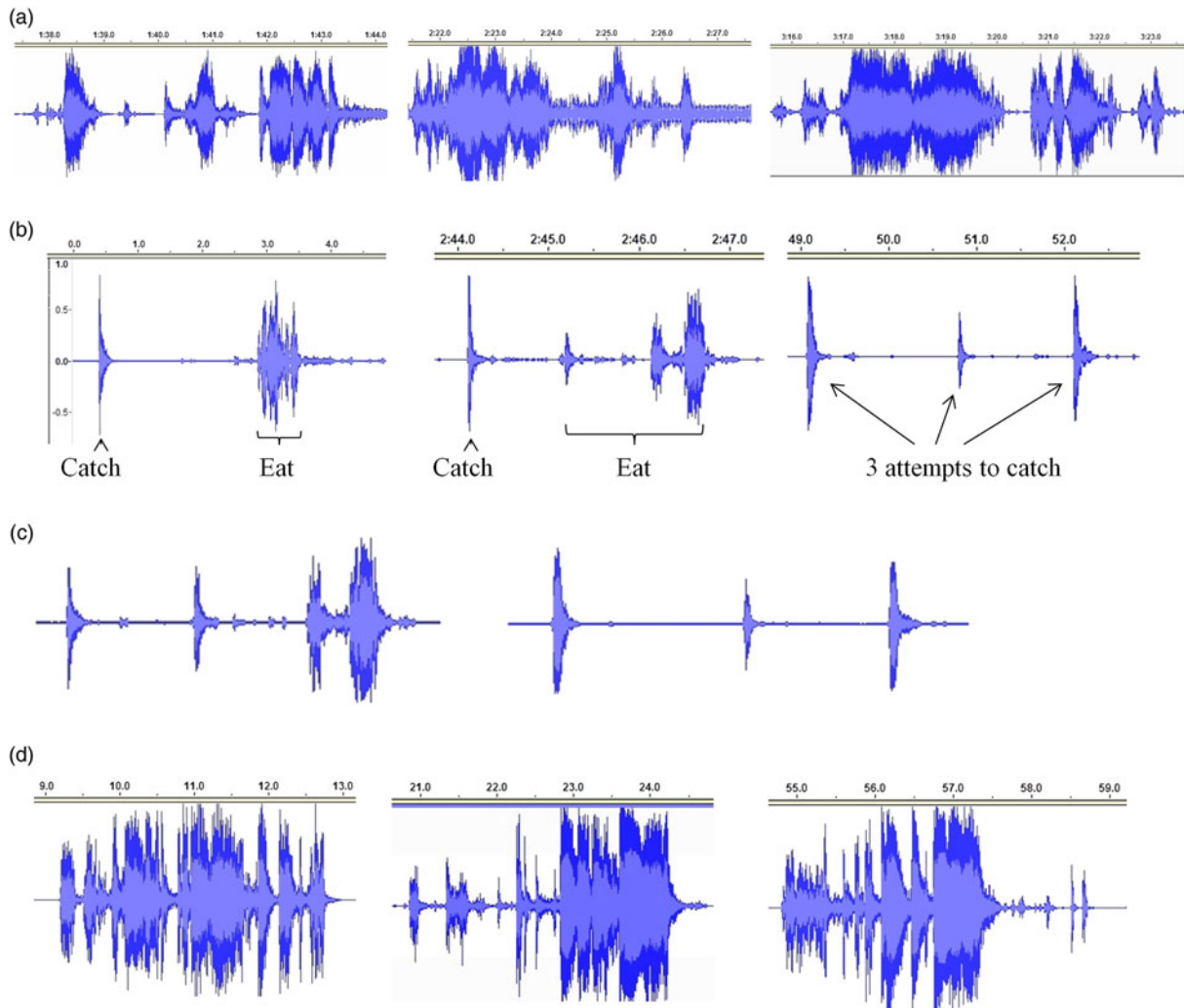


Fig. 1. Vibration patterns produced by: (a) swimming mosquito larvae; (b) dragonfly nymphs catching and eating mosquito larvae; (c) playback of dragonfly vibrations; (d) clicking a light switch.

confirm that these vibrations were being played back correctly during the experiments, the playbacks were rerecorded. Two of the recordings in [fig. 1b](#) are shown on playback in [fig. 1c](#). In contrast to the dragonfly vibration, a clicking sound produced by a light switch gave a very different vibration pattern, although of a similar intensity ([fig. 1d](#)). An initial study comparing 30 individual mosquito larvae exposed first to vibrations from swimming larvae and then to clicks from the light switch showed no significant difference (ANOVA,  $F = 0.18$ ;  $df = 1, 29$ ;  $P = 0.84$ ) between the two. The light switch clicks were thus not used in the main experiment.

#### Results for bottom feeding in three mosquito species

*C. quinquefasciatus* showed no bottom feeding in any of the experiments and so could not be analysed further. A GLM ANOVA of the other two species showed that *C. perexiguus* ([fig. 2b](#)) had a significantly higher level of bottom feeding (ANOVA,  $F = 12.0$ ;  $df = 1, 4$ ;  $P < 0.0001$ ) than *C. longiareolata* ([fig. 2a](#)). Within the five experiments, exposure to the mosquito vibrations in *C. longiareolata* significantly reduced

bottom feeding (Tukey  $P = 0.004$ ) from 49% in the negative control to 42%, but there was a much greater reduction (Tukey  $P < 0.0001$ ) in bottom feeding when exposed to any of the three dragonfly stimuli (all  $< 20\%$ ). These three dragonfly stimuli (prey-capture vibrations, kairomones and both combined) were not significantly different from each other. In *C. perexiguus*, bottom feeding in the negative control at 53% was significantly higher (Tukey  $P < 0.0001$ ) than the other four experiments. Again, the three dragonfly stimuli were not significantly different from each other. Since bottom feeding remained relatively high in all the experiments compared with *C. longiareolata*, with the lowest being 28% bottom feeding, then the effect of mosquito vibration were only significantly different from the effect of dragonfly vibrations ( $P = 0.003$ ), but not the dragonfly kairomones.

#### Results for active swimming in the three mosquito species

*C. quinquefasciatus* showed no active swimming in any of the experiments and so could not be analysed further. A GLM ANOVA of the other two species showed that *C.*

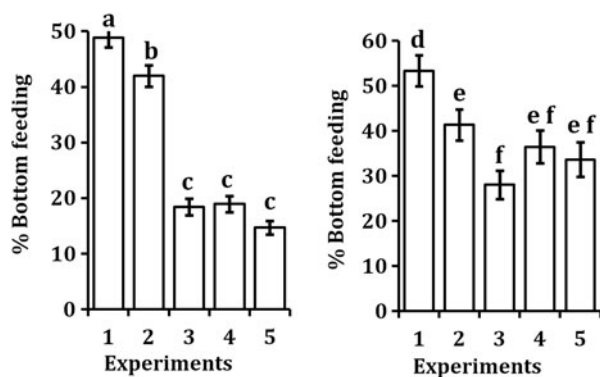


Fig. 2. % Bottom-feeding mosquito larvae (compared with surface filter feeding or actively swimming) for (a) *C. longiareolata* and (b) *C. perexiguus*. Where exp 1 = negative control, exp 2 = positive control (swimming vibrations), exp 3 = predator vibrations, exp 4 = predator kairomones, exp 5 = combined predator vibrations and kairomones. Bars show means  $\pm$  SE. Same letters above the bar = not significantly different (Tukey,  $P > 0.05$ ).

*longiareolata* (fig. 3a) had a significantly higher level of swimming activity in all experiments (ANOVA,  $F = 128.5$ ;  $df = 1,4$ ;  $P < 0.0001$ ) than *C. perexiguus* (fig. 3b). In *C. longiareolata*, exposure to the different predator stimuli significantly increased swimming activity (Tukey  $P < 0.0001$ ) to 10–12% compared with the two controls at 5–6%. Mosquito vibrations did not significantly (Tukey  $P = 0.81$ ) increase swimming activity compared with the negative control.

In *C. perexiguus*, only dragonfly vibrations significantly increased swimming activity compared with the negative control, and even then, swimming activity only rose to 4.1%.

## Discussion

In the area where mosquito egg rafts were being collected (Wadi Qurai), personal observation has shown that there are about 30 suitable pools for mosquito colonisation. Those in the wadi bottom, where there is an intermittent stream, contain *Aphanius dispar* fish and never had any mosquitoes. The rest are rain-filled pools and so only contain water for some weeks after rain. Roughly 60% of these are colonised by dragonfly nymphs (mainly *C. erythraea*) or notonectids, but which pools are colonised is continuously changing. Both dragonfly nymphs and notonectids are known to inhibit mosquito oviposition (Stav *et al.*, 2000; Eitam *et al.*, 2002), so the mosquitoes will avoid or reduce egg-laying when predators are present (Silberbush & Blaustein, 2011). However, if a pool is colonised by a predator after oviposition, then the mosquito larvae need strategies to avoid being eaten. This first requires predator detection, and preferably identification (to determine the degree of risk that the predator poses). Thus the highest predation risk comes from fish, which can eat all mosquito larvae in a  $25 \times 38 \text{ cm}^2$  pool in one day (Bond *et al.*, 2005). Dragonfly nymphs are a lower risk, because they mainly affect bottom-feeding mosquitoes (Roberts, 2012). Thus, identification of the specific predator allows the mosquito a more adapted response. Some mosquito species, such as *C. longiareolata*, can identify different predators by their chemical kairomones in the water (Roberts, 2014a). Being able to use an alternative predator identification mechanism would allow a

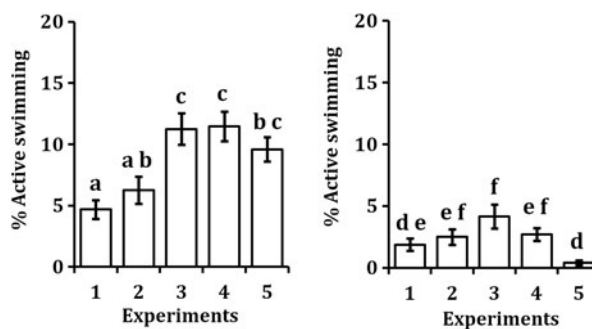


Fig. 3. % mosquito larvae actively swimming (compared with feeding) for (a) *C. longiareolata* and (b) *C. perexiguus*. Where exp 1 = negative control, exp 2 = positive control (swimming vibrations), exp 3 = predator vibrations, exp 4 = predator kairomones, exp 5 = combined predator vibrations and kairomones. Bars show means  $\pm$  SE. Same letters above the bar = not significantly different (Tukey,  $P > 0.05$ ).

more flexible response, for example in determining how close was the predator. Dragonfly nymph prey capture produces a very characteristic vibration pattern, which if detected would allow the mosquito to determine that a feeding predator is nearby and most probably on the bottom of the pool (being a dragonfly nymph).

*C. longiareolata* showed a significant response to prey-capture vibrations from dragonfly nymphs by reducing bottom feeding (and so switching to surface filter-feeding) and increasing prolonged swimming activity, which in nature would allow them to move away from the predator. This strong response was at a similar level to the equally strong effect of predator kairomones. Vibrations from other swimming mosquito larvae also significantly reduced bottom-feeding activity, although it did not increase swimming, but the activity change was only half the response shown from either dragonfly vibrations or kairomones. In this experiment, swimming activity was induced by crowding the mosquito larvae, so that there was constant interference between larvae when feeding. However in nature, swimming activity by many nearby larvae might be the result of a predator attack, so that switching to surface feeding would be a cautionary response to a bottom-living predator. Click vibrations that were equally loud to the dragonfly vibrations did not produce a decrease in bottom feeding that was significantly different from the vibrations of swimming larvae, although on some occasions, the larvae showed a startle response (brief swimming, before returning to bottom feeding). In contrast to the startle response, prolonged swimming always terminated in surface filter feeding. The mosquito larvae thus appeared to be able to specifically identify the prey-capture vibrations produced by dragonfly nymphs, from other vibrations in the water, and responded with a change in behaviour. Other researchers have shown a similar change in larval mosquito-feeding behaviour. Awasthi *et al.* (2015) showed that *Aedes aegypti* L. switched from bottom feeding to surface feeding in the presence of the copepod predator *Megacyclops*. In some species of the *Anopheles gambiae* Giles complex, both Gimonneau *et al.* (2012) and Roux *et al.* (2013) showed that in the presence of predators, the larvae switched from surface filter feeding to inactive resting against vertical surfaces.

Experiments on predator kairomones have shown that mosquito larvae can distinguish between different predators.

Thus, Sih (1986) and Kesavaraju & Juliano (2004) showed that mosquitoes responded to native but not alien predators, although Ferrari *et al.* (2007) showed that larvae could learn to recognise novel predators. Roberts (2014a) showed that *C. longiareolata* responded differently to kairomones from damselfly nymphs, dragonfly nymphs, Nepid nymphs and from fish (*Aphanius*). Similarly, when damselflies and dragonfly nymphs are prey, they can distinguish between different fish species and between larger dragonfly predators (Chivers *et al.*, 1996; Hopper, 2001; Stoks *et al.*, 2003). Thus their behavioural response to each stimulus depends upon the risk posed by that predator. Whether there is the same specificity with predator vibrations, such as the ability to distinguish between dragonfly and damselfly nymphs, has yet to be studied. However, preliminary studies on the response of mosquito larvae to vibrations from other potential predators, such as swimming *A. dispar* fish and tadpoles of *Bufo arabicus* Heyden, produced no significant response in any of the three mosquito species (D. Roberts, personal commun.). Although it is probably more difficult for a mosquito larvae to distinguish predatory fish from herbivorous fish, such as *Garra* species, just using their continuous swimming vibrations, whereas raptorial feeding insect predators produce characteristic sudden vibrations that could be identified.

Some terrestrial herbivores have been shown to identify the vibrations produced by different predators. For example, caterpillars responded specifically to leaf vibrations from walking predators (stink bugs) by dropping off the leaf using a silk thread, but did not respond to vibrations from other walking herbivores, and showed a different response to vibrations from flying predators such as wasps (Castellanos & Barbosa, 2006). Aphids respond to leaf vibrations by predatory coccinellid beetles by dropping off the leaf, but have a much weaker response to three species of predatory bugs (Losey & Denno, 1998). This response to the predator vibrations is greatly enhanced when alarm pheromones are present from other attacked aphids (Roitberg & Myers, 1978). Leaf miners are able to identify the vibrations produced by parasitoid wasps when drilling, and respond by evasive reactions (Djemai *et al.*, 2001). Apart from detection of predators, substrate vibrations are extensively used by insects and other arthropods for communication (Cocroft & Rodríguez, 2005; Hill, 2009), especially during courtship, transmitting information between social insects, and even during maternal behaviour among tree hoppers (Cocroft, 1999). Thus, many insects respond to surface vibrations, even though they have no hearing organs.

In contrast to *C. longiareolata*, *C. perexiguus* showed a very much smaller, although still significant, reduction in bottom feeding when exposed to dragonfly feeding vibrations. However, the bottom feeding was completely different in the two species. *C. longiareolata* actively moved over the bottom scraping biofilms, which would have put it at a high risk of being noticed by bottom-living dragonfly nymphs. *C. perexiguus*, however, lay ventral-side up on the bottom filter feeding. Its very inactive behaviour, coupled with its reluctance to swim, would have put it at a much lower risk of dragonfly detection.

*C. quinquefasciatus* showed no bottom feeding during these experiments, nor did it show any swimming activity, and thus no measurable response to dragonfly predators. Previous studies (Roberts, 2014b) showed some reduction in bottom-feeding in response to dragonfly kairomones, although the response was quickly habituated. In that experiment, the larvae were kept in groups of 30, so that conspecific interactions

would have occurred (if one starts to swim, others are likely to swim) and the group is thus more sensitive to any disturbance, such as the possible presence of a predator, than an isolated individual. *C. quinquefasciatus* is unusual in that it lives in water highly polluted with organic material. It is thus abundant in urban areas breeding in septic tanks (Menon & Rajagopalan, 1980), while in more natural conditions it is found in pools contaminated with animal faeces. This results in very high bacteria levels and thus low dissolved oxygen, so few predators can survive. Probably, anti-predator defences are thus of low importance to it. In contrast, the other two species were frequently found co-existing with especially insect predators, so their predation risk was very high.

In conclusion, both *C. longiareolata* and to a much lesser extent *C. perexiguus* were able to detect and identify vibrations from prey capture in dragonfly nymphs and responded by reducing bottom feeding on biofilms. *C. longiareolata* also showed strong swimming escape reactions. The dragonfly vibration responses were as great as dragonfly kairomone responses and there was no synergistic or additional effect, when the two factors were present together. *C. quinquefasciatus* did not show a response to either predator vibrations or kairomones.

Ideally, naive larvae would have been used in each experiment to ensure that no anti-predator response was carried over from an earlier experiment, but this was not feasible due to the short season in which larvae were available. Instead, a two h period was left between experiments.

### Acknowledgements

I thank Sultan Qaboos University for providing research facilities; Dr Michael Barry for assisting in dragonfly nymph field collection; and Dr Carlo Carboni for advising on the vibration detection and recording.

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