

# Effect of Alarm Chemicals and Predator Kairomones on the Behaviour of Two Species of Mosquito Larvae

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**ABSTRACT:** Mosquito larvae have developed a variety of responses to reduce the risk of predation, but this requires them to be able to identify the different species of predators and respond accordingly. We investigated the behavioural response of two mosquito species to three chemical signals: kairomones from two predators, and also to alarm semiochemicals from killed mosquito larvae. *Culex perexiguus* mosquito larvae are primarily surface filter-feeders. In response to all three chemical signals, they significantly reduced feeding by the high-risk active bottom scraping of biofilms in favour of the less active (and so lower predator-detection risk) surface filter feeding. Active escape swimming (instead of feeding) also increased for all three signals, but was much less for dragonfly nymph kairomones. Dragonflies are almost entirely bottom feeders and so are a much lower danger to surface feeding mosquitoes compared with damselfly nymphs, which feed at all depths. *Culiseta longiareolata* mosquito larvae normally have a high level of bottom-feeding. This was significantly reduced to all three chemical signals, but escape swimming only occurred for dragonfly kairomones (which are natural predators for the bottom-feeding larvae).

**Keywords:** *Culex perexiguus*; *Culiseta longiareolata*; Mosquito larvae; Predator kairomones; Anti-predator defense.

أثر المواد الكيميائية التنبيهية وكيرومونز الكائنات المفترسة على سلوك نوعين من يرقات البعوض

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**المخلص:** لقد طورت يرقات البعوض مجموعة متنوعة من الإستجابات للتقليل من خطورة الإقتراس وذلك بقدرتهم على التعرف على مختلف الأنواع المفترسة والإستجابة وفقاً لكل نوع. في هذه الدراسة تحققنا من الإستجابة السلوكية لنوعين من البعوض لثلاثة مواد كيميائية تنبيهية وإشارات كيميائية وهما نوعان من الكيرومونز من نوعين مفترسين ومن مواد شبيه كيميائية من يرقات بعوض مبيت. إن يرقات بعوض الكيولكسي بيركسيقاص في الأساس هي من الكائنات التي تتغذى عن طريقة الترشيح السطحي. كرد فعل للثلاث إشارات الكيميائية قللت يرقات البعوض إلى درجة عكسية من تغذيتها المعتادة عن طريقة الترشيح السطحي التي هي أقل خطورة عليها من الإقتراس إلى التغذية على البيوفيلم المتواجد في القاع الأسفل حيث تشكل هذه النوعية من التغذية خطر عليها من الإقتراس. وبدلاً من أن تقضي وقتاً أكبر في التغذية زادت اليرقات في حركة سباحتها هروباً من الإشارات الثلاثة الكيميائية ولكن تم ذلك لدرجة أقل في حالة حورية ذبابة التنين (اليعسوب) لمادة الكيرومونز. تتغذى ذبابة التنين (اليعسوب) كلياً على القاع لذا هي أقل خطورة من الإقتراس بالمقارنة بمقترنات الأجنحة التي لا تتغذى على القاع وإنما على الترشيح السطحي. إن يرقات بعوض كليسيبتا لونيقياربولاتا لمادة ما تتغذى على القاع بكثافة وقد إنخفضت تلك بشكل كبير عندما واجت الإشارات الكيميائية، في حين إن حالة الهروب سبحة حدثت لذبابة التنين (اليعسوب) لمادة الكيرومونز وهي في الأصل مادة افتراضية لليرقات التي تتغذى على القاع.

**الكلمات المفتاحية:** الكيولكس بيركسيقاص ، كيلوسيتا لونيقياربولاتا ، يرقات البعوض ، كيرومونز الإقتراس ، الحماية من الإقتراس.

## 1. Introduction

Many mosquito larvae live in very small pools of water where, due to their low activity, they are vulnerable to aquatic predators. However, not all predators are of equal danger. Fish in particular may eliminate all mosquito larvae from an aquatic habitat [1]. The larvae should thus have a phenotypic plasticity, responding differently to different predators depending on the risk that the particular predator poses to them. Thus *Culiseta longiareolata* Macquart strongly responds to fish, dragonfly and damselfly nymphs, but not to nepids [2], which do not feed on mosquito larvae. Similarly as prey, damselfly nymphs [3] and dragonfly nymphs [4, 5] alter their response to specific predators depending on the risk. Closely related mosquito species may show different responses to a specific predator, with no response to predators to which they are not normally exposed [6, 7].



## EFFECT OF ALARM CHEMICALS AND PREDATOR KAIROMONES

This phenotypic plasticity requires being able to identify specific predators. In many aquatic habitats, visibility is poor and the vision of mosquito larvae is very limited, so chemical kairomones given off by predators have many advantages to the mosquito. As the kairomone disperses through the water, it allows the larva to detect a predator that is not visible [8]. Mosquito larvae have been shown to respond differently to different predator kairomones and so are able to identify the predator and tailor their response accordingly [9]. They also respond to alarm semiochemicals from damaged conspecific larvae [10], which indirectly show the presence of predators.

Most mosquito larvae spend much of their time in relative inactivity by filter feeding at the surface, and part of their time in active moving over the bottom where they scrape biofilms. However, bottom-feeding is risky, as the constant movement attracts predators and also makes the larvae accessible to benthic predators such as dragonfly nymphs [11].

In this study, we investigate two different mosquito species having different behavioural responses to predators: a) reducing active bottom-scraping in favour of increasing the less active surface filter-feeding; b) actively swimming to escape away from the predator.

### 2. Materials and Methods

Eggs rafts of the two mosquito species, *Culex perexiguus* Theobald and *Cs. longiareolata*, were collected from temporary rock pools in Wadi Qurai near Sumail about 60km from the University in northern Oman. After hatching, the larvae were fed daily with yeast powder. The two predators, dragonfly *Crocothemis erythraea* Brullé and damselfly *Ischnura evansi* Morton nymphs, were collected from small fish-free pools in Wadi Al-Khod about 5 km from the University and then kept separately in small jars. Each was fed daily on larvae of the mosquito species being tested. Mosquitoes and predators were kept in a lab temperature of 23 °C.

During the experiment, the predators were individually kept in 4 L water containers. Fourth (final) instar mosquito larvae (initially *Cx. perexiguus*, later *Cs. longiareolata*) were individually kept in 16 polystyrene jars. Each 8 cm diameter jar contained 400 ml of distilled water. These jars each had water pumped into them using 16 peristaltic pumps (WPX1 from Welco Ltd, Tokyo, Japan) at a calibrated flow of 17 ml/h from the four 4 L containers (so each container connected to four mosquito jars) (Figure 1).



**Figure 1.** Experimental set up showing: peristaltic pumps at the top; mosquito jars in middle row; predator containers alternating with waste jars on bottom row.

There was thus no visual contact between predator and mosquito, but the mosquitoes were receiving water containing either dragonfly or damselfly kairomones. The mosquito jars then overflowed into waste jars.

Exp. 1: Control. The experiments started on day 1 with all 16 mosquito jars having distilled water pumped into them as a control. After leaving for 30 min to allow the larvae to settle, ten records were made at 10 minute intervals on the behaviour of each larva. Over a 5 sec period, it was recorded whether the larva was feeding or actively swimming, and whether the feeding was surface inactive filter feeding or active feeding on the bottom by scraping of biofilms.

Exp. 2: Mosquito alarm chemicals. After completing the 1<sup>st</sup> experiment, each of the water containers had added to it four finely chopped up mosquito larvae releasing alarm semiochemicals: the water containing these chemicals was then pumped into the 16 jars of mosquito larvae. After a 5 minute interval to allow mixing of the water, 10 recordings at 10 minute intervals were made as previously. At the end of the experiment, the mosquito jars were flushed with distilled water overnight and the mosquito larvae were fed.

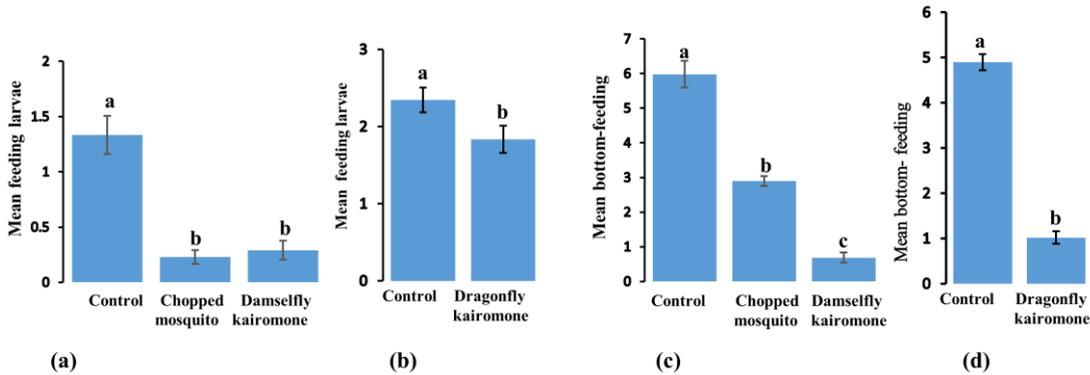
Exp. 3: Predator kairomones. Dragonfly nymphs were kept unfed in each of the four 4 L containers overnight to allow a build-up of predator kairomones. The following morning, this water was pumped into the jars containing the same mosquito larvae as previously. The larvae were allowed to settle for 30 min, then activity and feeding type recordings were made as previously.

These experiments were repeated for a total of three replicates using fresh mosquito larvae and fresh predators in each replicate, so that the responses of a total of 48 larvae were recorded. The experiments were then repeated using

damselfly nymphs to replace the dragonfly nymphs. They were then further repeated using *Cs. longiareolata* mosquito larvae, to see the effect of both dragonfly and damselfly kairomones, as well as the effect of alarm semiochemicals.

The data were arcsine transformed and then analyzed using a univariate analysis of variance (ANOVA) with SPSS (ver 21, SPSS, Chicago, Ill, USA). Significant differences were separated by Tukey's HSD (honestly significant difference) test. The graphs used back transformations of the data to calculate mean number  $\pm$  SE of bottom feeding and of active swimming larvae.

### 3. Results

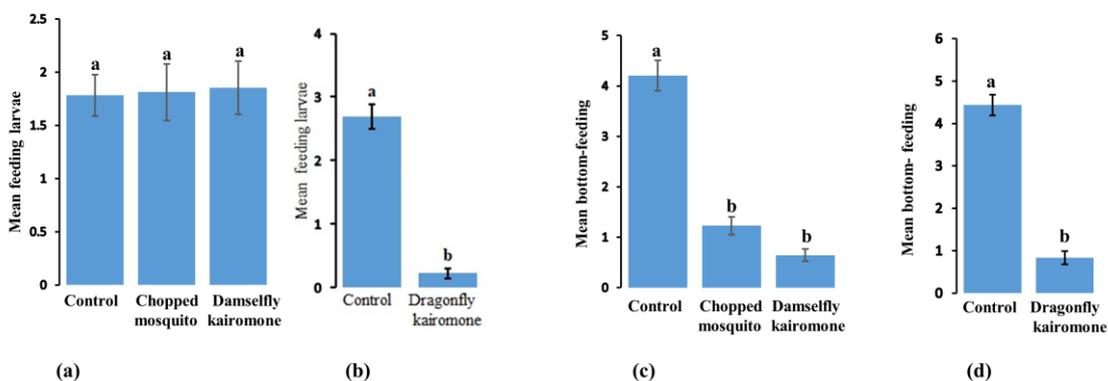


**Figure 2 (a,b).** Mean number ( $\pm$  SE) of feeding (non-swimming) *Cx. perexiguus* larvae for controls; exposed to alarm chemicals; damselfly kairomones and dragonfly kairomones.

**Figure 2 (c,d).** Mean number ( $\pm$  SE) of bottom-feeding *Cx. perexiguus* larvae for controls; exposed to alarm chemicals; damselfly kairomones and dragonfly kairomones. Letters show Tukey differences in the ANOVA.

*Cx. perexiguus* larvae (Figure 2) significantly (ANOVA,  $F = 28.4$ ;  $df = 2$ ;  $p < 0.0001$ ) increased their swimming escape activity in response to alarm semiochemicals from chopped up mosquito larvae and to kairomones from predatory damselfly nymphs (with Tukey showing no difference between the two responses). They also showed a smaller increase in escape swimming in response to dragonfly nymphs ( $F = 4.9$ ;  $df = 2$ ;  $p = 0.5$ ). Surface filter-feeding (at the expense of reduced bottom feeding) significantly increased in response to both alarm semiochemicals ( $F = 83.1$ ;  $df = 2$ ;  $p < 0.0001$ ) and damselfly kairomones (with a Tukey test showing that the response was much greater to damselfly kairomones), as well as to dragonfly kairomones ( $F = 147.9$ ;  $df = 2$ ;  $p < 0.0001$ ).

*Cs. longiareolata* larvae (Figure 3) showed no response in swimming activity to either chopped mosquito alarm semiochemicals or to damselfly kairomones ( $F = 0.05$ ;  $df = 2$ ;  $p = 0.95$ ),



**Figure 3 (a,b).** Mean number ( $\pm$  SE) of feeding (non-swimming) *Cs. longiareolata* larvae for controls; exposed to alarm chemicals; damselfly kairomones and dragonfly kairomones. **Figure 3 (c,d).** Mean number ( $\pm$  SE) of bottom-feeding *Cs. longiareolata* larvae for controls; exposed to alarm chemicals; damselfly kairomones and dragonfly kairomones.

Letters show Tukey differences in the ANOVA, but did increase swimming escape responses to dragonfly nymph kairomones ( $F = 48.3$ ;  $df = 2$ ;  $p < 0.0001$ ). Bottom-feeding was significantly reduced by the presence of both alarm semiochemicals ( $F = 70.6$ ;  $df = 2$ ;  $p < 0.0001$ ) and damselfly kairomones (the frequency of responses to these two stimuli were not significantly different from each other) as well as to dragonfly kairomones ( $F = 52.7$ ;  $df = 2$ ;  $p < 0.0001$ ).

#### 4. Discussion

Mosquitoes have developed a number of strategies to reduce their predation risk, since the larvae may be trapped in small pools of water with no possibility of escape. At the time of oviposition, the adult females carefully test the available water bodies for chemical kairomones indicating the presence of predators and then avoid those where the kairomones are present [12, 13, 14]. However, their response may depend on the risk posed by a particular predator, and so they may still oviposit where less dangerous predators are present [15].

This does not solve the problem of predators arriving after oviposition. The larvae thus have various possible strategies to reduce their risk. A common response in all aquatic prey is to reduce activity, since most predators are better at seeing movement rather than detail [16, 17]. However, inactivity will affect the efficiency of feeding resulting in a trait compensation, in which the rate of larval development is slowed down. Thus *Culex sinaiticus* has been found to prolong its larval development by reducing feeding in the presence of dragonfly nymphs, but not to respond to damselfly nymphs [11]. Similar results were found in *Anopheles quadrimaculatus* [18] and *Culex pipiens* [19]. This strategy, however, extends the period in which the larva is exposed to the predator. A few species such as *Cx. perexiguus* shorten the larval development in the presence of predators, but this results in smaller and so most probably less competitive adults [20].

Less drastic behavioural changes available to the larva include escape swimming, in which the larva spends several minutes swimming to a new location away from the predator, and in reducing bottom-feeding. Many mosquito species feed both at the surface by filter-feeding on floating microorganisms and on the bottom by scraping biofilms covering surfaces. Their choice depends on food availability, but also the preferred feeding method varies between species. However, bottom-feeding is more risky, because the high level of activity attracts the attention of predators, especially dragonfly nymphs sitting on the bottom. The reduction of bottom-feeding in the presence of predators has been shown to occur in a number of studies [21, 22, 23], and has been recorded for *Cs. longiareolata* [11]. This behavior was found for both species in the present study. Similarly, both species reduced bottom-feeding in response to alarm semiochemicals from chopped up larvae, but in *Cx. perexiguus*, this was at a significantly lower level compared with the response to predator kairomones. Alarm chemicals from damaged conspecific larvae are known from other studies to enhance antipredator defenses [21, 24]. Stopping feeding and actively swimming away from a predator source was shown by *Cx. perexiguus* to all three alarm signals, but to a much smaller extent to dragonfly kairomones, possibly because dragonflies sit on the bottom and so can be avoided by surface feeding. In contrast, *Cs. longiareolata* (a major bottom-feeder) only swam in response to dragonfly kairomones, and so is more selective in its response.

#### 5. Conclusion

Both *Cs. longiareolata* and *Cx. perexiguus* mosquito larvae reduced the more risky bottom-feeding in preference for an increase in surface filter-feeding (which is less visible to a predator) in response to kairomones from both dragonfly and damselfly nymphs, as well as to alarm pheromones from damaged conspecific larvae. However, in *Cx. perexiguus*, this response was significantly greater when to either of the predator kairomones than to the alarm pheromones, but in *Cs. longiareolata*, there was no significant difference between them.

Actively swimming to escape from predator kairomones or alarm pheromones was shown by *Cx. perexiguus*, but *Cs. longiareolata* only swam in response to dragonfly kairomones, and not to the other two factors.

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