# Aspects of nutrition and oviposition in the endemic rockpool mosquito *Opifex fuscus* Hutton (Diptera: Culicidae)

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## ABSTRACT

*Opifex fuscus* is a New Zealand endemic coastal species of mosquito. It has primitive taxonomic characteristics making it an interesting species from both ecological and evolutionary perspectives, although there is limited information available on its biology. This preliminary investigation examines evidence for a link between autogenous oviposition and adult nutrition, with some observations on longevity. Our data indicate that only sugar-fed adult females are capable of laying their first batch of eggs autogenously. This species was also observed to exhibit skip oviposition in the laboratory. Females fed sugar and laying eggs survived markedly longer than both females fed sugar and not laying eggs, and females not fed sugar.

## KEYWORDS

Longevity, survival, nutrition, autogeny, oviposition

## INTRODUCTION

*Opifex fuscus* Hutton (Diptera: Culicidae) is a New Zealand endemic coastal mosquito species, which inhabits saline/brackish rock pools above the high tide mark (Belkin 1968). This species is widespread along the coasts of both the North and South Islands (Nye 1962), but is no longer present in the southeast of the South Island around the Otago Peninsula (R. Cane, unpubl. data. 2007), where it has possibly been displaced by the established Australian mosquito, *Aedes australis* (McGregor 1965). *Opifex fuscus* is never found away from its coastal rocky habitats (Miller & Phillips 1952).

The genus *Opifex* is unique to New Zealand and possesses primitive taxonomic characteristics of the tribe Aedini (Belkin 1968). This genus was monotypic until recently when Reinert *et al.* (2004) reassigned *Aedes chathamicus* as *Opifex chathamicus*, although this change and many others suggested by these authors are not yet widely accepted.

*Opifex fuscus* exhibits unusual mating behaviour, which occurs before, during or soon after female emergence from the pupal case (Kirk 1923; McGregor 1965; Marks 1958; Slooten & Lambert 1983). The males have a pair of large tarsal claws on each of the front legs which they use to grip the female pupae (Marks 1958; Slooten & Lambert 1983). There is vigorous competition for mates, and several males may set upon a single female at once (Marks 1958; Slooten & Lambert 1983). This behaviour is useful in laboratory studies, as it is relatively easy to get the mosquitoes to mate.

This species is reported to overwinter as larvae (Belkin 1968), but will pupate and emerge during periods of fine warm weather in milder winters (Kirk 1923; Belkin, 1968). The larvae are large relative to other New Zealand mosquito species as they have the ability to store fat reserves (Haeger & Provost 1964). They generally eat more as a larva than other mosquito species, developing more slowly over a longer period (Haeger & Provost 1964). Colonies of *Op. fuscus* are easily established in the laboratory (Haeger & Provost 1964; 1965), as larvae collected in the field during cooler winter weather are able to be reared to adults at a suitable temperature.

*Opifex fuscus* females exhibit autogeny, i.e. during the first gonotrophic cycle their ovaries can develop eggs in the absence of blood feeding (Clements 2000). This is a useful trait for rearing in the laboratory as blood is not required for the production of viable eggs. Haeger and Provost (1964) reported that their laboratory colony of *Op. fuscus* adult females originally sourced from the Wellington region did not require sugar or blood meals to complete the first gonotrophic cycle. Females of this species will take blood meals, but only after first depositing an autogenous egg batch (Haeger & Provost 1964; 1965). Telang and Wells (2004) found in the autogenous species *Aedes atropalpus,* that 100% of females produced their first batch autogenously regardless of whether they were fed water or sugar, post emergence. They cannot develop a second egg batch without then taking a blood feed (Haeger & Provost 1964). *Op. fuscus* females are reputed to be aggressive with a painful 'bite' (Miller & Phillips 1952) and have been recorded biting during the day in summer (Watt 1978) and early autumn (M. Disbury, pers.comm. 2007).

We report on a laboratory investigation into the

influence of adult nutrition on autogenous egg production with observations on the longevity of *Opifex fuscus* females. This also provided an opportunity to observe oviposition habits. Skip oviposition is a behaviour observed in adult female mosquitoes whereby they do not oviposit their entire batch of eggs at one time in one location, but oviposit a small number of eggs at a number of different sites over a period of several days (Mogi & Mokry 1980). Mogi & Mokry (1980) suggested that this behaviour may be common in container breeding species which lay their eggs singly (such as *Op. fuscus*). This phenomenon has been reported for species laying autogenous eggs, but not for *Op. fuscus*.

## MATERIALS AND METHODS

Three separate experiments (E1-E3) were set up using field collected larvae reared in laboratory conditions.

### *Field collections*

*Opifex fuscus* larvae were collected from one of two geographic locations. The first collection of 3rd and 4th instar larvae was made from coastal rock pools at Moa Point, Wellington during July 2006. No early instars or adult mosquitoes were observed in the field. The second and third collections were made from rock pools at Hicks Bay, East Cape in August 2006 (3rd- 4th instars) and January 2007 (2nd- 4th instars). Female *Op. fuscus* were actively biting during the summer field collection, but were not during either winter collection.

A survey of coastal vegetation was undertaken at the Moa Point site during February 2007 to identify potential sources of sap and nectar in the immediate vicinity of the *Opifex* breeding habitat (within 20m of the rock pools). Identifications were based on Crowe (1997).

### *Laboratory rearing*

All larvae were placed in a large plastic container covered with fine mesh in preparation for rearing to the adult stage. The laboratory had a natural light/dark cycle and an average room temperature of 18°C for July and August and 20-24°C for January. The larvae were fed daily with a small pinch of ground lucerne-based rabbit pellets (E1 only), fish food flakes (VitaPet Goldfish Flake Plus, Premium Mix, VitaPet Pty Ltd, NSW, Australia) and dog food pellets (E1, E2, E3). All three food types were used initially in E1 as larval food, however it was found that the rabbit pellets tended to cause a surface scum which made the rearing difficult to manage. Fish food and ground dog food pellets only was used for E2 and E3.

The rearing container was supplemented with additional habitat water (collected from the Wellington

rock pools) when required. Pupal development and adult emergence was monitored daily.

### *Adult emergence*

The rearing container was checked daily for newly emerged adults. In view of the species' mating behaviour, females were assumed to be mated on emergence and were removed from the container using an aspirator. When large numbers of males accumulated, a proportion was removed to prevent overcrowding. Overcrowded males often drowned the females as pupae or newly emerged adults during their competitive aggressive mating behaviour.

Each female was placed in a styrofoam cup labelled with a unique number and date of emergence. The containers had been previously ¼ filled with seawater of 16-18 ppt and lined with a folded piece of paper towel as the oviposition substrate. The bottom edge of the paper towel was positioned below the water surface, so the entire piece remained moist. Each cup was covered with a piece of 0.5 mm mesh to retain the mosquitoes while allowing them to remain visible. The seawater in each cup was topped up as required.

### *Experiment parameters*

E1 – A single cotton dental wick soaked in a 15% sugar solution was inserted through the mesh top of each oviposition cup. Observations were only carried out during weekdays. The rearing container was also provided with sugar soaked dental wicks.

E2 - Oviposition cups were provided with either sugar solution soaked wicks or nothing. The rearing container was not supplied with sugar, but was checked daily (including weekends). Some males needed to be removed from the rearing container for a short time to feed, so they remained healthy.

E3 - Same as for E2, except the oviposition cups were provided with either sugar solution or water soaked dental wicks.

All cotton wicks soaked in either 15% sugar solution or water were refreshed daily (weekdays only for E1). The females were checked each morning between 8 and 10am and each afternoon between 2 and 4pm for the presence of eggs (except during weekends of the 1st experiment). Each experiment continued until all mosquitoes collected had been reared through to the adult stage and all females collected had died. As females died, each was frozen in a labelled Petri dish.

### *Egg collection*

Once a female had oviposited, she was aspirated out of the cup and the paper towel (with the eggs) was removed, blotted to remove excess water and sealed in a

**Table 1.** Oviposition by fed and non-fed *Opifex fuscus*

| | sugar-fed females | | non-fed females | |
|---|---|---|---|---|
| Expt. | No. tested | No. (%) that oviposited | No. tested | No. (%) that oviposited |
| E1 | 42 | 19 (45%) | - | - |
| E2 | 34 | 22 (64%) | 30 | 0 |
| E3 | 131 | 25 (19%) | 130 | 0 |
| **Total** | 206 | 66 (32%) | 160 | 0 |

labelled, plastic zip-lock bag. A new piece of towel was placed in the cup and the female reintroduced. The date of oviposition and the number of eggs were recorded for that female and notes made on the condition of the eggs.

### Data analysis

The longevity data obtained from experiments 2 and 3 were subjected to two independent samples t-tests. The first was a comparison of females that laid eggs with those that didn't lay eggs (sugar-fed females only). The second was a comparison of the nutrition treatments for only those females that didn't lay eggs. Experiment 1 data were not included in these analyses as a proportion of the data were affected by the lack of monitoring during weekends.

## RESULTS

### Oviposition

In each of the three experiments, autogenous eggs were laid only by females which had been provided with the 15% sugar solution after emergence (Table 1). This was consistent for females from both Wellington (experiment 1) and Hick's Bay (experiments 2 & 3).

All eggs produced were from the first (autogenous) egg batch, as no blood meals were provided. The proportion of females not ovipositing may have been related to factors not considered here.

Of 66 ovipositing females, 30% (20) laid more than 50 eggs. The largest total egg count from an individual female was 100, with 98 of those deposited during a single 24-hour period. The mean number of eggs laid was 30 ± 32 (mean ± standard deviation) (range 1-100), 27 ± 21 (range 2-68) and 19 ± 17 (range 1-57) for experiments E1, E2 and E3 respectively.

The average period of time from emergence to start of oviposition was 11.7 and 13.5 days for experiments 2 and 3 respectively. The minimum number of days was seven, while the maximum was 31 after which a single egg was laid. Females were observed to lay most of their eggs during the morning.

A minimum period of 24 hours between oviposition events was defined as skip oviposition (or successive laying events), which appeared to occur in all three experiments. A total of 38 females laid eggs on more than one occasion, with three individuals ovipositing 6, 7 and 8 times respectively (Table 2).

The length of time between successive laying events varied considerably between females, ranging from 1-8

**Table 2.** Number of laying events for individual females (skip oviposition).

| Number of laying events | Expt 1 n = | Expt 2 n = | Expt 3 n = | Total |
|---|---|---|---|---|
| 1 | 9 | 5 | 14 | 28 |
| 2 | 4 | 9 | 6 | 19 |
| 3 | 2 | 1 | 2 | 5 |
| 4 | 2 | 3 | 3 | 8 |
| 5 | - | 3 | - | 3 |
| 6 | 1 | - | - | 1 |
| 7 | 1 | - | - | 1 |
| 8 | - | 1 | - | 1 |
| **Total** | **19** | **22** | **25** | **66** |

**Table 3. Average longevity of females in experiments 2 and 3.**

| Experiment | Nutrition | n | Mean life span of ovipositing females (days) | n | Mean life span of non-ovipositing females (days) | LSD 5% | Total n |
|---|---|---|---|---|---|---|---|
| **2** | 15% sugar solution | 21* | 36.0 | 12 | 18.2 | **8.8** | 33* |
| | nothing | - | - | 28* | 10.2 | | 28* |
| | | | **LSD 5%** | | **7.8** | | |
| **3** | 15% sugar solution | 24* | 33.5 | 95* | 27.9 | **6.2** | 119* |
| | water | - | - | 124* | 6.6 | | 124* |
| | | | **LSD 5%** | | **2.7** | | |

**\*Reduced number of females as some escaped and therefore weren't included. LSD – least significant difference**

days. The majority of females laid eggs only once, or on two successive occasions.

### Longevity

In experiment 2, the sugar-fed females which laid eggs lived significantly longer than those which did not lay eggs ($p<0.001$) (Table 3). In both experiments, non-egg laying females lived significantly longer when sugar-fed than when given either nothing or water ($p<0.05$ and $p<0.001$ respectively).

### Botanical Survey

Flowering plants as potential sources of sap and nectar adjacent to *Op. fuscus* breeding sites at Moa Point were identified as: Pohuehue/wire vine, *Muehlenbeckia complexa*; Taupata, *Coprosma repens*; Yellow bush/Tree lupine, *Lupinus arboreus*; Shore groundsel, *Senecio lautus*; European marram grass, *Ammophila arenaria*; Hares tail, *Lagurus ovatus*; Knobby club-rush, *Ficinia* (=*Isolepsis) nodosa*; American sea rocket, *Cakile edentula*; Kokihi/beach spinach, *Tetragonia trigyna*; Iceplant, *Carpobrotus edulis*; Horokaka/native iceplant, *Disphyma australe*; Pinatoro/native daphne, *Pimelea prostrata*; Wharariki/mountain flax, *Phormium cookianum*. These species comprise a mix of low growing, salt and wind tolerant shrubs, herbs and grasses typical of coastal habitat around Wellington area.

## DISCUSSION

Our results indicate that only *Op. fuscus* females that are fed sugar as adults are capable of laying their first batch of autogenous eggs. This is contrary to the findings of Haeger and Provost (1964) who reported that their laboratory colony of *Op. fuscus* adult females, originally sourced from the Wellington region, emerged from the larval stage with large energy reserves, and did not require sugar or blood meals to complete the first gonotrophic cycle. This also differs from the results of Telang and Wells (2004) for *Aedes atropalpus*, which were observed to oviposit regardless of provision of adult nutrition or water. One possible explanation is that the larvae in our experiment did not have sufficient energy reserves for oviposition without post-emergence sugar feed supplementation. The amount of food available to the larvae prior to collection is not known, but once in the laboratory the daily supplements were at least equal to those reported by Haeger and Provost (1964). It would now be of interest to compare energy reserves in emerging adults with different levels of larval nutrition provided, and with obligate blood feeding species.

A range of flowering plants, mostly native to New Zealand, were identified in close proximity (c. 20 m) to *Op. fuscus* breeding habitat. These could provide a source of sugars from sap, and periodically nectar, for adult mosquitoes living in an otherwise harsh coastal environment.

The average length of time until first oviposition was longer in our experiments than that of Haeger & Provost (1964; 1965) who recorded oviposition beginning on the sixth day after emergence in constant room temperatures of 25°C and 20°C respectively. Mosquito life cycles are well known to increase with temperature in both laboratory and field environments; our lower laboratory temperatures explain the longer time period before oviposition was observed.

Some of the *Op. fuscus* females exhibited skip oviposition in the laboratory. This behaviour, while possibly ensuring a greater distribution of progeny from an individual female and reduced sibling competition,

uses greater maternal energy reserves and may increase the risk of adult female mortality (Harrington & Edman 2001). This would also have ecological implications in terms of spreading the risk of egg-laying in temporary or tidal pools. A molecular approach using individual markers could potentially elucidate this in the field.

The natural vertebrate hosts of this species before human settlement of New Zealand were probably marine mammals and perhaps also seabirds. Autogeny combined with skip oviposition, may be of significance in that marine mammals, once extremely abundant around much of the coastline, are on land and thus available as hosts, sporadically. The wide variation observed in the length of time between successive oviposition events may be related to this but additional data is required to enable any significant conclusions to be made. Given their readiness to bite humans, we may be a substitute host.

The increased longevity of the female mosquitoes that were provided with sugar relative to those without, indicates that access to a source of sugar may be a key factor in determining the size of each new generation of *Op. fuscus*. It might also be concluded from these data that any mated female which fails to find a food source will not survive long and will be unable to deposit any eggs. It would be of interest in this regard to examine the minimum duration and frequency needed for food availability to enable skip oviposition to occur in the laboratory. The variation in the longevity of the sugar-fed females which did not lay eggs might be associated with larval nutrition, but requires further investigation.

## ACKNOWLEDGMENTS

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