DOES POLARIZED LIGHT GUIDE CHIRONOMIDS TO NAVIGATE TOWARD WATER SURFACES?

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With 4 figures

ABSTRACT: The effect of container colour, phytoplankton and soluble organic matter on female's choice was investigated in field experiments. Sixty-litre barrels filled with water were exposed to natural populations of mosquitoes and non-biting midges, and the numbers of laid egg-masses/day were calculated. Treatment applied included Black, Green, White and shiny silver. Significant differences in number of egg masses per treatment were found. The preferred choice for oviposition by non-biting midges (chironomids) was Black > Green > White > Shiny Silver. To further confirm that polarized light can be an important factor for females in selecting habitat, we used light traps with polarized and unpolarized light. Traps with polarized light attracted significantly more females than unpolarized traps. Organic matter did not change the choice by females, (in contrast to Culicidae females) but blooming phytoplankton did so. Phytoplankton intensifies the light reflection and polarization of the water surface. The results indicate that chironomid females recognize different degrees of polarized light reflected from the water surfaces and select the water surface which reflects the most intensive polarized light to lay their egg masses.

Keywords: Chironomidae, egg-laying, vision, oviposition site, polarized light

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RESUMO: Foi investigado o efeito da cor do recipiente, fitoplâncton e matéria orgânica solúvel na escolha da fêmea em experiências de campo. Um barril de sessenta litros de água foi exposto a populações naturais de mosquitos e mosquitos não-picadores e foram calculados os números de ovos depositados por dia. As cores dos tratamentos aplicados incluíram o preto, o verde, o branco e a prata brilhante. Foram encontradas diferenças significativas no número de massa de ovos por tratamento. A escolha preferida para a oviposição dos mosquitos não-picadores (quironomideos) foi a cor preta>verde> branco> prata brilhante. Adicionalmente para confirmar a importância de luz polarizada na selecção do habitat pela fêmea, usamos armadilhas de com e sem luz polarizada. As armadilhas com luz polarizada atraíram significativamente mais fêmeas que as armadilhas de luz não polarizada. A matéria orgânica não afectou a escolha das fêmeas de quironemideos em contraste com as fêmeas de Culicidae, mas o fitoplâncton florescente sim, uma vez que a densidade de fitoplâncton afecta a reflexão da luz e a polarização à superfície de água. Os resultados indicam que as fêmeas de quironemideos reconhecem diferentes graus de luz polarizada reflectidos pelas superfícies de água e seleccionam superfícies de água que reflictam a luz polarizada mais intensa de forma a fazerem a oviposição.

INTRODUCTION

Non-biting midges (Chironomidae) spend most of their lifetime in an aquatic environment. Adults emerge from their pupae in the water, usually at sunset and only for a short time**,** in order to mate and oviposit. Females return to the water only once (in most of the species) to lay eggs. Gelatinous egg masses are attached to objects at water level (PINDER, 1980; ARMITAGE *et al*., 1995). The objective of the present research was to verify whether and how females select the breeding habitat.

Use of yellow sticky traps and direct observations revealed quite a high population of adults swarming above the ramps between adjacent ponds in Tiv'on Waste Stabilization Ponds (WSP). The various ponds include primary, secondary and polished sewage ponds, as well as rehabilitated water ponds, adjacent to each other. Sampling of egg masses in ponds were correlated with water quality (BOD, COD, Detergent, etc.). Strong negative correlation ($r = -0.33$, $P < 0.001$) between BOD, COD, detergents and eggs laid was found (GAHANAMA & BROZA unpublished). It is well known (BENTLEY & DAY, 1989; SPENSER, BLAUSTEIN & COHEN, 2001) that female mosquitoes select the egg-laying habitat by sensing the water and responding to its chemical and biochemical nature. Accordingly, we may ask what is the case with female chironomids? After swarming and mating near the water bodies, do they deliberately select their breeding habitat, or randomly return to the nearest water body? If they do select the habitat, what is the exact mechanism, an olfactory or a visual stimulus?

MATERIALS AND METHODS

Sixty-litre plastic barrels were used for choice experiment at two locations: the edge of a spring, and near WSP. Twelve to 24 barrels were used for each observation, and the following treatments were conducted (each replicated X4); black barrels (B) with tap water only, black barrels with decaying plant stems (cane) or only extract of cane stem as well as green (G), white (W) and shiny silvered (S) barrels. In later stages water from the oxidation pond was used (the shiny silver colour was obtained by covering the bottom and walls of the barrels with a shiny plastic with metal spattering). The barrels with water were exposed to flying adult chironomids and mosquitoes. Egg masses were counted after 24 and 48 h.

Experiment I, Elroy Spring. Located on the southern edge of Lower Galilee. The site included the spring outlet**,** which flows into a shallow pool (500m^2). Sixteen barrels were arranged in four rows near the water body**.** The barrels were alternately organized, 1 metre apart**,** in four treatments (in four replicates) as follows: **Bc,** Black barrels – water with fermented cane stem; **Bex,** black barrels – water with cane extract; **B,** black barrel with water only; **G,** green barrels with water only. Egg masses were recorded 30 times during the summer of 2005.

Experiment II, Waste Stabilization Pond, Tiv'on (=TWSP). Fifteen barrels placed in a row, 1.5 m. apart. Three treatments (in five replicates); **B,** black barrels with water only; **G,** green barrels; **W,** white barrels. **Experiment IIa**, was carried out early in the season (2006) during the build-up of adult population (low population) and, **IIb**, at the time of the fully active population. Egg-masses were recorded on five consecutive days.

Experiment III, TWSP. Twenty-four barrels arranged in two rows, one row on the top of the pond dam ("high row"), the other 1 m below ("low row"). Three types of barrels (in four replicates), **B,** black; **G,** green; and **S,** silver, were arranged alternately (1 m apart) in each row. All the barrels contained a mix of oxidation pond water loaded with phytoplankton in tap water, (1:3). **Exp. IIIa:** the first two days; diluted phytoplankton. **Exp. IIIb:** day 11, during phytoplankton blooming. The phytoplankton in the barrels consisted of unicellular green algae *Euglena* sp., and diatoms, *Navicula* sp. Many diatoms were attached to the inner surface of the barrels. Phytoplankton density was recorded, using a haemocytometer under the light microscope.

Experiment IV, TWSP, light trapping. Four Mini light traps, a product of "Nature Discovery", USA (10X15 cm trapping chamber, 32mm diameter of top opening, 2.5-V incandescent lamp) were adapted for use as polarized (diffuser covered with polarized filter) and non-polarized (polarized filter covered by diffuser) traps, respectively (see Fig. 2.). Light intensity was equal in all traps. Traps were laid, on their side, above

Styrofoam board floating near water edge. In each observation day, traps were opened for 5 consecutive periods, 10 min. each. At the end of each 10 min. period midges were counted and removed.

Statistical analysis. One-way ANOVA was used for Experiments: 1, 2 and 3A, while two-way ANOVA was used for experiment 4. For experiment 3B we used non-independent T-test.

RESULTS

Experiment I: Mosquitoes made a clear choice: higher mean numbers (76.9, 14.6 and 1.25) of rafts per barrel were collected from the three "black" treatments: Bc., Bex and B, respectively (fig 1; P< 0.05 for all groups). All black barrels differed significantly from G (0.5).

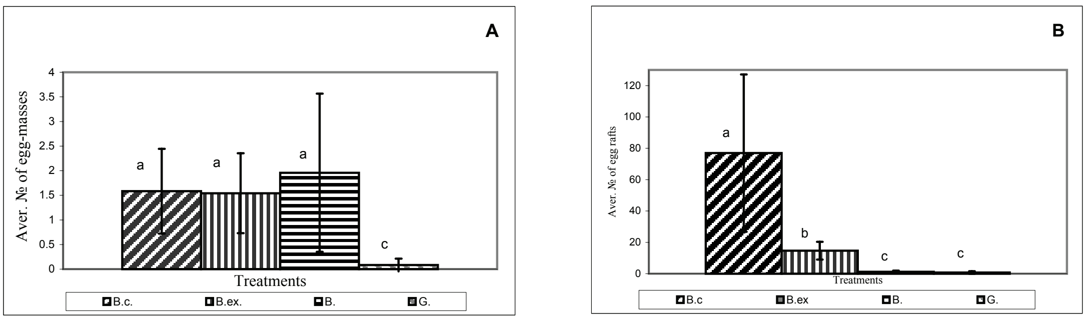

Fig. 1. Mean number of chironomid egg-masses (A) and culicid rafts (B), Elroi Spring. Bars with same letter are not significantly different. A P<0.001. B P< 0.05. (B) Black barrel/ tap water. (Bc) black cane plant added. Black (Bex) extract of cane added. Green (G) green barrels with tap water.

The low number of Chironomid egg masses indicated that total chironomid population at this place was quite low but results indicate that females responded equally to all three black choices (B.c. 1.58; B.ex. 1.54; B. 1.96 (P>0.05)), but avoided the green barrels (G; 0.2). For all black and green barrels (P< 0.05). Female mosquitoes responded both to the chemical nature of water and the barrel colour. Non-biting midges responded to the visual stimulus only in present conditions.

Experiment II. The next experiments were carried out in Tiv'on Waste Stabilization Ponds (WSP), a habitat with high population levels of Chironomidae. Results are summarized in Fig. 2. In this figure are shown observations in a typical midseason month. Oviposition was significantly higher (P<005) in the black (21.5) than in the green (7.44) barrels, and in the green than in the white (1.0) barrels. Early in the season, despite the limited number of egg masses, the attraction of the different barrels remained as above. In fact, the least attractive barrel, W (white), almost did not attract any females (Fig. 2A).

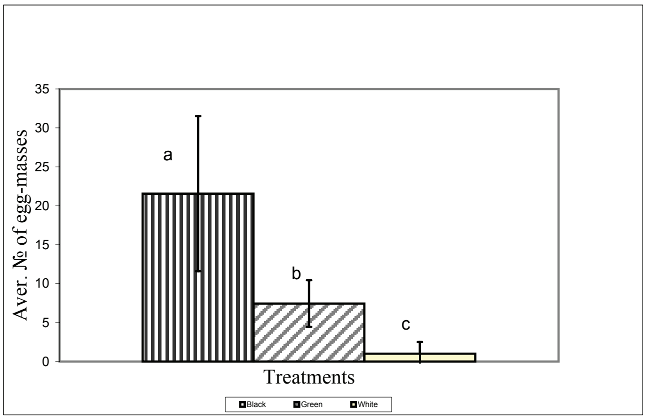

Fig. 2. Experiment near Waste Stabilization Ponds (WSP). Mean Number of egg-mass in - Black, Green and White barrels with tap water. Bars with different letters are significantly different (P< 0.001).

Experiment III. Barrels were filled with diluted (1:3) oxidation pond water. *Euglena* sp., the green unicellular alga, was the dominant phytoplankton (almost exclusive). Algae density on day 2 post-dilution was $7.5*10^3$ cells/L., but by day 11 it had reached these levels: B, black: $1.5 * 10^4$ / ml; G, green: $1.5 * 10^5$ / ml; S, silver: $2.5 * 10^5$ / ml.

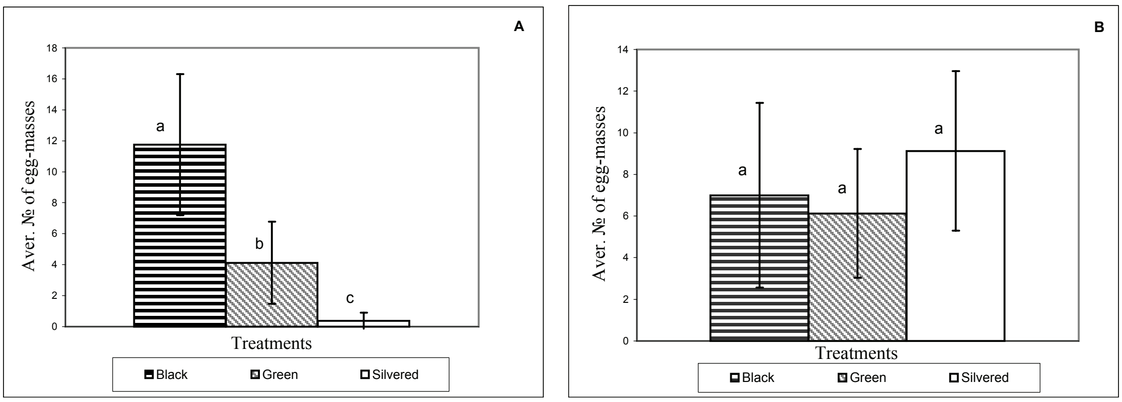

Fig. 3. Oxidation pond water experiment. Mean Number of egg-masses/day collected in Black, Green and silvered barrels filled with diluted (1:3) pond water. A data on day 2 (diluted phytoplankton) P<0.05). B day 11, during phytoplankton blooming, P> 0.05. Bars with same letter are not significantly different.

The load of egg-masses collected on days 2 and 11 of the experiment are shown in Fig. 3A and 3B. In 3A it is seen that females made a distinct choice in favor of the black barrels (21.6) rather than the green (6.9), and only 1.6 of the silver (P< 0.05). After 9 days all barrels yielded similar numbers of egg masses (P> 0.05). Moreover, shiny silver barrels attracted even higher numbers of ovipositing females (18.25) than the other two: black (13.5) and green (12.25). At this stage light rays were reflected from the shiny bottom and wall above and below the water surface (on S) and were non-polarized.

Intensity of this light was higher than intensity of polarized light reflected from the water surface. It reduced the effect of polarization. The numbers of laid eggs descended in the order black > green > white. Ten days later (Fig. 3B) phytoplankton reached the blooming stage, which was more intensive in the shiny silver barrels that absorbed maximum sun radiation (Fig. 4). Results indicate that at the early stage (Fig. 3A) females responded to light reflected from the barrel walls according to its colour, at the second stage (Fig. 3B) females' vision responded to the polarized reflected water surface rays.

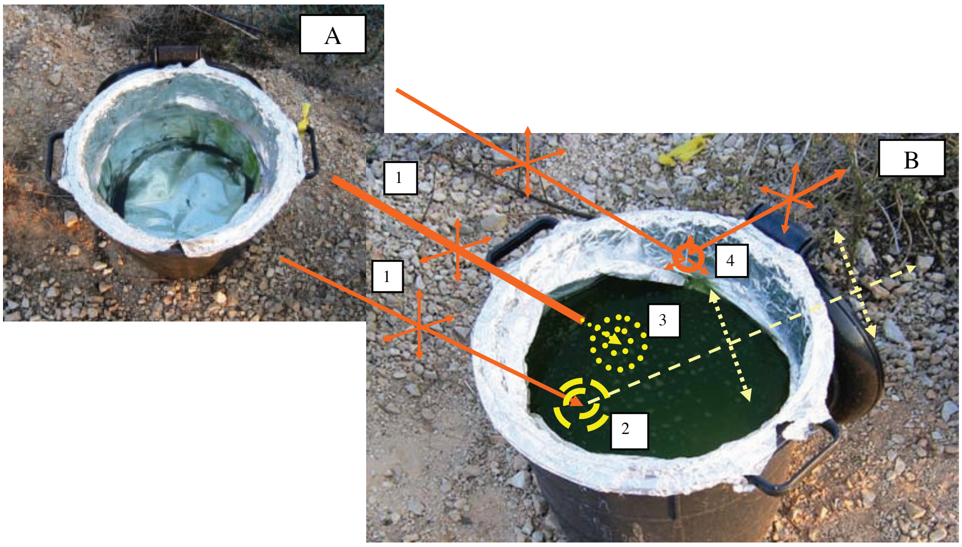

Fig. 4. A. Clean water with very low concentration of algae. All rays were reflected from the shiny walls and bottom of barrel are non polarized. B. Silvered barrel, filled with pond water after development of *Euglena* algae en masse. (1) Non-polarized rays hits water surface and barrel walls above and below water. (2) Dark shiny water surface reflected horizontally polarized light. (3) Rays inserted into barrel depth absorbed by dark water. (4) Rays hit the aluminum foil above water surface, reflected without polarization.

Experiment IV. In the prior experiments we confirmed that differential reflection of light through the different treatments/colours support females' site selection. However, although we assumed that this was related to sensing horizontal polarized light we could not exclude light intensity as being important. This question was resolved at the next stage (Fig 4). In the experiment with light traps significantly higher numbers of females were captured in polarized than non-polarized traps (fig 4; mean numbers: 42.0 and 25.75). The number of males trapped in both polarized and non-polarized traps, which were opened very close to water surface, was three times lower than the number of females. However, the difference in the numbers of males trapped by polarized and non-polarized traps was insignificant (11.25 and 8.25).

DISCUSSION

Recent investigations of the structure and function of the compound eye of various insects from adult Ephemeroptera to Diptera showed that they can detect polarized light, and that this capacity is indispensable for orientation and navigation toward their target (Horvath, 1994; Homberg, 2004). Specific ommatidia within compound eyes, usually arranged at the dorsal rim, are responsible for perception of the polarized rays (MEYER & DOMANIKO, 1998; LABHART & MEYER, 1999). An early and well known observation by KARL VON FRISCH(1948) explained the use of the vertically polarized skylight by honey bees returning to their beehives (DACKE *et al*, 2002).

Horizontally polarized light was confirmed by SCHWIND (1991) to be an effective signal in various aquatic insects for emergence from a water body. On the other hand, migrating swarms of desert locusts followed crossing Egypt, the Sinai Peninsula and southern Israel, were found to avoid flying above seawater, being able to detect horizontal polarized light and associate it with the water surface (SHASHAR *et al*., 2005). Several authors have reported that aquatic insects missed their natural water habitats and laid their egg-masses on artificial black surfaces, such as oil lakes (HORVATH & ZELL, 1996) or black roads after rain (THIENEMANN, 1954, cited by NOLTE 1993), mistaking wet roads for water bodies, which appear darker than land, as would be seen by eyes that recognize horizontally reflected polarized light. DATHANARAYANA & DASHPER (1986) listed Chironomidae as a family that can recognize polarized light, but they did not refer to the functional use of this ability in the chironomids' life. In the present experiments we proved that (A) chironomid females select their breeding habitat and males play no part in this stage; (B) selection is solely by visual sensing; (C) polarized light is the main factor involved in habitat selection.

We suggest that the use of polarized light is an optimal choice for ephemeral adults, acting as a speedy compass. Chironomini species observed by us in Israel must emerge from the water, mate, and return to the waters edge to lay their eggs. In our observations they either completed full reproductive activity during sunset before darkness, or reached the water's edge but waited for sunrise the next day to oviposit. We speculate that females discriminate reflected light of water while still copulating. Chironomids share a similar mode of life with the Ephemeroptera, despite the wide taxonomic distance between them. The adults of both groups live are shortlived and do not feed (with few exceptions), relying on energy accumulated during the benthic larval stage. Both form swarms near water bodies in order to mate, and females must rapidly find a water body before energy reserves expire. Mayflies and non-biting midges both mistake anthropogenic habitats – namely wet asphalt roads - for the dark water surfaces (see NOLTE 1993, citing THIENEMANN 1954 for chironomids, and KRISHKA & HORVATH, 1998 for mayflies). It has already been indicated that mayflies can detect water by reflected polarized light (KRISKA, BERNATH *et al*., 2006) and in the present research we widened the scope to include Chironomidae in such behaviour.

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