Competition among Aedes aegypti larvae in microcosms

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Abstract

Female larvae of the Aedes aegypti mosquito outcompete male larvae for food. Male larvae pupate earlier and at smaller masses in vials where competition is most intense. Competition at the higher densities causes the food/larva to appear lower than the equivalent mg/larva at lower densities for both sexes. The total food per vial appears to change the nature of the exploitative competition among females, which affects the competition among males. No interference competition was observed; females may dominate the competition by their larger size (increased filtering ability) and by retaining food particles within their guts to extract a larger fraction of their nutrients. Pupal mass for both sexes is primarily affected by food/larva; density affects the pupal mass through competition and the total food/vial (the interaction between food and density) and this interaction is different for the two sexes. Age at pupation is more affected by density than by food/larva and the interaction between food and density also differs across the two sexes for this variable. The nature of competition between and within the sexes at various food levels and densities is described. The implications of this complex response to food level and density among larvae on the ecology of this species is considered.

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Guidelines

It is easier to create a standardized solution of baker's yeast and dilute it down to the concentration necessary to add the calculated total mg per vial for each treatment. However, the yeast must be completely dissolved in the solution and it must be repeatedly stirred to keep it from clumping or settling.

Protocol

Step 1.
For experiment 1: Obtain mosquito eggs from an A. aegypti colony after feeding females on a mouse.

Step 2.
Hatch the eggs in distilled water, immersing them for at least 2 hours.

Step 3.
Assign random numbers to treatments: 20 treatments (4 food levels crossed with 5 densities, see below) with 5 replicates each.

Step 4.

Number 100 flat-bottomed shel vials (25 mm diameter x 95 mm tall) with random number and treatment.

Step 5.
Fill shell vials with 20 ml distilled water in each.

Step 6.
Weigh out baker's yeast and dissolve it in distilled water to achieve the following concentrations: 2 mg/larva, 3 mg/larva, 4 mg/larva and 5 mg/larva crossed with 5 densities (4, 5, 6, 7 and 8 larvae per vial). [2 mg/larva with 4 larva per vial requires a total amount of yeast of 8 mg, dissolved in 0.5 ml distilled water and added to the 20 ml in the appropriate vials—5 replicates of each treatment, for 20 treatments.]

Step 7.
Count 4, 5, 6, 7 or 8 larvae into each vial according to the treatment marked on the vial.

Step 8.
Arrange vials by number (randomized sequence) and cover with cheese cloth to prevent food or other mosquitoes from entering the vials.

Step 9.
Store vials in a room at ambient temperatures (in Florida) with a temperature range of 18 degrees C to 33 degrees C.

Step 10.
Day zero is the day the experiment begins. Check the vials for pupae beginning on day 4 (this is the day before any larvae are expected to pupate).

Step 11.
Remove each pupa into a holding shell vial with 10 ml distilled water. Record the original vial number, the treatment, the date (day of pupation). To pick up a pupa without disturbing the treatment vial, use a fire-polished wide mouth glass pipette (about 2 mm diameter) to capture the pupa at the water surface and transfer it to the holding vial. Capillary forces will capture the pupa, but you may have to gently blow the pupa out of the tube into the holding vial. Put only 1 pupa in each holding vial.

Step 12.
Remove each pupa from the holding vial by pipette as described, blot it on a piece of paper towel, pick it up gently with fine-tipped forceps and place it on a balance. Weigh it to the nearest 0.01 mg. Record the weight. Transfer it to the stage of a 10 x stereoscopic microscope and determine the sex of the pupa. Record the sex. Transfer it back to a vial of water to complete pupation (and eventually to rejoin the mosquito colony).

Step 13.
Continue until all larvae have pupated or died.

Step 14.
For experiment 2: Obtain mosquito eggs from an A. aegypti colony after feeding females on a mouse.

Step 15.
Hatch the eggs in distilled water, immersing them for at least 2 hours.

Step 16.
Assign random numbers to treatments: 4 treatments (2 food levels crossed with 2 densities, see below) with 40 replicates each.

Step 17.
Number 160 flat-bottomed shel vials (25 mm diameter x 95 mm tall) with random number and treatment.

Step 18.
Fill shell vials with 20 ml distilled water in each.

Step 19.
Weigh out baker's yeast and dissolve in distilled water to achieve the following concentrations: 3 mg/larva and 4 mg/larva crossed with 2 densities (5 and 6 larvae per vial). [3 mg/larva with 5 larva per vial requires a total amount of yeast of 15 mg, dissolved in 0.5 ml distilled water and added to the 20 ml in the appropriate vials—40 replicates of each treatment, for 4 treatments.]

Step 20.
Count 5 or 6 larvae into each vial according to the treatment marked on the vial.

Step 21.
Arrange vials by number (randomized sequence) and cover with cheese cloth to prevent food or other mosquitoes from entering the vials.

Step 22.
Store vials in an insectary at 26 degrees C and 12/12 light/dark cycle for the first four days and overnight thereafter.

Step 23.
Day zero is the day the experiment begins. Check the vials for pupae beginning on day 4 (this is the day before any larvae are expected to pupate).

Step 24.
Remove each pupa into a holding shell vial with 10 ml distilled water. Record the original vial number, the treatment, the date (day of pupation). To pick up a pupa without disturbing the treatment vial, use a fire-polished wide mouth glass pipette (about 2 mm diameter) to capture the pupa at the water surface and transfer it to the holding vial. Capillary forces will capture the pupa, but you may have to gently blow the pupa out of the tube into the holding vial. Put only 1 pupa in each holding vial.

Step 25.
Remove each pupa from the holding vial by pipette as described, blot it on a piece of paper towel, pick it up gently with fine-tipped forceps and place it on a balance. Weigh it to the nearest 0.01 mg. Record the weight. Transfer it to the stage of a 10 x stereoscopic microscope and determine the sex of the pupa. Record the sex. Transfer it back to a vial of water to complete pupation (and eventually to rejoin the mosquito colony).

Step 26.
Continue until all larvae have pupated or died.