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Competition in Aedes aegypti larvae: the effects of distributing food inputs over time

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ABSTRACT

This protocol details the three experiments analyzed in the manuscript, "Competition in Aedes aegypti larvae: the effects of distributing food inputs over time.

Experiment one

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

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

3 Assign random numbers to treatments: 16 treatments (2 food levels, 2 densities, 2 aliquot treatments, 2 timespan treatments; see below) with 10 replicates each.The 2 food levels are 16 mg/test tube or 32 mg/test tube delivered according to the aliquot and timespan treatments.The 2 densities are 4 larvae or 8 larvae per test tube.The aliquot treatment divides the total food into 2 or 4 aliquots (portions).The timespan of food delivery is either 3 days or 6 days, and the aliquots are spread evenly across the interval with the first aliquot on day 0 and the last aliquot on day 3 or day 6.The 4 aliquot treatment adds less food on day 0 and on the last day, but adds two more aliquots during the course of the experiment.

4 Label 160 new, disposable, round bottomed test tubes (25 mm diameter x 150 mm tall) with random number and treatment.

5 On day 0, fill test tubes with 20 ml distilled water in each.

6 Weigh out baker's yeast and dissolve it in distilled water to achieve the following concentrations: 8 mg/ml; 16 mg/ml; 32 mg/ml.Dissolve the dried yeast completely before carrying out further dilutions; there should be no residual clinging to the bottom or sides of the mixing vessel.

7 Add 0.5 ml of yeast suspension to each of the 160 test tubes according to treatment:treatments 3, 4, 7, 8, receive 0.5 ml of the 8 mg/ml suspension (4 mg food in each); treatments 1, 2, 5, 6, 11, 12, 15, 16 receive 0.5 ml of the 16 mg/ml suspension (8 mg food in each); treatments 9, 10, 13, 14 receive 0.5 ml of the 32 mg/ml suspension (16 mg food in each).

8 Count 4 or 8 larvae into each test tube according to the treatment marked on the test tube.

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

10 Store test tubes in a room at ambient temperatures (in Florida) with a temperature range of 18 degrees C to 33 degrees C.

11 Day zero is the day the experiment begins.

12 On day 1 weigh out baker's yeast and dissolve it in distilled water to achieve the following concentrations: 8 mg/ml; 16 mg/ml.Do not keep the yeast suspension from the previous day; always measure out fresh yeast.Add 0.5 ml of the 8 mg/ml suspension to treatments 3 and 7.Add 0.5 ml of the 16 mg/ml suspension to treatments 11 and 15.

13 On day 2 weigh out baker's yeast and dissolve it in distilled water to achieve the following concentrations: 8 mg/ml; 16 mg/ml.Do not keep the yeast suspension from the previous day; always measure out fresh yeast.Add 0.5 ml of the 8 mg/ml suspension to treatments 3, 4, 7 and 8.Add 0.5 ml of the 16 mg/ml suspension to treatments 11, 12, 15 and 16.

14 On day 3 weigh out baker's yeast and dissolve it in distilled water to achieve the following concentrations: 8 mg/ml; 16 mg/ml; 32 mg/ml.Do not keep the yeast suspension from the previous day; always measure out fresh yeast.Add 0.5 ml of the 8 mg/ml suspension to treatments 3 and 7.Add 0.5 ml of the 16 mg/ml suspension to treatments 1, 5, 11 and 15.Add 0.5 ml of the 32 mg/ml suspension to treatments 9 and 13.

15 On day 4 weigh out baker's yeast and dissolve it in distilled water to achieve the following concentrations: 8 mg/ml; 16 mg/ml.Do not keep the yeast suspension from the previous day; always measure out fresh yeast.Add 0.5 ml of the 8 mg/ml suspension to treatments 4 and 8.Add 0.5 ml of the 16 mg/ml suspension to treatments 12 and 16.

16 On day 5 there is no food added to any test tube.

17 On day 6 weigh out baker's yeast and dissolve it in distilled water to achieve the following concentrations: 8 mg/ml; 16 mg/ml; 32 mg/ml.Do not keep the yeast suspension from the previous day; always measure out fresh yeast.Add 0.5 ml of the 8 mg/ml suspension to treatments 4 and 8.Add 0.5 ml of the 16 mg/ml suspension to treatments 2, 6, 12 and 16.Add 0.5 ml of the 32 mg/ml suspension to treatments 10 and 14.

18 Check the test tubes for pupae beginning on day 4 (this is the day before any larvae are expected to pupate).Remove all the pupae before adding any more food.

19 Remove each pupa into a numbered holding shell vial with 10 ml distilled water. Record the original test tube number, the treatment, and the date (day of pupation). To pick up a pupa without disturbing the treatment test tube, 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 numbered holding vial.

20 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).

21 Continue until all larvae have pupated or died.

Experiment two

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

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

24 Assign random numbers to treatments: 12 treatments (4 food levels crossed with 3 densities, see below) with 5 replicates each.The food levels are 2 mg/larva, 3 mg/larva, 4 mg/larva and 5 mg/larva, for 3 densities: 1 larva/vial, 2 larvae/vial, and 3 larvae/vial.Note that the first and third experiments used food/test tube or total food as the treatment, while the treatments in the second experiment are food/larva.

25 Label 60 flat-bottomed shell vials (25 mm diameter x 95 mm tall) with random number and treatment.

26 Fill shell vials with 20 ml distilled water.

27 Weigh out baker's yeast and dissolve in distilled water to achieve the following concentrations:2 mg/larva, 3 mg/larva, 4 mg/larva and 5 mg/larva crossed with 3 densities (1, 2, and 3 larvae per vial).[2 mg/larva with 1 larva per vial requires a total amount of yeast of 2 mg, dissolved in 0.5 ml distilled water.At the other extreme, 5 mg/larva for 3 larvae requires 15 mg of yeast dissolved in 0.5 ml distilled water.The appropriate total amount of yeast for each treatment is added to the 20 ml in the 60 individual vials—5 replicates of each treatment, across the 12 treatments.]

28 Count 1, 2 or 3 larvae into each vial according to the treatment marked on the vial.

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

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

31 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).

32 Remove each pupa into a numbered holding shell vial with 10 ml distilled water. Record the original vial number, the treatment, and 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 numbered holding vial.

33 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).

34 Continue until all larvae have pupated or died.

Experiment three

35 For experiment 3: Obtain mosquito eggs from an A. aegypti colony after feeding females on a mouse.

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

37 Assign random numbers to treatments: 6 treatments (3 second food inputs, 2 delay treatments; see below) with 25 replicates each.Each treatment receives a first food input of 1 mg dry weight of yeast in 0.5 ml distilled water.The three second food inputs are 1 mg, 2 mg or 3 mg dry weight of yeast in 0.5 ml distilled water.The second food inputs are delivered on either day 6 or day 8 of the experiment.

38 Label 150 new, disposable, round bottomed test tubes (25 mm diameter x 150 mm tall) with random number and treatment.

39 Fill test tubes with 20 ml distilled water in each.

40 Weigh out baker's yeast and dissolve it in distilled water to deliver 1 mg of yeast in 0.5 ml of distilled water.All 150 test tubes receive 1 mg of yeast on day 0.

41 Place 1 larva into each of the 150 test tubes.

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

43 Store test tubes in a room at ambient temperatures (in Florida) with a temperature range of 18 degrees C to 33 degrees C.

44 Day zero is the day the experiment begins.

45 On day 6 weigh out baker's yeast and dissolve it in distilled water to achieve the following concentrations: 2 mg/ml; 4 mg/ml; 6 mg/ml.Do not keep the yeast suspension from the previous days; always measure out fresh yeast.Add 0.5 ml of the 2 mg/ml suspension to treatment 1.Add 0.5 ml of the 4 mg/ml suspension to treatment 2.Add 0.5 ml of the 6 mg/ml suspension to treatment 3.

46 On day 8 weigh out baker's yeast and dissolve it in distilled water to achieve the following concentrations: 2 mg/ml; 4 mg/ml; 6 mg/ml.Do not keep the yeast suspension from the previous days; always measure out fresh yeast.Add 0.5 ml of the 2 mg/ml suspension to treatment 4.Add 0.5 ml of the 4 mg/ml suspension to treatment 5.Add 0.5 ml of the 6 mg/ml suspension to treatment 6.

47 Check the vials for pupae beginning on day 4 (this is the day before any larvae are expected to pupate).Remove all the pupae before adding any more food.

48 Remove each pupa into a numbered holding shell vial with 10 ml distilled water. Record the original test tube number, the treatment, and the date (day of pupation). To pick up a pupa without disturbing the treatment test tube, 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 numbered holding vial.

49 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).

50 Continue until all larvae have pupated or died.