Manipulating predation risk

There are two usual contexts in which amphibian larvae (termed *assay tadpoles*) are exposed to chemical signals of predation produced by predators feeding on other tadpoles (termed *feeder tadpoles*). In one context, the predators are contained with a cage that floats or sits in the container where the assay tadpoles are held. In the other context, water containing predator chemicals is transferred into the container in which the assay tadpoles are held. Both methods work for all types of predators.

Context 1: Predators in cages

In this context, predators reside in cages that float within each mesocosm. The predators are fed regularly. Predators exude chemical cues (kairomones), the process of catching and consumes tadpoles produces chemicals, and there may be visual or hydraulic cues produced as well.

- The number of predators per mesocosm depends on your needs and the size of the mesocosms. I usually use between 0.0033-0.0125 predators/L (or 1.5-3.5 predators/m^2); higher for experiments in which high doses are part of the design.
- Each predator goes into an individual cage. We use 10-cm diameter plastic or PVC tubing (or well pipe), cut into 11-cm lengths. The volume of the cages is therefore about 1 L. Each cage contains a block of styrofoam (styropore). The two open ends of the cage are covered with fiberglass window screening (20×20 cm square), attached with two sturdy rubber bands (size 63 or 64 is best). If your rubber bands are narrower, you will need more of them.
- The styrofoam ensures that the cage floats. This simplifies the process of feeding predators (no need to reach way down to retrieve the cage). It also keeps the predators alive if oxygen is depleted during warm weather.
- In my experience, the cage design does not have to be modified for different kinds of predators. Even sticklebacks (*Gasterosteus aculeatus*) and large-ish adult newts (*Triturus alpestris*) do fine in these small cages.
- Check rubber bands carefully every time you feed. If the predator escapes, you have lost that mesocosm. Rubber bands will break after a little while in the sun. Ensure that both openings of the cage have at least two rubber bands, so that there is no problem if one breaks before the next feeding occasion.
- Feeding is usually done several times per week, because the predator releases chemical signals for only about 2-3 days after eating a tadpole. Fish kairomones probably don’t depend on recent consumption of tadpoles.
- The feeding is done as follows:
    - Decide how much food each predator will get. I feed 300 mg wet mass of feeder tadpoles per predator on each feeding occasion, three times a week (900 mg/week).
    - Lay out cups to hold the feeder tadpoles, one per predator plus a couple extras just in case. A small amount of water goes into each cup.
    - Count out three groups of 10 feeder tadpoles into three cups. Weigh each group and determine the mean mass of a tadpole. Decide how many tadpoles each predator receives to ensure the correct amount of food.
    - Count out the feeder tadpoles into the cups.
    - Take the cups out to your array of mesocosms. I always place them on a "feeding table" next to the experiment. It is good to be able to stand comfortably while doing the feeding, and you don’t want to open and close the predator cages right at the side of a mesocosm

(occasionally a feeder tadpole or a predator gets airborne!).

- Tadpoles are very sensitive to chemical cues of predation. The water in one mesocosm must never be allowed to drip into any other mesocosm. Wash the surface of the feeding table and rinse your hands off between treatments.
- I handle the cages in a very standardized way during feeding. Cages are turned over, dipped up and down twice, and then removed from the mesocosm. Turning over the cages is important because it forces you to inspect each rubber band every four days. Dipping the cages mixes the water a bit, and also standardizes the amount of disturbance.
- At the feeding table, remove the screen from one end of the cage, check that the predator is okay, and add the feeder tadpoles. All tadpoles may not be eaten from the previous feeding during periods of cold weather or when a dragonfly is about to moult. I do not normally worry about this; predators always eat all the tadpoles eventually.
- When feeding fish, I keep a 6-L bin with water on the feeding table, and submerge the predator cage in the water while doing the feeding. This somewhat reduces the stress for the fish. Other predator species do not mind a short time out of water.
- As you return the cage to the mesocosm, check that neither the predator nor any feeder tadpoles are stranded on top of the styrofoam block.
- I always rotate cages within treatments, to even out any differences among individual predators. Early in the experiment, when the tadpoles are small, you must inspect the cages very carefully to avoid carrying along small tadpoles with the rotating cages.
- If you are using dragonfly larvae, watch animals closely for signs that they are approaching metamorphosis. Those signs include swollen wing pads (in the final instar, wing pads will be 10-13 mm long in aeshnids), increasingly visible color in the side of the thorax and dorsal surface of the abdomen, and unwillingness to feed. Obviously the animal will die if it attempts to emerge in the cage or in a holding cup. You must release dragonflies when they approach metamorphosis.

<u>Context 2: Water transfer</u>

In this context, predators are held individually in 1-L or 5-L bins, or else predators are placed into individual cages that are suspended within a larger container (typically an 80-L plastic tub). In both cases, water from the predators is removed periodically and transferred into the containers where the assay tadpoles live.

- Water transfer should be done every day, if you can manage it. The reason is that predator cues break down fairly quickly. The behavior of assay tadpoles is no different from control tadpoles 48 hours after exposure to predator water.
- If the predators are kept in individual bins, be sure they cannot escape. Remember that *Aeshna* and adult newts will crawl out, fish can jump, and *Notonecta* can fly away even from the water surface.
- If the predators are kept in cages within larger tubs, fill up twice as many tubs as you need to hold the predators. If the predators are kept individually within small bins, lay out twice as many bins as you need.
- Each predator is fed at time *x* on day 1. The next day at time *x*, the predator is moved into clean water (aged 24 h from the previous day). The predators are then fed in their clean water. The water in which they spent the previous 24 h is added to the experiment. After the water is used, refill the extra predator containers so that the water is aged 24 h by the next day.
- The amount of predator-water added to the assay containers, and the amount of food fed to

each individual predator, requires you to perform some simple calculations. I rarely feed more than 2 mg tadpole wet mass per L of assay water per day. If you are feeding predators less often than daily, this comes to no more than 14 $mg \cdot L^{-1} \cdot week^{-1}$. Typical concentrations in my experiments are about 3-11 $mg \cdot L^{-1} \cdot week^{-1}$. If you know the volume of water in the predator containers and the mass of the feeder tadpoles, you can calculate how much water to add to each assay container.

- Predator-free treatments receive an equal volume of aged tap water added at the same time.
- You may want to impose a predator-replacement policy. For example, you might swap out the predator for a new one if it consumes less than half of the feeder tadpoles within 24 h.

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April 2012