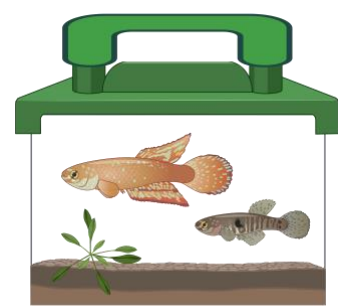


## Using Your Killi-Kit – The Official Step-by-Step Protocol



### Observation of killifish eggs:

- Killifish eggs come in a small plastic container filled with moist peat moss. Eggs will be sitting on top of this layer of peat. You should be able to see them with the naked eye.
- Using your paintbrush, gently pick up killifish eggs from on top of the peat and place in petri dish. Peat can be cleaned off the egg with the brush.
- Use the smart phone microscope to observe the tiny fish inside the egg. It might move, and you can see its heartbeat!
- Once you can see that the developing fish has large, sparkling eyes and a slow heartbeat, they are ready to hatch and have entered a dormant or diapause stage much like a deep sleep. They are waiting to hatch in water.



### Preparing the food:

- When the fish are ready to hatch, you should set up the brine shrimp hatchery 24 hours before you hatch the killifish. No hurry, killifish can wait in diapause for days to weeks to months!
- Cut a 1L plastic bottle into 2 pieces making the cut slightly above the base and invert the top of the bottle inside the base cap side down (see pamphlet and video link below).
- Add in half a liter of tap water to the bottle along with 1 tbsps. of non-iodized table salt and ¼ tsp. of brine shrimp eggs included in the kit.
- Plug in the air pump included with the kit and attach the airline tube to it (also included).
- Place the other end of the airline tube into the bottle. Wait 24 hours for the shrimp to hatch in the bubbling water. Adding a warm light over the shrimp may speed up hatching.
- 24 hrs. later, remove the tube from the bottle and wait 10 min. The hatched shrimp will sink to the bottom of the bottle near the cap and the shrimp eggshells will float.
- Use the pipette to collect the shrimp at the bottom and feed to the baby killifish.
- New brine shrimp should be hatched every 24-48 hrs. after cleaning out the brine shrimp hatchery.

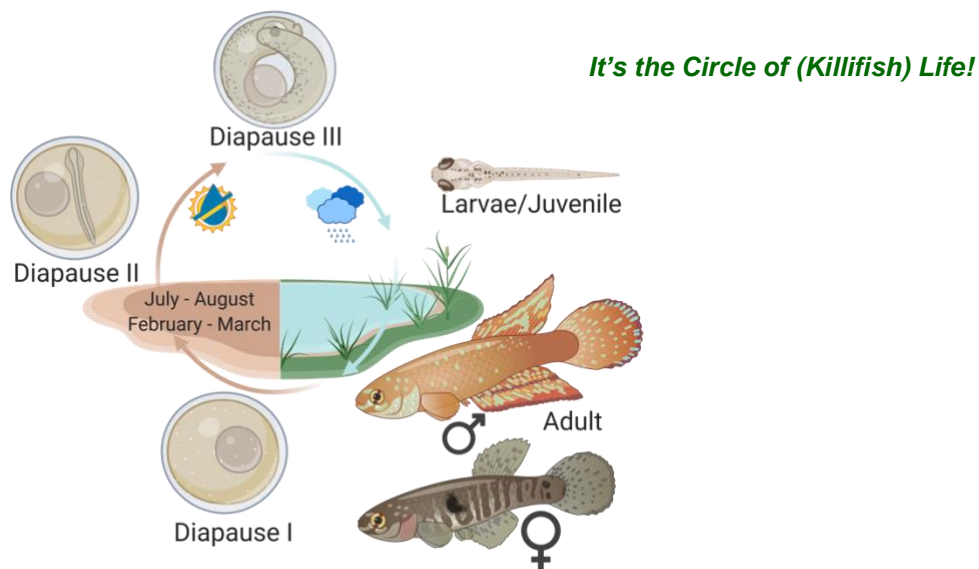
### Hatching your killifish:

- When you are ready wake up the baby killifish and hatch them out, simply add the entire contents of the container with eggs and peat to about 2-3 inches of water. **MAKE SURE the water is dechlorinated** with by adding dechlorinator to the water. You will see babies hatching and swimming around within 1-24 hours.
- Peat can be left in the water- it will help the fish hatch and give them places to hide.
- Babies can and will eat hatched brine shrimp as soon as they hatch out and require half to one pipette of food per day.
- If the fish do not hatch out after 24 hrs, recollect the peat and dry in it a paper towel until it is just slightly moist. Put the peat back in the sealed container and try hatching again in a week or two.



## Raising your killifish:

- Killifish will do well at room temperature as long it is not too cold (near an air conditioner or a basement) and ambient room lighting. Light from a window will be enough but do not keep the tank within direct sunlight.
- After hatching, successively raise the water level over the course of several days, adding about 2 inches per day. Then change 25% of the water each day with water that is about the same temperature as that in the tank. Remove any uneaten food with the pipette.
- Killifish will grow quickly and eventually need a larger aquarium in a couple weeks.
- Killifish can keep eating the hatched brine shrimp and once they are larger, starting around 2 cm/1 in., they will also eat dried or frozen fish food like adult brine shrimp or bloodworms (from the pet store).
- **DO NOT** release fish into the wild. If you can no longer care for them contact us or your local pet store.
- Males and females look very different, and they are very colorful. There are over 750 species of killifish. Which one did you hatch out?



## We would love to hear from you!

Questions and photos of you enjoying your kits can be sent to our email:  
[killi.kits@gmail.com](mailto:killi.kits@gmail.com)

For a video instructional guide see: <https://youtu.be/NDlhZ2liuKI>

For the Killi-Kit information pamphlet see:  
<https://drewt1023.wixsite.com/andrewwthompson/killi-kits>

Follow us on Twitter:

[@nematolebias](https://twitter.com/nematolebias)

[@fishevodevogeno](https://twitter.com/fishevodevogeno)

The Killi-Kit is an outreach project for citizen scientists of all ages, developed by Andrew W. Thompson (Dr. Drew) and Ingo Braasch from the **Fish Evo Devo Geno Lab** (Department of Integrative Biology, Michigan State University), and funded by the *NSF BEACON Center for the Study of Evolution in Action*. Images created with BioRender.com

