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We are still developing and optimizing this protocol

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DIY Intervention Testing in Daphnia: A simplified way to test lifespan interventions at home

Forked from DIY Lifespan Intervention Testing in Daphnia magna: A simplified way to test lifespan interventions at home

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ABSTRACT

This guide is for amateur science enthusiasts who are looking to conduct safe and easy animal experiments at home. This can be done with the aim of educating yourself and others about ecology, aquaculture, and pharmaco-biology. Additionally, by using a safe and accessible organism, you could contribute effort and data to the community science movement. We provide accessible ways for obtaining Daphnia, keeping daphnids at home, conducting experiments, troubleshooting, recording parameters of health and lifespan, and reporting the results. You will be able to begin immediately and build up gradually. A typical experiment might take 4-8 hours a week and last 1-2 months. The basic setup cost is around $50, depending on how food and equipment are sourced. We strongly encourage the reader to contact us with any suggestions and feedback to help further improve and develop this guide.

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Grandmother culture generation, your Daphnia reserve

1 **First steps**

> **Note**
>
> If you already have your grandmother culture and you can skip and go to the mother culture section.

1.1 Before introducing Daphnia, take a large spring water jug and cut off the top in order to have a light transparent bucket with a side handle.

> **Note**
>
> Best practice is to collect some rainwater (if not possible well/spring water is a good alternative) mark the water level and let it stand in indirect sunlight.

1.2 Add a drop/granule of any plant mineral fertilizer to generate “green water” which will serve both as food and provide autogenous biological filtration.

2 Once the water begins to turn, green Daphnia may be added.

> **Note**
>
> This process may be expedited by adding a small amount of soil.

3 Once the grandmother culture is established and stabilized the only regular water quality maintenance required is to compensate for evaporation by adding rain or distilled water to the original level.

Mother generation, the experimental set up

4 Once your grandmother culture is stabilizied, you can start the experimental set up.

Note

This section will need to be redone for each experiment.

5 **How to choose containers for the experiment**

Note

- Small, identically sized, clear containers (e.g. yogurt container or baby food jar). Ideally made of glass or a minimally porous plastic (acrylic, polycarbonate).
- The transparency will let light in and allow for photosynthesis of the algae as well as maintain a standard day/night cycle.
- The containers will need to be cleaned before and between uses and a porous or scratchable material will make it challenging to do this effectively.
- Do not use containers that may have an oily residue, this can inhibit algal growth and cause Daphnia to become trapped to the surface of the water.
- Be mindful of where these containers are placed during experiments, as they need to have equal and adequate sunlight and water temperature.

6 Identify and remove three large females, preferably ones with neonates visible in the brood chamber.

7 Isolate each of these females in their own container.

8 Feed these females spirulina every other day and complete 100% water changes on the other days.

Note

Within a few days, the isolated grandmothers should be laying clutches.

9 **Feeding**

Note

When feeding experimental groups consistency is key.

9.1 Create a mixture of 1 tsp (3g) freeze-dried spirulina in 1/3c (75mL) water (scale up proportionally).

Note

Ensure that it is mixed thoroughly and then filter through a fine fish net or similar to remove any fragments that have not gone into the solution.

9.2 Mix again and immediately pour into a silicone tray for making small ice cubes and then freeze.

9.3 A few hours before feeding the required amount of cubes can be thawed and remixed to ensure a constant concentration of food is used for the duration of your experiments.

Note

It is recommended to feed 4 drops or approximately 40uL per animal (if you have many animals every 10 drops is approximately 1 mL) but this may need to be adjusted depending on other conditions.

9.4 Check if there's uneaten food collects at the bottom, if so, try feeding less.

Note

Note that it is normal for detritus and biofilm to accumulate in this culture.

10 Water Changes and Removal of offspring

10.1

Pipette out the mother or mothers and transfer them into a new container with new water.

10.2 Check the container to ensure that no neonates have been transferred accidentally.

> **Note**
>
> If a suitable clutch has been collected, it may be raised as a group receiving regular feeds and water changes.

10.3 Once individuals in this group begin to show signs of sexual maturity you may split them and house them individually or in pairs.

> **Note**
>
> Any neonates the mother generation produces may be used for experimentation. Alternatively, if your mother generation is not producing enough neonates for your required sample size, create additional mother groups. It is acceptable to use the progeny of the mother generation (instead of the grandmother culture) to make these groups.

Daughter cultures, the execution of the experiment

11 Before starting the experiment, these conditions have to be checked:

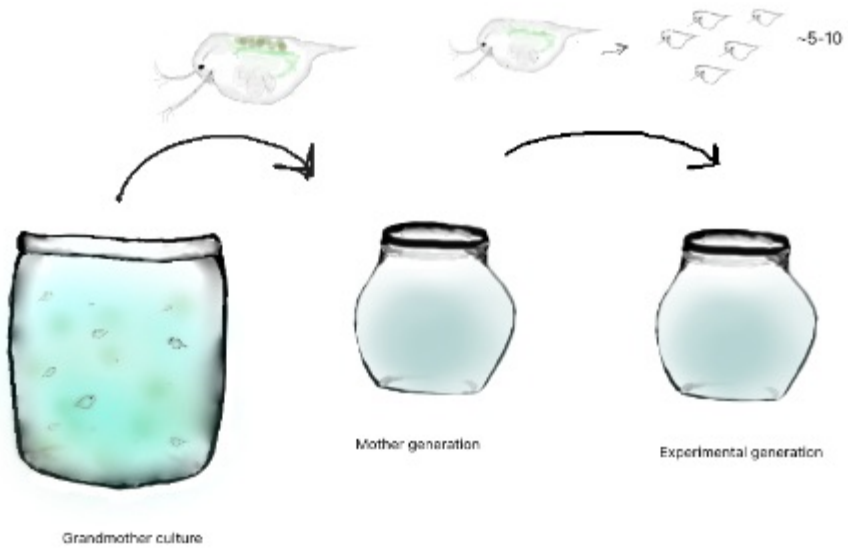
- The “mother generation” containers contain 1-2 mothers that can have clutches of 5-30 neonates each. (3.d)
- 10 mL per individual Daphnia so the volume of experimental containers will be the number of individuals multiplied by 10mL to make sure you have the correct number for your container or the correct container for the number. (3.b) We recommend only filling the volume of the container ⅔ of the way so that the media is not easily spoiled when the containers are being moved.

- In longevity experiments, adequate statistical power for conditions that may extend the life span by 10% or more typically require at least 50 individuals per group when followed throughout their lifespan (more is better, statistically). Consider this when setting up the mothers for your experiment.

Note

Please be sure to have enough of a control (at the very minimum 30 individuals and 4 jars, better more) , there should be at least five replicates

12 Synchronizing age



Using the sexually mature progeny of the grandmothers, set up as many jars of isolated mothers as will meet the needs of your experiment.

Note

Use the technique of removal of mothers as seen in step .

12.1

Try to pull females at the same egg stage to have a better chance of synchronizing neonate emergence.

12.2

Overfeed these mothers and perform water changes at least every 3 days (so waste wont build up from the increased feeding), this will lead to larger, more frequently produced and healthier clutches.

Note

Once female Daphnia reach sexual maturity they can produce a clutch every molt (approximately 5 days) and can produce up to 30 offspring depending on the conditions and maternal age. Mothers are significantly larger than the neonates are so the next few days is the ideal time to separate them!

12.3 Let the clutches reach sexual maturity to confirm that they are all female.

Note

You will want to use clutches born on the same day (or close) to have a consistent age across your experiment. To manage variation between the clutches, combine these clutches, they are now considered a single age cohort that can be split into experimental groups.

12.4

Note

If the experimental design requires it males can be used, however this can be a little more difficult and can require a microscope or magnifying glass for the immediate identification. The “mother generation” of male clutches may also need a different treatment in order to stimulate the production of males such as controlled stress like increased salinity, alkalinity or alcohol juvenile growth hormone III.

12.5 Reach the ideal density in the experimental groups of 10mL of water per daphnid.

Note

The size of your experimental groups will be dependent on the number of synchronized clutches you have as well as the volume of your containers.

13 **Removal of offspring without water changes**

Note

If you can do 100% water change, use the same technique as step 5 for the mothers, otherwise follow these steps:

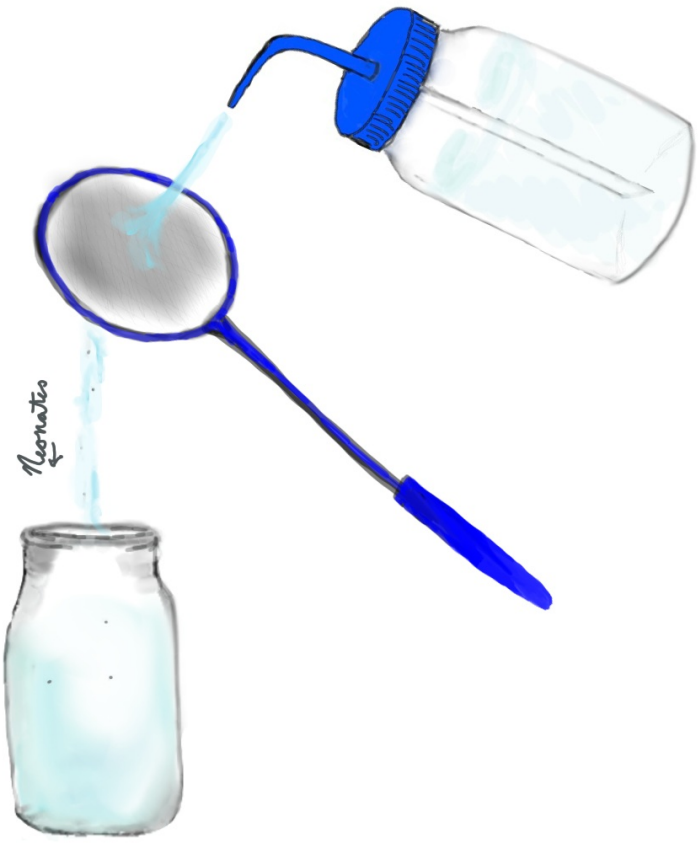
13.1

Temporarily remove individuals in the experimental group with a pipette. For groups of only a few daphnia you may leave them in the water in the pipette. For larger groups you should transfer them into your makeup water.

13.2

Filter the water through a mesh small enough to capture the offspring and animal molts while allowing the experimental solution to move into a separate, clean container.

13.3



If the offspring are being kept for counting or any other reason, quickly rinse them from the mesh into a new container.

Note

Do not use offspring from experiments for stock culture or any other experiments unless you specifically want to observe the effect of the current experimental conditions on offspring.

13.4

Reintroduce the experimental group into the water that is now free of the offspring.

Data collection workflow

14

Note

Suggested measurements and notes to take:

- Life span
- Number of offspring or fecundity
- Physical changes
- Behavioral changes

15

Life span

15.1 Start with Daphnia born on the same day and ensure that the neonates from each mother are split evenly between groups, refer to step to see how to achieve this best.

15.2

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
	Experiment 1) Cohort start date of birth xxfxxfxxxx														
	date	Jar 1 (alive 1yes/0no)	neonates y/n	# neona	notes	date	Jar 2 (alive 1yes/0n	neonates y/n	# neona	notes	date	Jar 3 (alive 1yes/0n	neonates y/n	# neona	notes
	5-May	1	n	0		5-May	1	n	0		5-May	1	n	0	
	6-May	1	n	0		6-May	1	n	0		6-May	1	n	0	
	7-May	1	n	0		7-May	1	y	7		7-May	1	n	0	
	8-May	1	y	15		8-May	1	n	0		8-May	1	y	5	
	9-May	1	n	0		9-May	1	n	0		9-May	1	n	0	
	10-May	1	n	0		10-May	1	n	0		10-May	1	n	0	
	11-May	1	n	0		11-May	1	n	0		11-May	1	n	0	
	12-May	1	n	0		12-May	1	y	23		12-May	1	n	0	
	13-May	1	y	22		13-May	1	n	0		13-May	1	y	9	
	14-May	1	n	0		14-May	1	n	0		14-May	1	n	0	
	15-May	1	n	0		15-May	1	n	0		15-May	1	n	0	
	16-May	1	n	0		16-May	1	n	0		16-May	1	n	0	
	17-May	1	n	0	eph	17-May	1	y	28		17-May	1	n	0	
	18-May	1	n	0		18-May	1	n	0	red body	18-May	1	y	11	
	19-May	1	n	0		19-May	0	n	0		19-May	1	n	0	
	20-May	1	n	0		20-May	0	n	0		20-May	1	n	0	
	21-May	1	y	20		21-May	0	n	0		21-May	1	n	0	
	22-May	1	n	0		22-May	0	n	0		22-May	1	n	0	
	23-May	1	n	0		23-May	0	n	0		23-May	1	y	10	
	24-May	1	n	0		24-May	0	n	0		24-May	1	n	0	
	25-May	1	y	25		25-May	0	n	0		25-May	1	n	0	
	26-May	1	n	0		26-May	0	n	0		26-May	1	n	0	
	27-May	1	n	0		27-May	0	n	0		27-May	0	n	0	
	28-May	1	n	0		28-May	0	n	0		28-May	0	n	0	
	29-May	1	y	27		29-May	0	n	0		29-May	0	n	0	
	lifespan	25					14					22			
	fecundity			109					58					35	

Note this day as day one for your cohort as well as the day of death for each individual along with their condition (experiment specifics, control etc.).

Note

Life span in Daphnia can be easily measured in days given that the average life span is around 20-30 days. However, it is normal for there to be significant deviation from this usually ranging between 10 and as many as 100 days. This is why it is very important to know the baseline for your particular culture and the conditions of your control groups (cohorts kept in identical conditions of experimental groups but without interventions like drugs).

16 Fecundity

Note

Fecundity is when and how many offspring are produced; here are some things to remember when measuring it:

- Daphnia can have offspring at every molt. For healthy animals, molting happens every 3-5 days and the neonates grow very quickly. You do not want to skip offspring removal for very long or they will grow and become indistinguishable from the group being studied. Offspring will also change the conditions in the container (food availability, waste accumulation, etc.). This creates additional variation between your experimental groups.
- Neonates are very small when they first emerge and are challenging to visualize and count. First, separate them from the mothers and allow them to grow for a couple of days when this is easier.
- Fecundity is likely to change over the lifespan of an individual as they age. The timing and rate of this change may be as interesting to study as the average total fecundity between groups.

Fecundity reflects the health, stress, age, and functionality of Daphnia. Changes in fecundity within replicates can reflect unseen variables that are much more difficult to control at home.

17 Physical changes

17.1

Note

Color changes are the easiest parameter to observe in a meaningful way. Daphnia will turn red when in water with low dissolved oxygen as it triggers the production of hemoglobin. The extra hemoglobin makes them better able to absorb and use oxygen
Daphnia can also appear brownish or orange when they are building up lipid droplets as fat storage.

17.2

Check the color of the gut tract.

Note

The greenish color of the gut tract or if it is throughout the gut or not can be indicative of Daphnia health. If it is partially brown or completely clear this is a sign of health issues that could stem from low food availability, inability to eat properly, not digesting correctly, or other health issues, which could be useful to monitor and track.

17.3 Check the general shape of the Daphnia.

Note

Daphnia can experience developmental problems or altered body shape under certain conditions. Body shape changes are easier to observe under magnification than with the naked eye.

17.4 Check if there's an abnormal proportion of males.

Note

The production of male neonates can be indicative of stress or damage to the mother. See the above “Female vs. male” for more insight on what can cause this. If your experimental groups are making more males -- it is an interesting feature to track.

18

Behavioral changes

Note

Many behavioral changes may happen. Here we described only a few, common behavioral changes. You may find others in your experiment. As always, differences from your control are of particular interest, even though behaviors in the control can also matter to judge if conditions are correct.

18.1 Check if the Daphnia are swimming differently, less or more.

18.2 Check if an experimental group increased or decreased feeding rate compared to the control.

18.3 Check if the Daphnia are producing ephippia/resting eggs.

18.4 Check if the Daphnia started staying on the bottom of the jar or if they are occupying any specific vertical area in the jar.

18.5 Check if the Daphnia are still responding to light.

Start testing drugs !

19

Safety information

Properly labeling chemicals is always important but at home, there are added safety risks. Ensure that all equipment (chemical and chemical measuring tools) are clearly labeled and kept completely separate from home goods. Items like measuring cups, tablespoons, and stirrers should never be shared. All drugs and drug solutions must be kept out of reach of children and pets.

20 **Selecting and storing drugs**

Note

Restrict your analysis to water-soluble drugs so that Daphnia are exposed uniformly in the intervention jars.

20.1 Search online for a drug's water solubility.

20.2 If your drug is in tablet form then thoroughly crush it before trying to get it into solution.

> **Note**
>
> Though it is not the case with simple compounds like table salt and baking soda, many medications will lose potency if they are not stored under conditions specific to their composition. For this reason, it is recommended that drug solutions be mixed fresh before each water change.

21 Find your drug's toxic dose in literature

21.1 Check this paper if your drugs has a known EC50 and LC50 (or LD50) in Daphnia : https://doi.org/10.1016/j.scitotenv.2020.143038

21.2 If your drug isn't listed, check the litterature for an available LC/EC 50 in Daphnia.

21.3 If you can't find it in the literature for Daphnia, check for another invertebrate or aquatic model (i.e. C. elegans and zebrafish).

21.4 If there's still no information, it is recommended that you perform at-home toxicology bioassays to help find your starting dose.

22 Short-term toxicity test: Find your EC048

22.1 Collect a large group of neonates and split them evenly between seven containers.

Note

As a dose that is lethal to 50% of animals is always going to be higher than a dose that has an effect on 50% of animals we recommend using different dilutions depending on if you are starting with an EC50 or LC50, for your drug.
EC 50:
0% (control), 0% (control), 100%, 50%, 20%, 10%, 5%
LC 50:
0% (control), 0% (control), 50%, 25%, 10%, 5%, 1%

22.2 Make 2 of these containers the controls and have the other five contain the LC50:

- 50% the concentration of the LC50
- 25% the concentration of the LC50
- 10% the concentration of the LC50
- 5% the concentration of the LC50
- 1% of the concentration of the LC50

22.3 Lightly feed your neonates and leave them in their solutions for 48 hours.

22.4 Check the containers after 48h and count the number of immobilized neonates. Your EC048 is the highest concentration at which there is no difference from the experimental to control group.

23 Prolonged toxicity test: Finding your EC07 Days

Do the same steps as for your EC048h but you leave your experimetal group exposed to the drug for 7 days.

23.1 Chekc for animal reproductive maturation (when do they start producing ephippia or neonates) and note the day that each group becomes reproductive.

Note

Reproductive fitness is an indicator of overall fitness and if none of your dosages reach reproductive maturity within 4 days of the control restart this trial at lower doses. If you have dosages that have comparable survival to the control after 7 days but do not reach maturity, you will want to extend your trial to determine this.

23.2 If the dose has animals reaching sexual maturity within 4 days of the control then it is an appropriate dose for a longevity experiment. Continue to maintain experimental conditions for the lifespan of the animal recording whichever findings are relevant to your investigation see section.

Note

If no group reaches sexual maturity within a difference of 4 days then the experiment should be re-run with lower doses.

24 **Full scale longevity testing**

Note

Once these dosages have been confirmed and initial findings have been noted you are ready to run a full-scale longevity experiment.
It is recommended that a second longevity experiment be run with these dosages which has a more robust number of replicates as described in section X . This will make any findings significant enough to be reportable.

Discussion and Future work

25 If you want to go a step further, you can do a smurf assay.
For more information check: https://bio-protocol.org/pdf/bio-protocol2722.pdf

26 Sharing data is an important part of this open science project, guidelines will be provided soon so stay informed !